Società Chimica Italiana
Divisione di Spettrometria di Massa

Lepore mare
Passione dal 1948

Torre Canne (Brindisi, Italy)

October 16-18, 2024

Scientific Programme

Book of Abstracts

8th MS FOOD DAY

October 16-18, 2024

Torre Canne (BR) - Italy

COMMITTEES

Scientific Committee

Gianluca Giorgi	*Università di Siena*
Giuseppe Avellone	*Università di Palermo*
Giuliana Bianco	*Università della Basilicata, Potenza*
Franco Biasioli	*Fondazione Edmund Mach, S. Michele A/A (TN)*
Lucia Bonassisa	*BonassisaLab S.p.A., Foggia*
Anna Cane	*Istituto Nutrizionale Carapelli, Firenze*
Chiara Dall'Asta	*Università di Parma*
Roberta Galarini	*Ist. Zooprof. Sperim. Umbria e Marche, Perugia*
Renzo Galli	*Fileni, Cingoli (MC)*
Davide Garbini	*Coop Italia, Casalecchio di Reno (BO)*
Luciano Navarini	*illycaffè, Trieste*
Michele Palmisano	*Lepore Mare, Fasano (BR)*
Michele Suman	*Barilla e Università Cattolica del Sacro Cuore*
Sauro Vittori	*Università di Camerino*

Organizing Committee

Gianni Lepore	*Lepore Mare, Fasano (BR)*
Nicola Camarda	*Lepore Mare, Fasano (BR)*
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Claudia Vatteroni	*Parmalat, Milano*

ORGANIZED BY

Società Chimica Italiana
Divisione di Spettrometria di Massa

Lepore mare
Passione dal 1948

WITH THE PATRONAGE OF

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SCIENTIFIC PROGRAMME

Wednesday, October 16, 2024

2:00 p.m. Registration

2:30 p.m. Welcome addresses

Gianni Lepore, Michele Palmisano
Lepore Mare spa, Fasano (BR, Italy)

Giuliana Bianco
University of Basilicata, President of the Division of Mass Spectrometry of the Italian Chemical Society

Gianluca Giorgi
University of Siena, 8 MS Food Day Scientific Committee

Awards & Fellowships Ceremony

3:00 p.m. **Session 1:** *Chairpersons: Franco Biasioli, Giuliana Bianco*

3:00 p.m. **PL1: *In vivo* aroma release and perception of complex food matrices**
Markus Stieger
Wageningen University, Wageningen (The Netherlands)

3:45 p.m. **OR1: Unraveling the gut volatilome dynamics and inter-individual variability during *in-vitro* digestion and fermentation of black beans**
Andrea Dell'Olio, Josep Rubert, Iuliia Khomenko, Martina Moretton, Vincenzo Fogliano, Franco Biasioli
Food Quality and Design, Wageningen University and Research, Wageningen (The Netherlands)

4:00 p.m. **OR2: Driving Innovation in Food Volatiles Analyses by Mass Spectrometry**
Jonathan Beauchamp
Department of Sensory Analytics & Technologies, Fraunhofer Institute for Process Engineering and Packaging IVV, Freising (Germany)

4:15 p.m. **OR3: Real-time wine off-flavor analysis with chemical ionization mass spectrometry**
Luca Cappellin, Felipe Lopez-Hilfiker, Luigi Ciotti, Manuel Hutterli
Dipartimento di Scienze Chimiche, Università degli Studi di Padova, Padova (Italy)

4:30 p.m. **OR4: Lycopene extraction with α-pinene, natural volatile deep eutectic solvent menthol-thymol and extra virgin olive oil**
Azzurra Stefanucci, Lorenza Marinaccio, Eulogio J. Llorent-Martinez, Adriano Mollica
Department of Pharmacy, University G. d'Annunzio, Chieti (Italy)

4:45 p.m. **OR5: Authentication of Italian monovarietal extra virgin olive oils through HS-SPME-GC-MS, HPLC-DAD-MS and sensory analysis in combination with a GA-LDA-HCA statistical approaches for their clustering**
Tommaso Ugolini, Federico Mattagli, Alessandro Parenti, Fabrizio Melani, Marzia Migliorini, Nadia Mulinacci, Bruno Zanoni, Lorenzo Cecchi

DAGRI – Department of Agricultural, Food, Environmental, and Forestry Sciences and Technologies, University of Florence, Florence (Italy)

Session 2: *Chairpersons: Linda Monaci, Giuseppe Avellone*

5:00 p.m. **OR6: Inert columns, a "passive" solution for an active problem**

Emanuele Ceccon

Restek, Cernusco S/N (MI, Italy)

5:15 p.m. **OR7: Analysis of the volatilome of virgin olive oils: quality grade evaluation and study of modifications during storage**

Rosalba Tucci, Enrico Casadei, Enrico Valli, Chiara Cevoli, Silvia Mingione, Francesca Baroccio, Sara Barbieri, Alessandra Bendini, Stefania Carpino, Tullia Gallina Toschi

Department of Agricultural and Food Sciences, Alma Mater Studiorum - Università di Bologna, Cesena and Bologna (Italy)

5:30 p.m. **OR8: Virgin olive oil authenticity assays in a single run using two-dimensional liquid chromatography-high resolution mass spectrometry**

Irene Caño-Carrillo, Bienvenida Gilbert-López, Cristina Ruiz-Samblás, Antonio Molina-Díaz, Juan F. García-Reyes

Analytical Chemistry Research Group, Department of Physical and Analytical Chemistry, University of Jaén, Jaén (Spain)

5:45 p.m. **OR9: Quality and safety of a traditional and healthy Algerian product**

Benedetta Sgrò, Qada Benameur, Nadra Rechidi-Sidhoum, Giuseppa Di Bella, Vincenzo Lo Turco, Angela Giorgia Potortì

Department of Biomedical and Dental Sciences and of Morphological and Functional Images (BIOMORF), University of Messina, Messina (Italy)

6:00 p.m. **OR10: Liquid chromatography coupled with mass spectrometry for multiple detection of hidden allergens in bakery-products: two case studies produced at pilot plant scale**

Anna Luparelli, Elisabetta De Angelis, Rosa Pilolli, Francesca Lambertini, Linda Monaci

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

6:15 p.m. **IP1: SFC-MS: a viable alternative to LC-MS in food safety analysis**

Paolo Redegalli

Shimadzu Italia, Milano (Italy)

6:30 p.m. End of session

6:45 p.m. Welcome cocktail

Thursday, October 17, 2024

9:00 a.m. **Session 3:** *Chairpersons: Marco Gaspari, Maria Assunta Acquavia*

9:00 a.m. **PL2: Presence of microplastic in food and beverage: do analytical methods matter?**

Stefania Gorbi

Univ. Politecnica delle Marche, Ancona (Italy)

9:45 a.m. **OR11: Food-borne thermally induced contaminants: mass spectrometry platform for the determination of the S/R ratio of 3MCPD esters and for the identification of glycidyl esters in oils and fats**

Alessia Lanno, Michela Torrelli, Simone Stefano, Sofia Ghironi, Alice Passoni, Alessandra Roncaglioni, Renzo Bagnati, Elena Fattore, Enrico Davoli

Laboratory of Mass Spectrometry, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan (Italy)

10:00 a.m. **OR12: A method for determining PFASs in bovine meat and tuna fish by UHPLC- HRMS Orbitrap**

Andrea Roviaro, Aurora Scarduzio, Sara Savini, Anna Sannino

Chemical Safety Department, Stazione Sperimentale per l'Industria delle Conserve Alimentari (SSICA), Parma (Italy)

10:15 a.m. **OR13: Recycled and virgin PET bottles and plastic cups: migration potential of contaminants**

Laura Barp, Sabrina Moret

University of Udine, Department of Agri-Food, Environmental and Animal Sciences, Udine (Italy)

10:30 a.m. **OR14: Quantitative LC-MS analysis of mycotoxins in plant-based protein isolates**

Romain Gabioud, Sasa M. Miladinovic

Institute of Life Sciences, University of applied sciences Western Switzerland, Sion (Switzerland)

10:45 a.m. **IP2: DART-MS for food analyses: new trends and developments**

Giuseppe Labella

Bruker Italia, Milano (Italy)

10:55 a.m. Poster session, Coffee break

11:45 a.m. **Session 4**: Joint session with the Divisione di Chimica degli Alimenti-SCI
Chairpersons: Nadia Mulinacci, Sauro Vittori

11:45 a.m. **OR15: A multiparametric study for the monitoring of contaminants in biological fluids**

Michele Spinelli, Carolina Fontanarosa, Stefano Lorenzetti, Rosita Gabbianelli, Alessandra Perna, Angela Amoresano

University of Campania "Luigi Vanvitelli", Department of Physical and Mental Health and Preventive Medicine School of Medicine and Surgery, Naples (Italy)

12:00 p.m. **OR16: Two years (2023-2024) of Perfluoroalkyl substances (PFASs) results from Italian official food control plans: what are the raising issues?**

Tamara Tavoloni, Arianna Stramenga, Ester Lucidi, Martina Ciriaci, Francesco

Griffoni, Arianna Piersanti

Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Ancona (Italy)

12:15 p.m. **OR17: CP-MIMS: a new frontier for the real-time monitoring of hazardous chemical migration from food contact materials**

Maurizio Piergiovanni, Monica Mattarozzi, Federica Bianchi, Nicolo Riboni, Cristian Maffezzoni, Veronica Termopoli, Viviana Consonni, Davide Ballabio, Maria Careri

Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma (Italy)

12:30 o.m. **OR18: Optimising QuEChERS for the analysis of mussels: efficient extraction of Emerging Contaminants in environmental samples**

Julia Gambetta Vianna, Barbara Benedetti, Marina Di Carro, Emanuele Magi

Department of Chemistry and Industrial Chemistry, University of Genoa (Italy)

12:45 p.m. **IP3: Tecniche avanzate di arricchimento e di separazione combinata alla spettrometria di massa per la caratterizzazione dei profili aromatici nel settore food & beverage**

Daniele Morosini, Daniela Peroni

SRA Instruments, Cernusco sul Naviglio (MI, Italy)

12:55 p.m. Buffet lunch

2:30 p.m. **Session 5:** *Chairpersons: Luciano Navarini, Paola Montoro*

2:30 p.m. **PL3: Bitter taste and mass spectrometry**

Roman Lang

Technical University of Munich (Germany)

3:15 p.m. **OR19: rECOBIOpack project: optimization of lipid extraction for coffee silverskin valorization**

Chiara Scapuzzi, Tamara Chwojnik, Luisella Verotta, Luciano Navarini, Stefania Lupinelli, Stefania Marzorati

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

3:30 p.m. **OR20: Characterization of alkylpyrazines by HS-SPME-GC-MS on roasted coffee from different botanical species and *terroir***

Valentina Lonzarich, Elisabetta De Angelis, Luciano Navarini

illycaffè S.p.A., Trieste (Italy)

3:45 p.m. **IP4: Analisi degli Alimenti: il nuovo Triplo Quadrupolo Agilent 7010D**

Marica Beggio

AgilentTechnology Italia, Cernusco Sul Naviglio (MI, Italy)

3:55 p.m. Poster session, Coffee break

4:45 p.m. **Session 6:** *Chairpersons: Riccardo Flamini, Fabiana Piscitelli*

4:45 p.m. **OR21: Hazelnut products traceability through combined isotope ratio mass spectrometry and multi-elemental analysis**

Michele Suman, Giuseppe Sammarco, Mattia Rossi, Daniele Cavanna, Laura Viotto, Piero Pettenà, Chiara Dall'Asta, Paola Iacumin

Analytical Food Science, Barilla G. e R. Fratelli S.p.A., Parma (Italy)

5:00 p.m. **OR22: Assessment of Pomodoro Riccio metabolomic profile through a multimethodological approach for food safety**

Valeria Vergine, Alba Lasalvia, Christian Rolando, Donatella Ambroselli, Luisa Mannina, Giuliana Vinci, Cinzia Ingallina, Maria Elisa Crestoni

Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, Roma (Italy)

5:15 p.m. **OR23: All-ion fragmentation and high-resolution mass spectrometry as key tools for the untargeted profiling of glucosinolates in Brassica microgreens**

Andrea Castellaneta, Ilario Losito, Giovanni Cisternino, Beniamino Leoni, Pietro Santamaria, Cosima Damiana Calvano, Giuliana Bianco, Tommaso R.I. Cataldi

Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Bari (Italy)

5:30 p.m. **OR24: Antioxidant reactivity of anthocyanins in red cabbage with an AAPH-incubating method using liquid chromatography coupled with high-resolution tandem mass spectrometry**

Claudia Lombroni, Lucrezia Angeli, Giovanna Ferrentino, Matteo Scampicchio, Ksenia Morozova

Università di Torino, Department of Agricultural, Forest and Food Sciences, Grugliasco (TO, Italy)

5:45 p.m. **OR25: Optimization of HPLC-HRMS method for the detection of antioxidant Maillard reaction products and study of reaction conditions for their production as food preservatives**

Sara Bolchini, Ksenia Morozova, Matteo Scampicchio, Roberto Larcher, Tiziana Nardin

Faculty of Agricultural, Environmental and Food Science, Free University of Bolzano, Bolzano (Italy)

6:00 p.m. **OR26: Characteristics and critical issues of packaging intended for dairy products**

F. Cozzolino, F. Pratesi, M. Vitulli

Food Contact Center s.r.l, Serravalle Pistoiese (PT, Italy)

6:15 p.m. **OR27: Compounds isolated from licorice root (*Glycyrrhiza glabra L.*) as natural antioxidants in prevention of lipid oxidation**

Ksenia Morozova, Lucrezia Angeli, Maria Concetta Tenuta, Umme Asma, Giovanna Ferrentino, Matteo Scampicchio

Free University of Bozen-Bolzano, Faculty of Agriculture, Environmental and Food Sciences, Bolzano (Italy)

6:30 p.m. **OR28: The impact of industrial peeling on the lipidome of canned cherry tomatoes (*Solanum lycopersicum* v. cerasiforme): a HILIC-ESI-FTMS study**

Davide Coniglio, Giovanni Ventura, Cosima D. Calvano, Adriana Spinelli, Ilario Losito, Tommaso R.I. Cataldi

Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Bari (Italy)

6:45 p.m. End of session

8:30 p.m. Social dinner

Friday, October 18, 2024

9:00 a.m. **Session 7:** *Chairpersons: Michele Suman, Cosima D. Calvano*

9:00 a.m. **PL4: Benchtop volatilomics and machine learning – principles, applications, opportunities**

Philipp Weller

Mannheim University of Applied Sciences, Mannheim (Germany)

9:45 a.m. **OR29: Exploring monacolins in red yeast rice: monitoring, quantification and cytotoxicity assessment**

Paola Nezi, Vittoria Cicaloni, Mattia Cicogni, Alessia Prete, Paolo Etiope, Barbara Barlozzini, Rita Pecorari, Laura Tinti, Laura Salvini

Fondazione Toscana Life Sciences, Siena (Italy)

10:00 a.m. **OR30: A method to measure the qualitative level of durum wheat and milk raw material by High Resolution Mass Spectrometry**

Angela Di Capua, Maria Assunta Acquavia, Nicola Zamboni, Rosanna Ciriello, Filomena Lelario, Carmen Tesoro, Roberto Rubino, Giuliana Bianco

Università degli Studi della Basilicata, Dipartimento di Scienze, Potenza (Italy)

10:15 a.m. **OR31: Empowering veterinary clinical diagnosis in industrial poultry production by ambient mass spectrometry**

Carmela Zacometti, Alessandra Tata, Andrea Massaro, Simona Ceroni, Sonia Falappa, Roberto Piro, Salvatore Catania

Istituto Zooprofilattico Sperimentale delle Venezie, Laboratorio di Chimica Sperimentale, Vicenza (Italy)

10:30 a.m. **OR32: Novel food production: towards a sustainable approach within a circular economy framework**

Behixhe Ajdini, Irene Biancarosa, Silvia Illuminati, Anna Annibaldi, Federico Girolametti, Matteo Fanelli, Lorenzo Massi, Gloriana Cardinaletti, Cristina Truzzi

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

10:45 a.m. **OR33: Nontarget Screening Workflow (NTS) for the analysis of Per and Polyfluoroalkyl Substances (PFAS) in animal products using QTof**

Andrea Perissi

Waters, Sesto San Giovanni (MI, Italy)

11:00 a.m. Poster session, Coffee break

11:45 a.m. **Session 8:** *Chairpersons: Rita Petrucci, Arianna Piersanti*

11:45 a.m. **OR34: Automazione nell'analisi dei pesticidi in GC-MS/MS tramite µ-SPE online**

Antonello Laricchiuta

Thermo Fisher Scientific, Rodano (MI, Italy)

12:00 p.m. **OR35: Exploring nitrosamine formation in meat: impact of cooking, digestion, and preservation**

Tiziana Nardin, Jakob Franceschini, Francesca Martinelli, Roberto Larcher

Fondazione Edmund Mach, Technology Transfer Centre, S. Michele all'Adige (TN, Italy)

12:15 p.m. **OR36: Radical neutralization reaction using pre-column reactor and high resolution mass spectrometry**

Lucrezia Angeli, Maria Concetta Tenuta, Ksenia Morozova, Matteo Scampicchio, Giovanna Ferrentino

Free University of Bozen-Bolzano, Bolzano (Italy)

12:30 p.m. **OR37: Assessing chemical drivers of acidity in *arabica* coffee using a flavoromics approach**

Agnese Santanatoglia, Edisson Tello, Sauro Vittori, Devin G. Peterson

Chemistry Interdisciplinary Project, ChIP, University of Camerino, Camerino (Italy)

12:45 p.m. **OR38: Unveiling Diversity in Amino Acid Stable Isotope Profiles for Classifying Italian Rice Varieties, Refining Types and Cultivation Methods**

Alberto Roncone, Zoe Giannioti, Luana Bontempo

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

1:00 p.m. Concluding remarks & Arrivederci!!

POSTER COMMUNICATIONS

P1 **Honey, I found the metals! The paradigm of heavy metals in honey. A comparative study of European and Argentine honey using a validated ICP-MS method**

Giacomo De Corso, Denise Decarli, Alessia Buco, Cecilia Mariani, Michela Burico, Claudio Marzio Quintiero, Mattia Gianni, Luisa Mattoli

Metabolomics & Analytical Sciences, Aboca SpA, 52037 Sansepolcro (AR, Italy)

P2 **Inorganic elements of different honeys from Algeria: safety and health effects**

Vincenzo Nava, Sofiane Derrar, Angela Giorgia Potortì, Giuseppa Di Bella, Vincenzo Lo Turco

Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF), University of Messina, Messina (Italy)

P3 **Black tea infusions: mineral element transfer, safety, and health effects**

Ambrogina Albergamo, Vincenzo Lo Turco, Vincenzo Nava, Giuseppa Di Bella, Angela Giorgia Potortì

Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF), University of Messina, Messina (Italy)

P4 **Inorganic elements in Italian and Moroccan carobs (Ceratonia siliqua L.)**

Vincenzo Lo Turco, Michelangelo Leonardi, Ambrogina Albergamo, Giuseppa Di Bella, Angela Giorgia Potortì, Irene Maria Spanò

Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF), University of Messina, Messina (Italy)

P5 **Analysis of Ultrashort- and Short-Chain Per- and Polyfluoroalkyl Substances (PFAS) in vegetables: method development and preliminary validation study**

Carolina Barola, Elisabetta Bucaletti, Danilo Giusepponi, Roberta Galarini

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

P6 **One method with dual detection for the analysis of PBDEs, HBCDs and eBFRs: levels in fish from the Adriatic Sea**

Tamara Tavoloni, Arianna Stramenga, Ester Lucidi, Martina Ciriaci, Francesco Griffoni, Paolo Palombo, Arianna Piersanti

Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Ancona (Italy)

P7 **Contaminanti ambientali nei prodotti ittici del Mediterraneo. qualità dell'ambiente marino in relazione alla salute umana**

Chiara Maggi, Giulio Sesta, Gianfranco Diletti, Michele Palmisano

ISPRA, Centro Nazionale per la rete nazionale dei laboratori, Fondazione Santa Lucia IRCCS, Roma (Italy)

P8 **Long term study (2012-2021) of Marine Biotoxins in mussels from NW Adriatic Sea (Italy). Is rainfall a suitable forecasting tool?**

Simone Bacchiocchi, Melania Siracusa, Giulia Diomedi, Laura Ferroni, Carmen Maresca, Francesca Barchiesi, Erica Calandri, Arianna Piersanti

Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", Ancona (Italy)

P9 **Off-odours in recirculated aquaculture systems fish**

Matteo Egiddi, Andrea Buettner, Jonathan Beauchamp

Fraunhofer Institute for Process Engineering and Packaging IVV, Freising (Germany)

P10 **Metabolite profiling of sea buckthorn fruits (*Hippophae rhamnoides* L.) through UHPLC-Q-Orbitrap-MS/MS analysis**

Annunziata Paolillo, Maria Assunta Crescenzi, Paola Montoro, Sonia Piacente

Department of Pharmacy, University of SalernoFisciano (SA, Italy)

P11 **Occurrence of an emerging class of per- and polyfluoroalkyl substances (PFAS) in hen eggs**

Elisabetta Bucaletti, Carolina Barola, Simone Moretti, Fabiola Paoletti, Danilo Giusepponi, Roberta Galarini

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

P12 **Analysis of perfluoralkyl substances (PFAS) in meat, edible offal and eggs using Xevo TQ-XS**

Luca Moscon

Gruppo Veronesi Via Valpantena 18/G, 37142 Quinto di Valpantena (Verona, Italy)

P13 **Development of a direct mass spectrometry approach for the real-time monitoring of PFAS release from cookware and food contact materials**

Cristian Maffezzoni, Maurizio Piergiovanni, Federica Bianchi, Nicolò Riboni, Monica Mattarozzi, Maria Careri

Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma (Italy)

P14 **Ultra-sensitive PFAS analysis according to EU regulations in food and environment**

Diego Martin, Javier Lopez, Miguel Angel Perez

Bruker Espanola S.A., Madrid (Spain)

P15 **Determination of phthalates in bottled water and purifier water by SPME-GC/MS**

Claudia Lino, Giuseppe Avellone, David Bongiorno, Serena Indelicato

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

P16 **Clams from Sicilian transitional water zones: mineral elements and safety**

Angela Giorgia Potortì, Benedetta Sgrò, Giuseppa Di Bella, Giovanni Bartolomeo, Salvatore Giacobbe

Department of Biomedical and Dental Sciences and of Morphological and Functional Images (BIOMORF), University of Messina, Messina (Italy)

P17 **Fingerprinting of green and roasted coffee (*Parainema* and *Obata*) volatile organic compounds (VOCs): HS-GC-IMS and GC-MS**

Matteo Bordiga, Gianluca Piana, Andrej Godina, Cesare Rossini, Marco Arlorio

Dipartimento di Scienze del Farmaco, Università degli Studi del Piemonte Orientale, Novara (Italy)

P18 **Effect of fermentation type on VOCs profile of table olives: a case study on 'Bella di Cerignola' cultivar using PTR-MS**

Antonia Corvino, Iuliia Khomenko, Emanuela Betta, Vittorio Capozzi, Franco Biasioli

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

P19 **Evaluating the effect of different cooking methods on polycyclic aromatic hydrocarbons formation in hamburger samples by means of GC-MS/MS**

Mariateresa Ingegno, Valeria Nardelli, Anna Accettulli, Giovanna Berardi, Ines Della Rovere, Marco Langianese, Anna Calitri, Marco Iammarino

Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia (Italy)

P20 **Phenols and VOCs fingerprint as a reliable tool for craft beer classification based on wheat origin**

Maria Assunta Acquavia, Angela Di Capua, Rosanna Ciriello, Filomena Lelario, Carmen Tesoro, Rocco Bochicchio, Saverio Laurenza, Emanuela Gregori, Roberto Rubino, Giuliana Bianco

Università degli Studi della Basilicata, Dipartimento di Scienze, Potenza (Italy)

P21 **First steps of a journey to detect antibiotic treatment biomarkers in pig chains**

Maria Pia Fabrile, Sergio Ghidini, Loris Alborali, Nicolò Riboni, Maurizio Piergiovanni, Monica Mattarozzi, Federico Scali, Maria Olga Varrà, Adriana Ianieri, Federica Bianchi, Maria Careri, Emanuela Zanardi

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

P22 **Veterinary drug residues in animal-origin food: an UPLC-MS/MS screening method for the determination of 21 beta-agonists in animal liver and lung**

V. Pieragostini, I. Pecorelli, I. Diamanti, C. Carloni, D. Boccia, R. Galarini, L. Fioroni

Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia (Italy)

P23 **Evaluation of toxicity profile of ClPFPECA-0,1 (N2) by lipidomics approach**

S. Moretti, E. Artino, G. Cruciani, L. Goracci

Department of Chemistry, Biology, and Biotechnology, University of Perugia, Perugia (Italy)

P24 **Targeted and untargeted metabolomics profiling and evaluation of fungal communities in Italian wheat grains to assess the food security**

Leonardo Lascala, Marzia Beccaccioli, Ilaria Montaina, Francesca Colais, Cesare Manetti, Massimo Reverberi

Department of Environmental Biology, Sapienza University of Rome, Rome (Italy)

P25 **Indirect recycling of fast food in insect feed: the use of UHPLC-MS/MS proteomics to ensure food safety**

M.C. Lecrenier, M. Aerts, A. Cordonnier, L. Plasman, V. Baeten

Walloon Agricultural Research Centre (CRA-W), Gembloux (Belgium)

P26 **Iterative data dependent analysis by LC-QTOF for untargeted approach in food matrices**

Simone Angeloni, Laura Alessandroni, Giovanni Caprioli, Gianni Sagratini, Massimo Ricciutelli

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

P27 **Aroma Profiling of Hops and Beer Using High-Capacity Sorptive Extraction with GC×GC–FID/TOF MS/SCD**

Laura McGregor, Anthony Buchanan, Daniela Peroni, Daniele Morosini

SRA Instruments, Cernusco sul Naviglio (MI, Italy)

P28 **Characterization of the natural vanilla samples of different botanical and geographical origin based on the aromatic profile**

Mauro Paolini, Long Chen, Alberto Roncone, Tiziana Nardin, Luana Bontempo, Roberto Larcher

Fondazione Edmund Mach (FEM), S. Michele all'Adige (TN, Italy)

P29 **Comparison of dried thyme from different geographical locations with GC-TOFMS and software tools designed to rapidly determine similarities and differences**

Elizabeth M. Humston-Fulmer, David E. Alonso, John Hayes, Joseph E. Binkley

LECO Corporation, Saint Joseph (MI, USA)

P30 Impact of stabilizers on ice cream's aroma release

Camila Cossettin Teixeira, Michele Pedrotti, Lorenzo Gennari, Andrea Cavallero, Iuliia Khomenko, Flavia Gasperi, Franco Biasioli

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

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ABSTRACTS

Plenary, Keynotes, Orals

PL1

In vivo aroma release and perception of complex food matrices

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The perception of food flavour is a dynamic and complex process which is influenced by numerous factors related to the properties of the food and the consumer. The composition, structure and physicochemical properties of foods, the structural breakdown during mastication and the oral processing behaviour of the consumer itself impact *in vivo* release of odour-active volatile organic compounds (VOCs) from the food matrix into the nasal cavity. *In vivo* aroma release influences food flavour perception which ultimately impacts food acceptability. In the last decades, our understanding of the relationships between *in vivo* aroma release and aroma perception advanced considerably revealing the fundamental physicochemical processes contributing to flavour perception. A multidisciplinary approach integrating *in vivo* aroma release [in-nose Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS)], oral processing behaviour, and dynamic sensory methods is needed to obtain a better understanding of how release, evolution, and fading of odour-active volatile organic compounds are perceived during consumption. This presentation outlines the relative contributions that food structure and oral processing behaviour have on *in vivo* aroma release and perception focusing on complex food matrices consisting of different components, so-called composite foods (i.e. breads with spreads) and plant-based meat analogues. The addition of solid foods to spreads increased *in vivo* aroma release (duration and intensity of aroma release) and decreased intensity of aroma perception. The addition of solid foods reduced the time to reach maximum intensity compared to when spreads were eaten alone for various odour-active volatile organic compounds. When spreads were combined with solid foods, consumer's ability to discriminate between foods was reduced. The processes of *in vivo* aroma release and perception of composite foods are multidimensional phenomena modulated by cross-modal texture-aroma interactions. The use of a multidisciplinary approach provides sensory profiles representing an ecological valid food consumption context that might offer new strategies to develop healthier foods.

OR1

Unraveling the gut volatilome dynamics and inter-individual variability during *in-vitro* digestion and fermentation of black beans

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Summary: *This study explores the effect of black beans on the gut volatilome through in-vitro digestion and anaerobic fermentation model systems. Two automated methodologies, HS-SPME-GC-MS and PTR-ToF-MS were employed to analyze VOCs longitudinally. The research highlights the highly dynamic nature of gut fermentation and emphasizes the potential of VOCs as personalized non-invasive biomarkers for real-time metabolic monitoring in gastrointestinal studies.*

Keywords: *gut fermentation, volatilomics, black beans*

Introduction

In Western countries, the increased prevalence of gastrointestinal illnesses poses a significant health burden. To address this issue, a comprehensive understanding of the gut microbiota is required. This knowledge is critical for developing effective methods to manipulate the gut microbiota and reducing the likelihood of metabolic disorders. Volatile organic compounds (VOCs) generated during intestinal fermentation play an important role in host-microbe interactions.[1] There is evidence that frequent consumption of highly fermentable dietary fibers modulates the microflora and gut-associated immune system through the production of gut microbiota metabolites (GMMs) like short-chain fatty acids (SCFAs).[1] Due to the extensive array of molecules produced by the gut microbiota, recent research has focused on identifying new key molecules that have pleiotropic roles. This study investigates the VOCs produced during the fermentation of black beans, a high-fiber, high-protein model food.

Methods

We applied two different automated, non-invasive, techniques: i) solid-phase micro-extraction (SPME) sampling coupled with gas chromatography-mass spectrometry (GC-MS) and ii) proton transfer reaction coupled with time-of-flight mass spectrometry (PTR-ToF-MS) to obtain a continuous monitoring of the gut colonic fermentation.[2,3] Black beans were subjected to static in vitro gastro-intestinal digestion, following the INFOGEST procedure suggested by Brodkorb et al..[4] The undigested pellet was freeze-dried and subjected to batch anaerobic in vitro colonic fermentation.[5] The digested material was fermented by three healthy faecal donors to measure inter-individual variability. Additionally, simulations of ascending and descending colonic conditions were conducted to understand the influence of colonic regions on black beans. Longitudinal multivariate time series analyses, such as empirical Bayes statistics (MEBA) and repeated measures ANOVA Simultaneous Component Analysis (RM-ASCA), were used to identify and select the most relevant VOCs.
The complete workflow is presented in Figure 1:

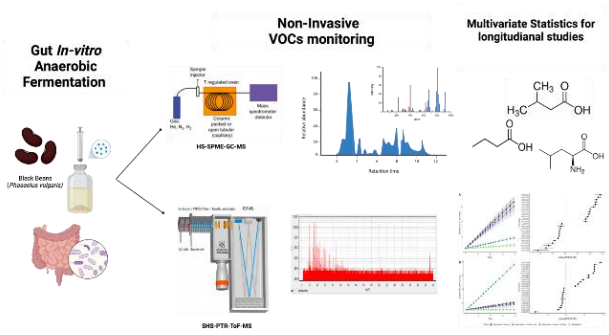

Figure 1*. Non-invasive VOCs monitoring coupled with multivariate statistics for longitudinal assessment of metabolic events taking place during gut microbial batch fermentation of black beans.*

Results and Discussion

By SPME-GC-MS fingerprinting, we detected a total of 156 VOCs. From this screening we identified several compounds exhibiting unique temporal clustering patterns across different donors and colon segments. Our analysis revealed an increased prevalence of sulphur-containing compounds specifically in samples obtained from the descending compartment of the colon. Additionally, by employing PTR-ToF-MS, distinct quantitative differences when comparing VOCs across individual donors were identified. These differences highlight the variability in microbial metabolic responses which is dependent on the type of dietary substrate but also the inherent microbial communities of faecal donors. The same substrate can lead to diverse metabolic profiles, potentially resulting in varied impacts on the host's health and metabolism. Overall, our findings underscore the significance of VOCs as personalized, non-invasive biomarkers for real-time metabolic monitoring in gastrointestinal research. The ability to longitudinally monitor these compounds provides valuable insights into individual metabolic responses and could serve in developing tailored dietary interventions and therapeutic strategies. This personalized approach could enhance our understanding of host-microbiome interactions and their implications for health and disease.

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OR2

Driving innovation in food volatiles analyses by mass spectrometry

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Summary: *Emerging developments in mass spectrometry, both in instrumentation and data processing, offer new opportunities in assessing food-related volatiles. This talk will review recent innovations in mass spectrometry applications in food science, from rapid mass spectral acquisitions to real-time packaging permeation characterisation and smart data processing for sample categorisation, amongst others.*

Keywords: *volatiles; food; packaging*

Mass spectrometry is central to food volatiles analysis. Throughout its long history in food science, mass spectrometry (MS) technology has undergone extensive developments to yield many flavours of instruments suited to different applications. These have been used in innumerable studies in food science to shed light on food compositions and biochemical processes, ranging from aroma characterisation to food processing, maturation and spoilage, as well as related applications to address questions concerning food packaging. A dominant form of mass spectrometry in the field of food volatiles analysis is its combination to gas chromatography (GC), often augmented with olfactometry (as in GC-MS/O) when odour-active aroma compounds are the subject of investigation [1,2]. GC-MS, including the extended configurations of GC-GC-MS and GC×GC-MS, offer comprehensive characterisations of food volatiles in terms of compound identities and quantities. Complementary analytical technologies in the form of direct injection mass spectrometry (DIMS), dominated by proton transfer reaction mass spectrometry (PTR-MS), offer the ability to characterise dynamic processes or provide high-throughput capabilities [3-6]. This talk will present recent innovations of GC-MS and PTR-MS technologies and their applications in food and packaging research, including smart odour assessments methods of GC mass spectra to rapidly categorise food samples [7], use of hyperfast-GC-MS in packaging applications, real-time characterisation of permeation rates through packaging films using PTR-MS, and direct quantitation of coffee aromas via liquid injection, amongst other featured applications.

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OR3

Real-time wine off-flavor analysis with chemical ionization mass spectrometry

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Summary: *Real-time wine off-flavor analysis could be an important innovative asset in wineries. No reliable real-time solution exists so far since the high ethanol challenges direct injection MS techniques. We show that innovative precursor ions make it possible to analyze selected off-flavors in real time.*

Keywords: *Direct injection mass spectrometry; online analysis; wine*

Introduction

Wine off-flavors are undesirable sensory attributes that can significantly affect the quality and consumer acceptance of wine. These off-flavors can originate from various sources, including microbial contamination, chemical reactions during fermentation, and storage conditions. Common off-flavors include earthy, musty, metallic, and sulfurous notes, which can be traced back to specific volatile compounds. For example, 4-ethylphenol is a significant off-flavor compound found in wine aged in barrels, and it can influence the wine flavor at concentrations as low as 10 µg/L (10 ppb). Rapid and accurate identification of these compounds is crucial for quality control in the wine industry.

Direct Injection Mass Spectrometry (DI-MS) has emerged as a powerful analytical approach for real-time monitoring and analysis of volatile compounds in complex matrices, also in industrial application relevant for the food industry [1,2]. DI-MS offers several advantages, including high sensitivity, rapid analysis times, and the ability to analyze samples with minimal preparation. However, the high ethanol content in wine poses a significant challenge for DI-MS analysis [3]. Ethanol, the primary alcohol in wine, can interfere with the ionization process in mass spectrometry. During the ionization step, ethanol molecules can titrate the primary ions, leading to ion suppression and reduced sensitivity for the target volatile compounds. This ionization interference complicates the detection and quantification of trace levels of off-flavor compounds, which are often present at parts-per-billion (ppb) or even lower concentrations. As a result, the development of effective strategies to mitigate ethanol interference is essential for the reliable application of DI-MS in wine off-flavor analysis. Proposed approaches include ethanol chemistry, high sample headspace dilution [3], fast gas chromatography pre-separation [4], argon injection in the DI-MS reactor [5].

In this study, we build on the idea that primary ions in chemical ionization can be (very) selective for specific volatile organic (or inorganic) compounds compared to other compounds. We report the first attempt to employ Br^- primary ions to avoid ethanol titration and have a very selective and efficient ionization of some wine off-flavors in real-time.

Experimental

The study employed a state-of-the-art Vocus AIM chemical ionization - mass spectrometer (CI-MS) manufactured by Tofwerk AG to analyze volatile organic and inorganic compounds in the air. The CI-MS system is integrated with a time-of-flight (TOF) mass spectrometer with a mass resolving power of 10000 (M/DM), providing high-sensitivity detection capabilities. The instrument can operate with many different primary ions, including positive and negative ions. Bromide primary ions (Br^-) were employed for this study.

Inside the ionization chamber, bromide ions (Br^-) selectively react with target analyte molecules via adduct formation at 50 mbar, producing negatively charged analyte-iodide clusters. These clusters were then introduced into the TOF mass spectrometer, where they were separated based on their mass-to-charge (m/z) ratios. Product ion fragmentation is negligible.

Wine samples (Rosé wine) were spiked with increasing concentrations of 4-ethyl-phenol. Moreover, a wine simulant with 20 % v/v ethanol in pure water was spiked with off-flavor concentration to produce calibration samples. All sample volumes were 50 mL placed in 100 mL vials. The vial headspace was directly sampled with a 1/4-inch Teflon tube connected to the instrument inlet. Sample air was drawn into the instrument at a flow rate of about 1.5 liters per minute for a few seconds. Data acquisition was performed in real-time (1 Hz), with continuous monitoring of the m/z range from 1 to about 550 Th.

Results

Ethanol and 4-ethyl-phenol were detected as adducts, namely $C_2H_6O{\cdot}Br^-$ and $C_8H_{10}O{\cdot}Br^-$, respectively. All expected isotopologues were present in the spectrum, but only the parent ions were considered in this study. Remarkably, the instrumental sensitivity of the bromide CI-MS to ethanol and 4-ethyl-phenol differed by several orders of magnitude. Therefore, despite the high concentration of ethanol in the matrix, no primary ion titration was observed, while 4-ethyl-phenol was detected in all samples in a split second. This is an unprecedented result for online mass spectrometry techniques, paving the way to the use of bromide CI-MS in industrial applications.

Figure 1 reports the results of rapid detection of 4-ethyl-phenol in the spiked wine. Notice that this aged wine has non-negligible content of 4-ethyl-phenol. Moreover, the addition of 10 mg/L of 4-ethyl-phenol was easily detected. The presence of two isotopologues of similar spectral intensity makes the detection more reliable. Isobaric product ions needed high mass resolving power to be separated. As a caveat, the presence of interfering isomers of 4-ethyl-phenol cannot be ruled out and shall be carefully evaluated in follow up studies. The instrumental response was linear in the tested 4-ethyl-phenol concentration range (from 10 µg/L to 400 µg/L).

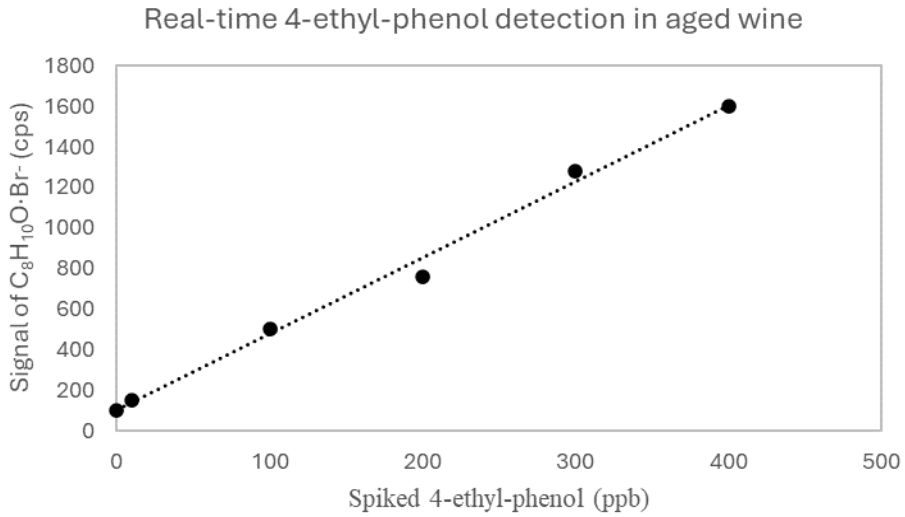

Figure 1. *Example of rapid 4-ethyl-phenol detection in spiked Rosé wine by bromide CI-MS. Reported cps correspond to the parent* $C_8H_{10}O \cdot Br^-$ *ion.*

Conclusions

This study unprecedentedly demonstrated the potential of bromide CI-MS for the real-time quantification of 4-ethyl-phenol in wine with sufficient sensitivity for industrial applications. The ionization mechanism was adduct formation and no product ion fragments were detected, demonstrating that this ionization mechanism is soft. High resolution mass spectrometry was used to separate isobaric interferences. Future developments include the evaluation of bromide CI-MS for other off-flavors and of other primary ions suitable for different off-flavors.

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OR4

Lycopene extraction with α-pinene, natural volatile deep eutectic solvent menthol-thymol and extra virgin olive oil

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Summary: *The development of new green approaches and techniques in chemistry is one of the main challenge of the last decades. Numerous researches have focused their attention on the discovery of novel solvents able to replace the conventional ones mostly known for their toxicity and high volatility.*

Keywords: *lycopene, extraction, EVO oil*

Introduction

A completely green, easy and no time consuming method to extract lipophilic compounds from food waste was developed using terpenes as green solvents and ultrasound-assisted extraction as green technique [1].

Experimental

The main finding of this research was the possibility to evaporate the DES solvent with the recovery of the dried extract similarly to the use of other solvents as *n*-hexane and α-pinene. The weakness of this method is the loss of lycopene during the evaporation. Despite this partial degradation, the quantity of lycopene in the dried extract is high. The application of this deep eutectic solvent is relatively unexplored, thus it can be used for the extraction of hydrophobic molecules less sensible to heat. Simultaneously, we used the ultrasound-assisted extraction with extra virgin olive oil as extraction solvent which lead us to the production of an enriched oil with improved antioxidant and radical scavenging activities.

Results and conclusions

We enriched an EVO oil with the aim to delineate a possible future nutraceutical application, opening new perspective in the use of food waste as source of beneficial compounds. For example, this enriched oil could be used as food seasoning. The ideal daily dose of lycopene is not defined yet, but according to different epidemiological studies, daily lycopene intake should be from 2 to 20 mg per day. For instance, to prepare the dish shown in the picture, we used about 10g of enriched EVO oil, so it would provide 9 mg of lycopene, a quantity that perfectly fits in the range established before.

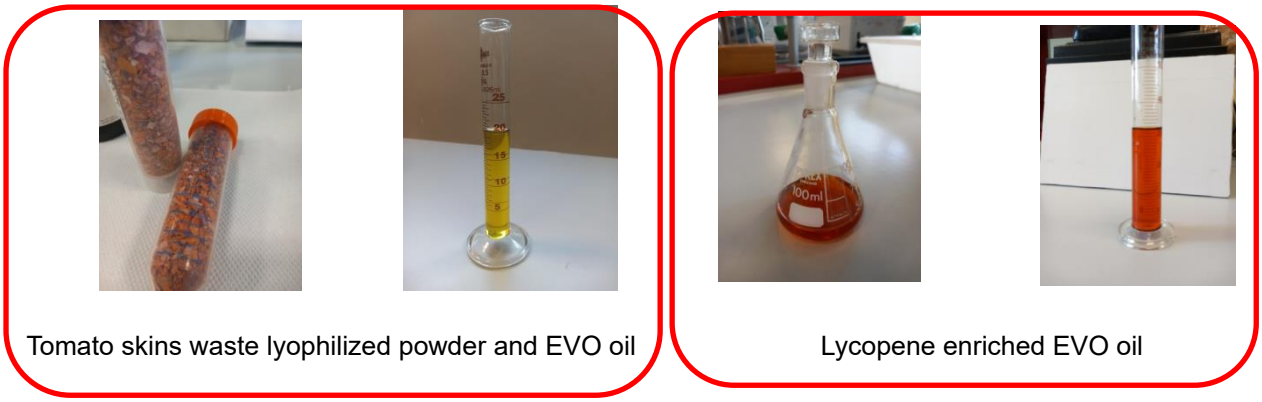

Figure 1*.Lycopene enriched EVO oil.*

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OR5

Authentication of Italian monovarietal extra virgin olive oils through HS-SPME-GC-MS, HPLC-DAD-MS and sensory analysis in combination with a GA-LDA-HCA statistical approaches for their clustering

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Summary: *With the purpose of Italian monovarietal EVOOs authentication and valorization, ≈ 400 samples of the main Italian cultivars were analyzed chemically (phenolic, volatile and terpene components) and sensorially. A Genetic Algorithm – Linear Discriminant Analysis – Hierarchical Cluster Analysis approach was employed for clustering cultivars based on their sensory and chemical features.*

Keywords: *HS-SPME-GC-MS, quantitation by multiple internal standards, chemometrics*

Introduction

Monovarietal extra virgin olive oil (MEVOO) is a niche product whose production has been constantly increasing in the last decades, in Italy as well as in the rest of the world, as also confirmed by its growing presence in the context of EVOO competitive awards [1,2]. MEVOOs of different cultivars are characterized by peculiar sensory attributes, which in turn influence their culinary applications and appeal to specific consumer segments. These unique characteristics lead to premium prices that discerning consumers are willing to pay. However, the very factors that make MEVOOs highly sought-after also render them more susceptible to fraudulent activities [3].

Because of these reasons, robust authentication and traceability measures are necessary to safeguarding consumer trust and to recognize cultivars whose MEVOOs show similar sensory and chemical features, in order to create different concourse classes and product segments.

MEVOOs of different cultivars are not only differentiated for their sensory attributes, but also for chemical ones. Phenolic compounds, terpenes and LOX pathway-derived VOCs are the molecules more capable of differentiating MEVOO [4], and mass spectrometry is an irreplaceable tool for their analysis. Therefore, in this study, a sensory method involving a new profile sheet was combined with HPLC-DAD-MS analysis of phenolic compounds and with two recently validated quantitative HS-SPME-GC-MS methods based on the multiple internal standard normalization (MISN) approach for the analysis of LOX derived VOCs [5] and terpenes [6], respectively.

The aim of this research was to characterize MEVOOs of different cultivars and to cluster them based on their chemical and sensory features, also searching for correlation among sensory and chemical data.

Experimental

Approximately 400 samples from 13 of the main Italian cultivars were collected from the 2020-2023 harvesting seasons and analysed with HPLC-DAD-MS (phenolic fraction), and HS-SPME-GC-MS (terpene and volatile fractions) using respectively the official IOC method and two different validated methods [5,6]; the same samples were also sensorially analysed by a professional panel using a profile sheet focused on the different nuances of positive attributes.

Sensory parameters were statistically analyzed with either a Genetic Algorithm (number of genes: 8; number of chromosomes: 200; number of couples: 25; number of generations: 25) or a Principal Component Analysis, then clustered by LDA-HCA. Genetic Algorithm was applied to the chemical data of samples divided in classes according to the clustering, and information on the most significant chemical parameters for the diversification of the cultivars was obtained. A correlation matrix was also built, in order to understand the correlation between chemical and sensory parameters.

Results

As showed by the overlapping chromatograms in Figure 1, the HS-SPME-GC-MS analysis of terpene and volatile profiles on MEVOO samples of different cultivars allowed pointing out clear chemical differences among them.

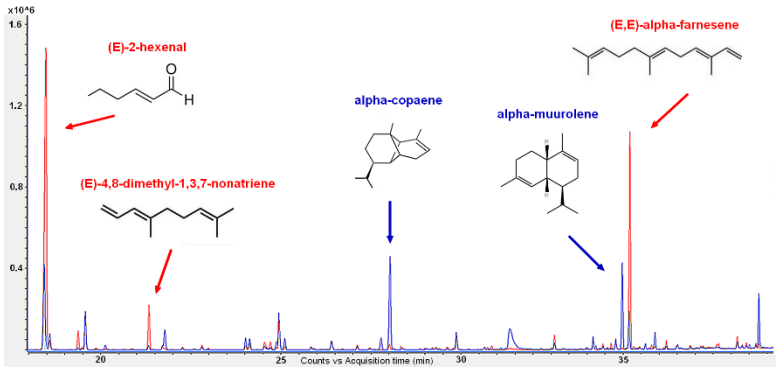

Figure 1. *Detail of overlapping gas-chromatographic profiles obtained from samples of two cultivars (red: Frantoio from Tuscany; blue: Nocellara del Belice from Sicily)*

By applying PCA/GA-LDA-HCA to the collected data, the clusters of cultivars in Figure 2 were obtained. Cultivars from different Italian regions were in the same cluster: in cluster 1, were classified cvs from central Italy and Apulia; in cluster 2, from Sicily, Marche and Lazio regions, while Bosana from Sardinia and Peranzana from Apulia are in cluster 3.

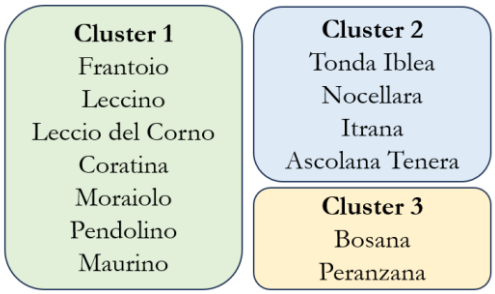

Figure 2. *Clustering of the Italian olive cultivars obtained with PCA/GA-LDA-HCA*

Both statistical approaches resulted in the same classification of the cultivars but, while PCA is more formally correct, the genetic algorithm allows obtaining more information, as the variables most often recurring in the best confusion matrices can be correlated to specific clusters of cultivars. For example, high values of bitterness characterized cluster 1 samples, while high values of tomato attribute characterized samples from both cluster 2 and 3. Application of the GA to the chemical data matrix also provided information on which chemical parameters are more important for the differentiation of the clusters. By restricting the number of parameters per gene to four, the following combination of variables resulted the most appropriate: Total phenolic content; *cis*-bergamotene; (*E*)-2-hexenal; 5-ethyl-2(5H)-furanone. By applying LDA-CV-LOO to classify an external group of samples in the defined clusters and using these parameters, the confusion matrix in Table 1 was obtained, providing a correct classification for 90.03% of the training set samples.

Table 1. *Confusion matrix obtained with the four best chemical variables*

	C1	C2	C3
C1	**94.7**	5.3	0
C2	12.1	**87.9**	0
C3	8.3	4.2	**87.5**
Error-Rate: **9.97%**			

Chemical analysis of these four parameters can therefore provide accurate information for the classification of an oil in one of the clusters, prior to the sensory analysis.

Conclusions

Combination of chemical (HPLC-DAD-MS and HS-SPME-GC-MS), sensory and statistical (GA-LDA-HCA) analysis can be proposed as a useful tool for MEVOOs valorisation in terms of consumer knowledge and acceptability. The same approach can as well be a step towards the creation of clusters of cultivars, which can potentially compete in different concourse classes, according to their sensory and chemical profiles.

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OR6

Inert columns, a "passive" solution for an active problem

Emanuele Ceccon

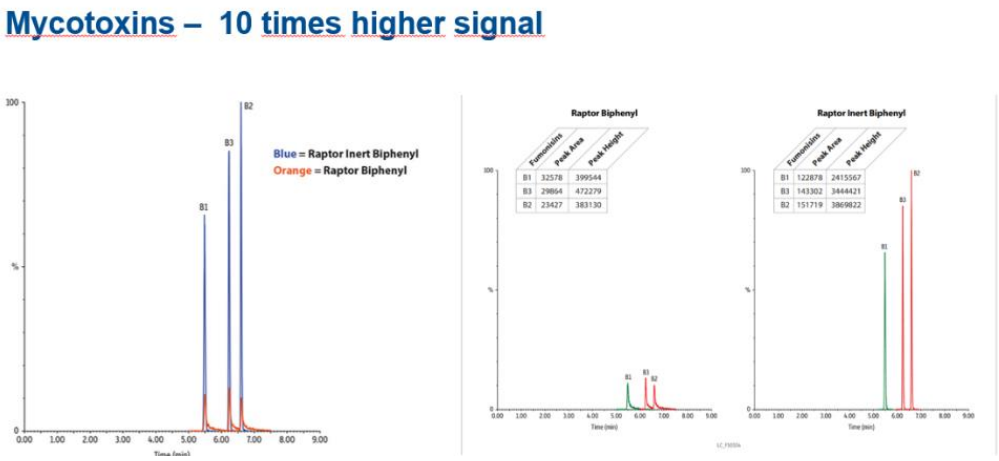
Restek, Restek, Cernusco S/N (MI, Italy)

Summary: *the more restrictive the limit are, the more we are going to ask the best from our chromatography, inert columns could be the future for some important applications*

Keywords: *micotoxins-Active molecules-Inert*

Introduction
Nsa and NSB (non specific adsorption and non specific binding) are interaction that could make our work harder then needed, what are NSa and NSB? Which are all the possible solutions?
All good question that we are going to address till explain how these problems are solved by Restek

Experimental
Demonstration of direct analytical improvement on mycotoxins, vitamins, pesticides.
Better peak shape, so better quantification, greater recovery, all of these are the real improvements you can obtain choosing the inert version of your standard column.



Results & Discussions
Do you need an inert column?
How to use it? Once that I use an inert column I do need an inert system?
This coating could affect you LCMS instrument? Is needed to use any specific additive into the mobile phases?
All question that we are going to address to close the explanation

OR7

Analysis of the volatilome of virgin olive oils: quality grade evaluation and study of modifications during storage

Rosalba Tucci,[1] Enrico Casadei,[1,2] Enrico Valli,[1,2] Chiara Cevoli,[1,2] Silvia Mingione,[3] Francesca Baroccio,[4] Sara Barbieri,[1] Alessandra Bendini,[1,2] Stefania Carpino,[4] Tullia Gallina Toschi[1,2]

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Summary: *Commercial virgin olive oils were sensory robustly classified by Panel test and analysed by HS-GC-IMS. The volatilome data were used to develop chemometric models to predict the commercial category, useful especially for borderline samples. Additionally, SPME-GC-MS analysis tracked volatile changes in selected samples over time.*

Keywords: *volatile compounds, virgin olive oil, chemometric models*

Introduction

To ensure legal compliance, extra virgin (EV) olive oil must maintain quality and purity parameters according to the Commission Delegated Regulation (EU) 2022/2104 and following amendments and integrations, throughout its shelf life, until the specified "best before" data [1]. Conformity evaluation includes organoleptic assessments by trained sensory panels, using the official method known as Panel test. Despite several modifications that occurred over the years, this official method still shows some weaknesses [2]. Therefore, the identification and quantification of volatile organic compounds (VOCs) in virgin olive oils (VOOs) is of great importance to assess their quality. Targeted and untargeted instrumental methods based on the analysis of these molecules can be considered an interesting tool to support sensory analysis [3]. In this framework, the use of headspace gas chromatography, coupled with ion mobility spectrometry (HS-GC-IMS), and solid-phase microextraction (SPME) in combination with gas chromatography (GC), coupled to mass spectrometry (MS), can improve the efficiency of official quality control processes in the olive oil companies and laboratories.

Experimental

A set of 67 commercial VOOs were sensory assessed by five professional and official Italian panels. A decision tree was used to robustly classify the samples based on the agreement among the panels regarding the commercial category and the most perceived defect [2]. At the same time, the samples were analysed by HS-GC-IMS in five different laboratories involved in the trial, equipped with the same instrument and applying the same analysis method, described in Valli et al. 2020 [4]. Starting from instrumental data, the commercial categories were estimated applying four PLS-DA calibration models previously built considering 15 selected volatile markers, both as monomers and dimers. At the same time, the shelf-life of 34 selected VOOs was evaluated using the SPME-GC-MS technique [5] to monitor potential qualitative and quantitative changes in VOCs over a 12-month period.

Results

The sensory evaluation conducted by five Italian panels on 67 samples was carried out using a decision tree, resulting in the following classifications: 13 EV, 35 virgin (V), 2 lampante (L) olive oils. A total of 17 samples were identified as borderline (BL), thus classified between EV and V. Particularly, 13 samples were classified as BL for the first type of misalignment, which regard disagreement of the commercial category among the panel. The remaining 4 samples were classified as BL due to the second type of misalignment, for a disagreement on the most perceived defect. By the application of the decision tree, 17 BL samples were subsequently subjected to the formative reassessment. The volatile compounds were analysed using HS-GC-IMS in five laboratories involved in the trial. The intensity values of 15 selected VOCs, as monomers and dimers, were used to estimate VOOs commercial categories based on four PLS- DA calibration models previously built [4] (Table 1).

Table 1. *Comparison between commercial categories predicted using four PLS-DA models (EV vs noEV; L vs noL; L vs V; EV vs V) and sensory commercial category in a representative set of 7 VOOs.*

Sample	Sensory classification	EV_noEV	L_noL	L_V	EV_V	Predicted category
1	V	0.05	0.09	0.06	0.13	V
2	V	0.90	0.07	0.10	0.89	EV
3	V	0.22	0.38	0.40	0.14	V
4	EV	0.87	0.28	0.84	0.53	EV
5	EV	0.74	0.18	0.46	0.29	BL (EV/V)
6	BL	0.72	0.07	0.06	0.56	BL (EV/V)
7	V	0.58	0.08	0.09	0.29	V

Through the combination of probability results obtained from the application of these four chemometric models, the commercial category of the 67 samples was estimated. In total, 36 samples were predicted as V, 14 BL, 13 EV, and 4 L (see the comparison for a representative set of 7 VOOs in Table 1). Moreover, the SPME-GC-MS technique was used to track changes in the VOCs of 34 selected VOO samples over a one-year storage period. For certain samples, a gradual reduction in the concentration of volatile compounds associated with the fruity sensory attribute (e.g., (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, 1-hexanol) was observed over a six-month storage period. Concurrently, there was an increase of volatile markers associated with rancid defect (e.g. hexanal). However, for some samples, the 12-month analysis is ongoing.

Conclusions

By applying four PLS-DA models, 80% of the analysed samples have been correctly classified in the commercial category established according to the herein developed robust sensory classification The results obtained are satisfactory in terms of prediction of commercial categories. The dataset will be implemented in the coming months to make the predictive models possibly even more robust and reliable to support the Panel test. Regarding the shelf-life study, the aim is to create a large dataset to provide a more robust and complete view of the phenomenon under investigation. Furthermore, by an integrated approach combining sensory analysis with screening (HS-GC-IMS) and targeted (SPME-GC- MS) analytical techniques, it will be possible to assess changes in the volatile compound profile of VOOs during the storage period.

Authors acknowledge the involved sensory panels, olive oil companies and laboratories, and Federolio for funding.
Dr. Enrico Casadei's research activity is funded under the National Recovery and Resilience Plan (NRRP) - NextGenerationEU "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security - Working ON Foods".

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OR8

Virgin olive oil authenticity assays in a single run using two-dimensional liquid chromatography-high resolution mass spectrometry

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Summary: *This work proposes a two-dimensional liquid chromatography/high-resolution mass spectrometry (2D-LC-HRMS) method for the simultaneous analysis of triterpenic alcohols, free sterols and steryl esters. To evaluate the content of these compounds, the proposed 2D-LC-HRMS system was applied to a set of different samples, including main commercial olive oil categories and sunflower oil.*

Keywords: *olive oil authentication; Two-dimensional LC (2D-LC); mass spectrometry*

The present study aimed to develop a derivatization-free method for the simultaneous analysis of triterpenic alcohols and sterols, both in their free and esterified form, trying to overcome some of the drawbacks found in the published methods for oil analysis. To this end, the use of two-dimensional liquid chromatography (2D-LC) is proposed as an alternative to the GCxGC separations described in the literature, but avoiding the derivatization process. Moreover, to simplify the sample treatment and to circumvent saponification, SPE was used to extract directly the sterols in their original form from the oil samples. The proposed strategy was to analyze the steryl esters in the ^{1}D and transfer the void volume, containing the free sterols and triterpenic alcohols, to the ^{2}D in a single cut. In addition, the developed approach allowed the acquisition of both MS dimensions in a single data file by coupling a selection valve to the 2D-LC setup. The system was evaluated with a mixture of 21 compounds, including triterpenic alcohols, free sterols and steryl esters. The determination of the compounds established in the official IOC method was studied on 25 oil samples, including olive oil of different commercial categories and sunflower oil. In summary, this paper presents for the first time the development of a 2D-LC-HRMS method for the simultaneous analysis of triterpenic alcohols, free and esterified sterols in oil samples. The main features sought with this approach are to obtain thorough information from the oil sample by determining the distribution of free or esterified sterols, as well as simplifying the procedure by skipping time-consuming steps such as saponification and derivatization.

OR9

Quality and safety of a traditional and healthy Algerian product

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Summary: *This study aims to evaluate the concentration of mineral elements by inductively coupled plasma mass spectrometry (ICP-MS) and direct mercury analyzer (DMA-80), together with the physico-chemical and compositional characteristics, of seven date syrup samples from Algeria.*

Keywords: *date syrup, minerals, potentially toxic elements, food safety*

Introduction

Date syrup, derived from the fruit of the date palm (*Phoenix dactylifera* L.), is a natural sweetener with a rich nutrient profile and numerous health benefits. This syrup is widely used in the Sahara regions, where it has been a staple in traditional diets for centuries, providing a crucial source of energy and nutrients (i.e., natural sugars, minerals, and antioxidants) and a valuable remedy for the treatment and prevention of various conditions such as diabetes, cardiovascular diseases, digestive disorders and nutritional deficiencies in these arid areas.[1,2] To ensure the benefits, date syrup must adhere to specific quality standards and be free of contaminants, which can arise from environmental factors and farming practices. Strict quality control and compliance with international safety standards are essential to address these risks. Moreover, nutritional labels on date syrup products are often either not provided or, when present, lack precision, leading to variability and uncertainty in assessing its exact nutritional value.

Experimental

Seven commercial date syrups from Algeria were examined to evaluate their quality by generating new or updated physico-chemical (pH, acidity, conductivity, total soluble solids) and compositional data (moisture, total sugar, ash, protein, total polyphenols). To ensure the safety and to evaluate the accuracy to nutrition labels of this products, mineral elements, including potentially toxic ones, were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) and a direct mercury analyzer (DMA- 80).

Results

The findings confirmed a high content of polyphenols (average value 13.98 ± 7.62 g/L) and minerals, except for one sample whose composition varied due to the addition of glucose, sugar, water, and caramel color. Potassium was the most abundant mineral (ranging from 1002.01± 93.80 to 2999.98 ± 344.51 mg/kg), followed by calcium and magnesium. Iron and zinc concentrations were also significant (average values 25.63 ± 12.59 and 9.57 ± 4.87 mg/kg, respectively). The same nutrition label is given in only two samples declaring a very wide range of essential element concentrations, so it appears inaccurate. In addition, a higher potassium, sodium, magnesium and calcium content and a lower manganese content than the samples analyzed is indicated.

As far as potentially toxic elements are concerned, very low levels have been detected. Nickel was found at concentrations below the analytical limit of quantification in all samples. Aluminum, arsenic, cadmium, lead, tin, and thallium were all present at concentrations below 0.10 mg/kg in all samples. Since there are no specified maximum levels for inorganic contaminants for this particular food, the results were compared with the maximum levels for honey, fruits, fruit juice concentrates, and food supplements [3]. None of the results exceeded these limits. Since date syrup is often used as a substitute for traditional sugar in Algeria, to evaluate the safety of date syrup for this purpose, the estimated daily intake of all minerals was calculated based on the amount of sugar consumed in Algeria and compared with their reference values [4]. The results indicated a high coverage of the reference value for cobalt (40-110%) and promising results for other essential elements. Additionally, there were no health risks from potentially toxic elements, as their reference values were not exceeded in any of the samples, confirming the safety of date syrups.

Conclusions

The results show a safe product rich in polyphenols and minerals. However, in order to reap the benefits of this product, it is necessary for consumers to be better informed about the nutritional information so it is hoped that the Algerian authorities will monitor the production and the commercialization of these products in order to enhance their value.

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OR10

Liquid chromatography coupled with mass spectrometry for multiple detection of hidden allergens in bakery-products: two case studies produced at pilot plant scale

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Summary: *The main objective of the study is to evaluate the applicability of a multi-target method for the analysis of allergenic traces in two different food matrices subjected to different technological and thermal treatments. The challenge is to find "universal" proteotipic peptides capable of tracing the culprit ingredients in baked goods despite the processing used for their production.*

Keywords: *multi-target methods; food allergens; hidden allergens detection; food processing*

Introduction

Currently there is no cure for food allergies and having at disposal sensitive and accurate analytical methods to detect tiny amounts of multiple allergens in foods represents a crucial point for the protection of allergic consumers. Food processing represents a critical issue in the development of robust and reliable methods for allergen quantification due to the numerous and different chemical/structure modifications that could occur at protein level,[1] often impairing detectability of the selected marker peptides.
In this study, the reliability and the transferability of a LC-MS/MS method developed on a Triple Quadrupole mass spectrometer platform for multiple detection of traces of eggs, milk, soy, nuts, peanuts and sesame in highly processed food was investigated. In details, rusk was chosen as highly processed food since its production involves technological phases harder than those used for other bakey production and analysis were performed on advanced High Resolution Mass Spectrometer platform. In detail, the sensitivity of the method in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ) along with a recovery estimation for each marker accomplished on samples enriched with each allergenic ingredient before processing at two different levels was performed. Moreover, the characteristics of the developed method were compared with the performance of a parallel method optimized by our group for detecting these six allergens in cookie matrix. This comparison also allowed to understand the applicability of these selected marker peptides to detect eggs, milk, soy, nuts, peanuts and sesame in bakery products produced at soft and harsh conditions, thus to be considered “universal” proteotypic peptides able to trace these culprit ingredients in bakery-foods despite the processing used for their production.

Experimental

The workflow for food allergens detection was based on protein extraction followed by tryptic digestion taking advantages of previous studies carried out from our research group on allergens analysis[2-4] and summarised in Figure 1.

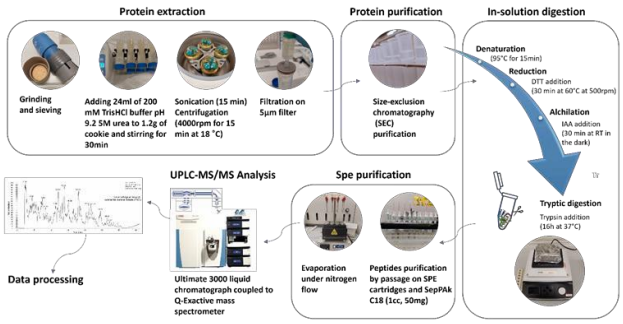

Figure 1. *Experimental workflow adopted for the bottom- up proteomics approach*

Analysis were accomplished on a Quadrupole- Orbitrap High-resolution hybrid platform coupled to a Ultimate 3000 liquid chromatograph and a calibration curve produced by adding increasing amount of the allergenic ingredient to allergen- free material along with rusk samples produced at two different levels of inclusion for the six allergens monitored (eggs, milk, soy, nuts, peanuts and sesame) were used.
Rusks were produced in a food pilot plant to provide a real and well-characterized matrix.

Results

The selected marker peptides reported in table 1 tracing for the different allergenic food ingredients were monitored in rusk chosen as complex food matrix. Calibration curves in the real food sample were obtained by progressively spiking the allergen free product with the 7 allergenic ingredients and the respective LOD and LOQ were calculated basing on the signal of the quantifier markers in order to test method sensitivity (Table 1).

Table 1. *Calculation of detection and quantification limits for the 7 allergenic ingredients obtained in TSIM/dda acquisition mode in Rusks matrix.*

Allergenic Ingredients	Peptide code	Matrix: Rusks	
		LOD (3*Sa/b) (µg/g)	LOQ (10*Sa/b) (µg/g)
Milk- Casein	FFV_MILK (2+)	2.8	9.2
	NAV_MILK (2+)	2.0	6.6
Milk- Whey	VLV_MILK (2+)	5.0	16.8
	IDA_MILK (2+)	7.7	25.6
Egg yolk	ATA_ EGG (2+)	N.D.	N.D.
	NIG_ EGG (2+)	3.7	12.4
Egg white	GGL_EGG (2+)	9.9	33.1
	ISQ_EGG (2+/3+)	5.3	17.8
Peanut	SPD_ARA (2+)	4.9	16.4
	TAN_ARA (2+)*	2.5	8.4
Hazelnut	ADI_ HAZ (2+)	6.5	21.6
	ALP_ HAZ (2+)	3.4	11.4
Soy	VFD_ SOY (2+)	3.2	10.6
	VLI_ SOY (2+)	7.6	25.2
Almond	TEE_ ALM (2+/3+)	6.7	22.2
	ADI_ ALM (2+)	3.4	11.5
Sesame	AFD_ SES (2+/3+)	4.3	14.3
	SPL_ SES (2+)	3.7	12.4

On the contrary, in Figure 2 the comparison between LODs calculated for each marker peptide detected in rusk and cookie is depicted. For both matrixes analysis were accomplished by working under t- SIM acquisition /DDA in positive ion mode and each calibration curve were prepared by diluting a 2000 µg total proteins/g matrix ("spike-stock" sample) in lab prepared with the allergen free samples in order to obtain 7 concentration levels (range 3-150 µg protein/g matrix for each allergenic ingredient). The LODs in the rusk matrix are results variable in the range 2-10 µg/g depending on the specific allergenic food/peptide e typically slightly higher than calculated with the cookie.

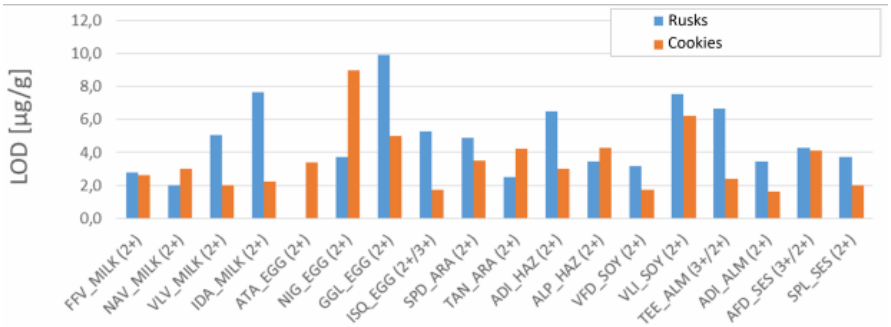

Figure 2. *Comparison of the LOD values calculated for the seven allergenic ingredients in the two matrices under investigation.*

Finally, method recovery was estimated by analyzing rusk samples produced at industrial level at two different inclusion levels, namely 24 and 48 µg total proteins/g of matrix.
At least two peptides for each food were detected in the rusk sample at 24 µg/g allergenic although with a very variable recovery from 2% to 90%.
The trend observed in the sample at 48 µg/g was similar with the confirmation of detection of all peptides integrated already at the lowest level and with the possibility of detecting and quantifying the ISQ peptide as well of egg white.

Conclusions
The multi-target method allowed the detection of traces of allergenic ingredients in rusks matrix with LOD values and within the range of 2-9.9 mg·kg−1 and LOQ values in the range of 6.6-33.1 mg·kg−1. The method, developed on a less processed matrix such as biscuits, is also applicable to hyper-processed matrices such as rusks with very interesting recovery values.

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PL2

Presence of microplastic in food and beverages: do analytical methods matter?

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The term Microplastics (MPs) was firstly introduced in 2004, when started studies on their occurrence in marine environments, and identified plastic polymer particles in the size ranged from 1 to 5000 µm, while Nanoplastics (NPs) ranged between 1-1000 nm. Since then, the worldwide distribution of MPs in aquatic and terrestrial environments was widely demonstrated and their ingestion by virtually all species was considered a matter of great concern, gaining considerable attention in the research community, as well as policy makers, governmental bodies, non-governmental organizations and the general public. This suddenly called for a major effort to obtains as many data as possible on their presence in several matrices, including food and beverage, considered as one of the major pathways for human exposure to MPs.

With this purpose a variety of analytical techniques has been developed and tested for the detection and characterization of polymer particles in the environment and next have been applied to the analysis of food matrices; however, to date, the field still lacks in methodology standardization, partially because no unique technique has been able to deal with the multidimensionality of the data necessary to describe MPs occurrence, including number of particles, particle size, distribution, shape and color, chemical identity, and mass.

Despite this complexity, a plethora of papers have recently published, demonstrating the presence of MPs/NPs in food (e.g salt, fruit and vegetables, seafood and fish, honey and sugar) and beverages (e.g. drinking water, soft and energy drink, beer, wine, milk), and available information has grown exponentially; however, comparison among those data is still limited due to the use of different protocols reporting units and confounding factors, especially critical in the case of fibers, which can easily be introduced during sample collection and processing, nonetheless researchers have been providing recommendations and frameworks to surpass this limitation. Moreover, in some of the studies, quality assurance to avoid contamination from the air and equipment is not described, and it is not always clear how a particle is identified as being a 'plastic'. As research diversified and the research community expanded, comparison between emerging datasets was hampered even further, and accuracy has become a key issue in recent publications.

Compared to other typologies of pollutants, analyses of MPs are generally complex and time- consuming, and the different applied approaches have demonstrated both advantages and weaknesses; procedures usually include adequate handling of different steps including: (i) sampling, (ii) isolation/extraction of MP/NP, (iii) separation/preconcentration, (iv) size determination, (v) chemical identification, and (vi) quantification. Although the type and number of treatments for isolation/extraction steps depend on the sample characteristics, a vacuum filtration is generally needed to concentrate on filters the MPs contained in different typologies of matrices (e.g. water sample, digested samples, supernatants obtained from a density gradient separations). Inspection of these filters to sort out suspected MPs using optical microscopy is a common practice, low cost and easy approach to provide information on shape, size, colour of particles and it represents a rational method for screening purposes or for non-professionals. On the downside, the manual selection of suspected MPs requires considerable time and big efforts and resources in terms of manpower to quantify a lot of particles, with a high risk of underestimation, especially due to failure to detect small particles.

Different approaches coupling non-destructive imaging and spectroscopic analysis were proposed for (automated) identification and chemical characterization of MPs on filters. Vibrational microscopy techniques, such as Fourier transform (FT) infrared (IR) and Raman microspectroscopy (µ-FTIR and µ-Raman), have proven efficient for the non-destructive identification of MPs in both biotic and abiotic matrices, providing spectral fingerprints for each type of plastic polymer based on its unique chemical structure. The main limitations of these techniques are related to spatial resolution for µ-FTIR (which prevents the identification of MPs smaller than ~ 10 − 20 µm) and to acquisition time for µ-Raman (> 20 h for 100 kpixels image), unless spatial resolution is smaller than 10 µm. Hyperspectral microscopy (HSM) has recently emerged as a further promising non-destructive, cost and time effective technique for the identification of MPs without heavy sample manipulations; it combines optical microscopy with spectroscopy, retrieving spectral images which contain spectroscopic information for each spatial point in the acquired 2D map of the measured sample.

The main limit of all these techniques is related to size detection, since they all exclude the possibility to detect lower size particles in investigated samples (i.e. particles less than ~ 3 – 5 µm).

Recently, thermo-analytical methods coupled with mass spectrometry, such as the traditional pyrolysis gas chromatography mass spectrometry (Py-GC-MS) and its evolution, the thermal extraction desorption gas chromatography-mass spectrometry (TED-GC-MS), were applied for the assessment of plastic in different

matrices, without intensive sample purification or with no purification at all. These techniques enable the identification and the mass-based quantification of synthetic polymers by relating their characteristic thermal degradation products to reference pyrograms and calibration curves of known virgin polymers. They can be also used to study volatile plastic-associated additives and contaminants, and detect very small particles, down to NPs, as they are not dependent on the particle size, provided that their number is high enough to exceed the (mass-based) detection limit. The main drawback of thermo-analytical approaches is that they do not provide information on the number, size and shape of plastic particles, although these are crucial to understand MPs bioavailability and toxicological effects. Furthermore, these methods are destructive, so that samples cannot be re-analysed or analysed by orthogonal techniques.

To date, is difficult to identify a single unique method able to deal with the multidimensionality of the data necessary to fully describe the occurrence of MPs/NPs in food; even the most wide-spread methods are still in development; there is a strong need to encourage and establish rigorous best practices in order to obtain reliable data and build a comprehensive knowledge. Rigorousness in method development ensuring accuracy, precision, validation and repeatability have to be considered fundamental for further development of the field.

OR11

Food-borne thermally induced contaminants: mass spectrometry platform for the determination of the S/R ratio of 3MCPD esters and for the identification of glycidyl esters in oils and fats

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Summary: *3-monochloro-1,2-propanediol and glycidol are thermally-induced food contaminants, classified as possibly and probably carcinogenic, respectively. As their toxicity is related to their different enantiomeric forms or their esterification, we propose a mass spectrometry platform for the quantification of 3-monochloro-1,2-propanediol enantiomers and the characterization of the glycidyl esters to better understand human exposure*

Keywords: *3-monochloro-1,2-propanediol, glycidyl esters, oils*

Introduction

3-monochloro-1,2-propanediol (3MCPD) and glycidol (gly) are food-borne and thermally- induced contaminants. They are typically formed during the refining process at high temperatures of vegetable oils, as well as during the manufacturing process of different foodstuffs (such as biscuits, bread, crisps, and spreadable cream). They exist in both free form and esterified with several fatty acids naturally present in food.[1]
In 2000, the International Agency for Research on Cancer (IARC) classified gly as a probable carcinogenic, placing it in Group 2A.[2] Subsequently, the Food Safety Commission of Japan, after conducting several studies, deemed gly as genotoxic.[3] In 2016, the European Food Safety Authority (EFSA) established a margin of exposure for gly at 25000.[4] The IARC also classified 3MCPD as a possible carcinogen, assigning it to Group 2B.[5] In 2018, EFSA updated the tolerable daily intake for 3MCPD to 2 μg/kg bw per day.[6] Notably, 3MCPD exists as two enantiomers with distinct biological activities: R-3MCPD has kidney toxicity, whereas S-3MCPD has an antifertility effect in males.[7] Consequently, in 2023 the European Union established maximum limits for gly and 3MCPD esters in oils (1000 and 1250 μg/kg, respectively) and infant formula (50 and 125 μg/kg, respectively).[8] Therefore, there is a crucial need for accurate analytical methods to measure the level of food-borne contaminants in foods and oils. The gold standard method to obtain occurrence data for the health risk assessment is the indirect method, which includes quantifying the total content of contaminants using GC-MS after hydrolyzing all esterified forms.[9] The risk assessment is carried out in the worst-case scenario, thereby assuming that all esters are hydrolysed in the stomach and that the free contaminants are thus absorbed. However, this approach does not differentiate between the two enantiomers of 3MCPD, thereby hindering the assessment of the specific dose achieved and the prediction of resulting toxicological effects. Moreover, this method does not permit the accurate characterization of each esterified form, which is critical because the fate of these forms in the gastrointestinal tract and their toxicity are still being investigated. Additionally, the inaccuracy of some hydrolysis methods has recently prompted the development of direct methods aimed at identifying the esterified form.[10]
Here, we propose a mass spectrometry platform to use together with the above-mentioned standard method to give us information about the S/R ratio of 3MCPD esters and the esterified forms of gly. The second approach can also be extended for the determination of the esterified forms of 3MCPD.

Methods

To determine the S/R ratio, a GC-MS method was employed, using a chiral column coated with γ- cyclodextrin. An indirect approach was chosen to assess the 3MCPD equivalents of the total ester content following hydrolysis. Fat and oil samples were extracted for analysis using a Multi-Purpose- Sampler (Gerstel) to ensure greater accuracy and reproducibility.
For the characterization of each esterified form of gly, a direct method in liquid chromatography coupled to high-resolution mass spectrometry (LC- HRMS) was proposed. In this approach, sample preparation required a liquid-liquid extraction using acetonitrile and a pre-concentration step. The presence of the gly forms esterified with the most abundant fatty acids was evaluated by acquiring spectra in data-independent acquisition mode (DIA). The identified structures were confirmed by comparing the obtained MS^2 spectra with the fragmentation pattern generated *in silico* with the software Mass Frontier (Thermo Scientific™). Additionally, an in-house database was developed to include the representative fragmentation and the specific retention time for each gly-ester.

Results

The complete resolution of S- and R-3MCPD was achieved in all the analyzed samples. The method was validated for accuracy and precision, demonstrating high reproducibility. Matrix effect was evaluated, showing a normalized matrix factor close to 1, indicating similar effects of the matrix on the analytes and the selected internal standards. In the samples analyzed thus far, detectable amounts of the two enantiomers were present in a 1:1 ratio, ranging from 0.176 to 1.648 µg/g. The combined amount of S- and R- enantiomers aligned with the total content of 3MCPD esters evaluated using a traditional indirect method and was within the limits established by the European Union [8]. The statistical analysis (Pearson correlation) confirmed the existence of a significant correlation between the sum of the two enantiomers obtained using this newly developed method and the total amount of 3MCPD esters evaluated through the indirect method ($r = 0.941$, $p<0.0001$).
Upon developing a method to determine the S/R ratio, the gly-esters were analyzed. The MS^2 spectra were compared with the fragments predicted with Mass Frontier, followed by confirmation of the retention times for all the esterified forms considered. For each analyte, two fragment ions, characteristic of the chemical structure, were selected. Glycidyl myristate was the most present ester (25 out of 30 samples), while glycidyl palmitate was found in the fewest samples (8 out of 30). A rice oil sample showed the highest contamination levels of glycidyl palmitate and linoleate (0.740 and 0.473 µg/g respectively), while one sample of refined olive oil had the highest contamination of glycidyl oleate (3.30 µg/g).

Conclusions

We developed an innovative method to isolate and quantify the two 3MCPD enantiomers, achieving complete resolution of the two isomers. This method demonstrated high accuracy, precision, and reproducibility. Notably, the analysis of oil samples demonstrated that 3MCPD esters exist as a racemic mixture in vegetable and fish oils, and margarines.
Additionally, we successfully characterized each esterified form of gly using HRMS. The analysis of the samples identified the presence of multiple esterified forms in each oil, comprising both saturated and unsaturated fatty acids. This novel approach enhances our ability to obtain accurate information regarding the type of esterification present in each sample. Ongoing studies are investigating the fate of the esterified forms in the gastrointestinal tract and their associated toxicity. Therefore, it is imperative to have an analytical method capable of quantifying each esterified form, as they may exhibit different toxicological properties.
In conclusion, this mass spectrometry platform offers enhanced information regarding the levels of each enantiomeric and esterified form. Integrating data from this platform with the one obtained from the traditional indirect method, can provide more accurate toxicological assessments, thereby advancing human health risk evaluation.

Acknowledgments

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OR12

A method for determining PFASs in bovine meat and tuna fish by UHPLC- HRMS Orbitrap

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Summary: *An analytical method for the quantification of twenty-four PFASs in canned bovine meat and tuna in olive oil was developed. The identification of PFASs in the two matrixes were analyzed by QuEChERS extraction, purification with dSPE follow by Solid Phase Extration protocol. The extracts were finally analyzed by UHPLC-HRMS Orbitrap.*

Keywords: *PFASs, QuEChERS, High resolution mass spectrometry*

Introduction

Per- and polyfluoroalkyl substances (PFASs), a class of compounds known for their persistence, ability to bioaccumulate and potential adverse health outcomes, pose a significant treat to environmental and human health.[1] The aim of this study was to evaluate and optimized a rapid method for determining PFASs in canned bovine meat and tuna in olive oil.

Experimental

A total of four samples (two canned bovine meat and two tuna in olive oil) were analyzed for monitoring the presence of PFASs. Samples were analyzed by QuEChERS extraction. Secondly, a dispersive solid-phase extraction was conducted to purify the samples. After a weak anion exchange solid phase extraction protocol was applied. Finally, the extracts were analyzed by UHPLC-HRMS Orbitrap in the ESI negative mode using an Hypersyl Gold aQ coloumn [2]. Quantification was performed using internal standards isotopically labelled analogues of the target analytes. Thermo Scientific TraceFinder 4.1 software was used for instrument control and data acquisition. In this method we explore sample preparation techniques using a QuEChERS extraction to achieve low ppb sensitivities and a subsequent solid phase extraction (SPE) for further sample clean-up and ppt sensitivities.

Results

Limits of quantitation (LOQs) were estimated on the signal observed at the lowest point of the calibration curve: these values were ranged from 0.010 to 0.025 μg kg^{-1}. The linearity was assessed on nine calibration levels for each PFASs over the respective range of 0.01 – 2.5 μg kg^{-1} of sample. Coefficient correlation ($R2$) was better than 0.9983 for all analytes. Recoveries were calculated for spiked sample products relative to the internal standard added to the sample before extraction. Recoveries at 2 levels of concentration: 0.1 and 1 μg kg^{-1} in tuna, 0.25 and 1 μg kg^{-1} in bovine meat matrix were found generally between 80 and 120% (Table 3) except for PFTrDA, PFTeDA, FOSA, PFNS and PFDS for which we didn't have the corresponding isotope labelled standards available (Table 1).

Table 1. *List of the 24 analytes and their retention times, target ions used for quantification and ISs*

Analyte	RT (min)	Target ion (*m/z*)	Internal standard
PFBA	4.34	212.9792	$^{13}C_4$-PFBA
PFPeA	7.14	262.9760	$^{13}C_5$-PFPeA
PFHxA	9.21	312.9728	$^{13}C_5$-PFHxA
PFHpA	10.63	362.9696	$^{13}C_4$-PFHpA
PFOA	11.71	412.9664	$^{13}C_8$-PFOA
PFNA	12.59	462.9632	$^{13}C_9$-PFNA
PFDA	13.33	512.9600	$^{13}C_6$-PFDA
PFUdA	13.95	562.9568	$^{13}C_7$-PFUdA
PFTrDA	14.92	662.9505	$^{13}C_2$-PFDoA
PFTeDA	15.26	712.9473	$^{13}C_2$-PFDoA
PFBS	7.74	298.9430	^{13}C3-PFBS

PFPeS	9.48	348.9398	$^{13}C_3$-PFHxS
PFHxS	10.76	398.9366	$^{13}C_3$-PFHxS
PFHpS	11.77	448.9334	$^{13}C_3$-PFHxS
PFOS	12.62	498.9302	$^{13}C_8$-PFOS
PFNS	13.33	548.9270	$^{13}C_8$-PFOS
PFDS	13.94	598.9238	$^{13}C_8$-PFOS
PFUnDS	14.45	648.9206	$^{13}C_8$-PFOS
PFDoDS	14.90	698.9174	$^{13}C_8$-PFOS
PFTrDS	15.24	748.9142	$^{13}C_8$-PFOS
FOSA	13.77	497.9462	$^{13}C_6$-PFDA
NaDONA	10.77	376.9689	$^{13}C_4$-PFHpA
9Cl-PF3ONS	13.00	530.8956	$^{13}C_8$-PFOS
11Cl- PF3OUdS	14.19	630.8892	$^{13}C_2$-PFDoA

Relative standard deviations were <10% for most PFASs. The applicability of the proposed method to detect and quantify PFASs has been demonstrated during a proficiency test with assigned values for PFHpS, PFHxS, PFOS, PFNA, PFUdA and PFOA (Table 2).

Table 2. *Overview of assigned values and obtained z- scores for the EURL-POP_2401 Proficiency test in bovine meat*

Compound	Assigned value (µg/kg)	Z-Score
PFNA	0.315	-1.6
PFOS	0.747	-1.4
PFHpS	0.239	-1.5
PFUdA	0.475	-1.4
PFHxS	0.0726	-2.0

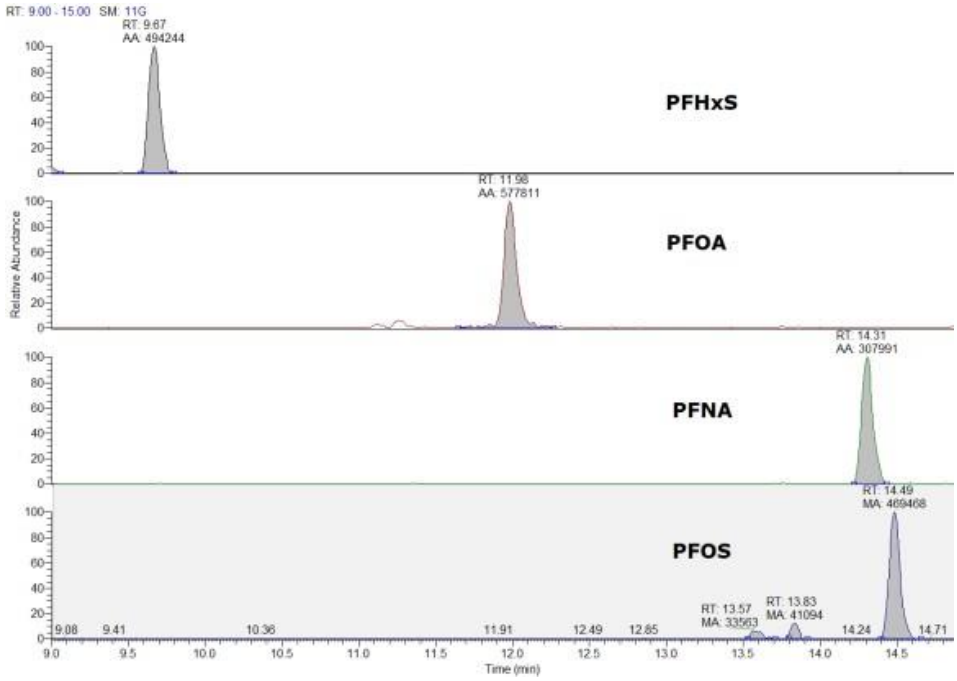

Figure 1. *Chromatograms of the 4 regulated PFASs*

Table 3. *Recovery % (% RSD)* of twenty PFASs spiked at two levels in a tuna in olive oil sample*

	Spiked level	
Compound	**0.1(µg kg^{-1})**	**1(µg kg^{-1})**
PFPeA	101 (4)	93 (2)
PFHxA	109 (10)	96 (1)
PFHpA	92 (6)	95 (2)
PFOA	120(11)	106 (6)
PFNA	99 (9)	96 (2)
PFDA	101 (5)	98 (4)
PFUdA	119 (8)	102 (3)
PFTrDA	5 (10)	53 (23)
PFTeDA	NA	14 (16)
FOSA	58(14)	52(18)
PFPeS	83 (3)	94 (7)
PFHxS	83 (3)	95 (0)
PFHpS	80 (3)	88 (7)
PFOS	107 (4)	100 (3)
PFNS	61 (7)	61 (9)
PFBS	89 (3)	94 (1)
PFDS	32 (6)	37 (11)
NaDONA	98 (9)	101 (2)
9Cl-PF3ONS	82 (1)	83 (4)
11Cl-PF3OUdS	89 (13)	92 (11)

* Each value is the mean of 4 determinations,
% RSD = Relative Standard Deviation

Conclusions

A method for the determination of 24 PFASs was developed in bovine meat and tuna in olive oil. The analytical strategy consisted in an extraction with H_2O/ACN 1:1 v/v in presence of $MgSO_4$ and NaCl follow by clean-up on SPE WAX-GCB cartridge. The sample extract after evaporation with nitrogen flow 0.8 mL of 60:40 H_2O/MeOH + 0.1% acetic acid were added prior to injection.[3] LC-HRMS Orbitrap was used to measure at trace levels these analytes. Application of this method on food matrix samples can permit in the future to collect data on PFAS contamination in food preserving.

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OR13

Recycled and virgin PET bottles and plastic cups: migration potential of contaminants

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Summary: *Food packaging is essential for protecting and preserving food, but it can also pose risks of chemical contamination through migration. Migration tests are crucial for assessing the safety of packaging materials by measuring the extent of chemical transfer into the food, ensuring compliance with regulatory standards*

Keywords: *food contact materials, migration, hydrocarbon contaminants*

Introduction

Food packaging serves multiple essential functions, including protecting and preserving food, extending shelf life, and providing information to consumers. However, it can also present risks of chemical contamination due to the potential migration of substances from the packaging materials into the food.
The extent of migration depends on various factors, including the composition and physicochemical properties of the packaging materials, interactions between the food and packaging materials, the physicochemical properties of the migrant, food composition, type of contact (gas phase or direct), packaging/food ratio, temperature and contact time.[1]

Experimental

This study examined the potential migration of different contaminants from virgin and recycled (30% and 100%) polyethylene terephthalate (PET) bottles, as well as cups made of high-density polyethylene (HDPE) and HDPE-low-density polyethylene (LDPE) in vegetable oil, the intended food contact for the packaging, and in food simulants. Accelerated migration tests at 60 °C for 10 days were conducted.
Volatile organic compounds (VOCs) were analyzed using head space solid phase microextraction and gas chromatography (GC) coupled to a mass spectrometer (MS), with two columns of different polarities. Phthalates (PAEs) were extracted with acetonitrile and analyzed by GC-MS. Hydrocarbon contaminants (HCs) were extracted in hexane and analyzed with an on-line liquid chromatography-GC system, quantified with a flame ionization detector and further characterized by a multidimensional GC approach.

Results

Analysis of VOCs from various PET materials showed primarily aldehydes with higher levels in 100% recycled PET, and ethylene glycol, that were only found in limited quantities after migration tests. Both virgin and recycled PET bottles were free of HCs, while plastic caps contained polyolefin oligomeric saturated hydrocarbons (POSH) with a high propensity to migrate almost completely into the oil (69-79% of the HCs present in the caps migrated into the oil after 10 days at 60 °C). The maximum migration (14 mg/kg) was found from LDPE/HDPE caps.

Conclusions

The profile of VOCs and HCs reflects the different composition of the starting material. The low concentration of VOCs in PET may be due to the super-cleaning process this material undergoes. PAEs and HCs were below the limit of quantification in PET bottles, but plastic caps contained POSH with a high propensity to migrate into the oil, although the low packaging-to-food ratio prevented high levels from being reached.

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OR14

Quantitative LC-MS analysis of mycotoxins in plant-based protein isolates

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Summary: *Optimized LC-MS methods for mycotoxin analysis in fava bean protein isolate is presented. Several extraction methods were investigated to ensure robust mycotoxin recovery. These findings highlight the importance of advanced extraction techniques and LC-MS parameters for accurate mycotoxin detection, enhancing food safety in plant-based proteins.*

Keywords: *Mycotoxin analysis, Fava bean protein isolate, LC-MS extraction technique*

Introduction

Mycotoxins, toxic secondary metabolites produced by certain fungi, pose significant health risks when present in food. Determining mycotoxin levels in food is crucial for ensuring food safety and protecting public health. Fava bean protein isolate, a valuable plant-based protein source, requires thorough analysis to ensure it is free from harmful contaminants. However, current extraction methods for mycotoxins from food matrices present substantial challenges, necessitating advancements in analytical techniques. Here, we present a multianalyte mycotoxin quantitative LC-MS analysis and extraction strategy for fava bean protein isolate**.**

Experimental

Standard samples of 11 mycotoxins were prepared by spiking known concentrations of working solutions of the mycotoxin mixture into blank fava bean protein isolate powder. The extraction procedures included organic liquid extraction,[1] solid-phase extraction (SPE),[2] the QuEChERS method,[3] and salting-out assisted solid-liquid extraction (SASLE).[4] For each extraction method, recovery and matrix factor were determined. Waters Oasis Prime HLB cartridges were used for SPE extraction.

Mycotoxins were separated using the Agilent 1290 Infinity II UPLC system equipped with a ZORBAX RRHD Eclipse (100×2.1 mm, 1.8 µm) C18 column. The LC system was coupled to an Agilent 6490 triple quadrupole mass spectrometer operating in MRM mode. MRM transitions were experimentally determined.

Results

To determine MRM transitions, standards of each of the 11 mycotoxins were recorded in MS1 and MS2 positive ESI modes. The MS1 and MS2 spectra of aflatoxins B1 and B2 are shown in Figure 1. For MRM transitions, the two most intense fragments were selected as quantifier and qualifier signals. The mixture of 11 mycotoxins was measured simultaneously in positive MRM mode. A representative chromatogram in the pure solution is shown in Figure 2.

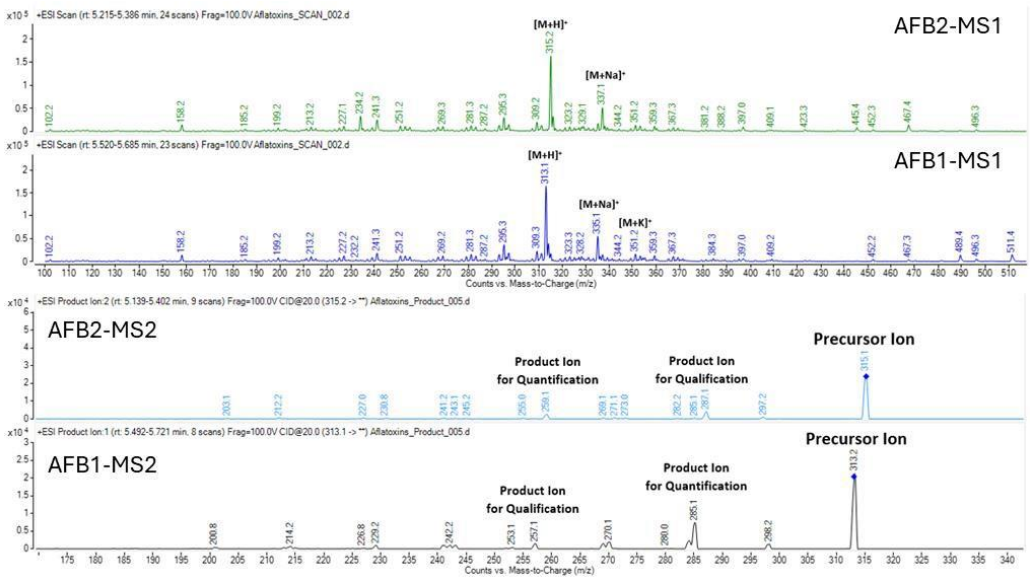

Figure 1. *Aflatoxin B2 and B1 MS1 spectra (upper section) and MS2 spectra (lower section)*

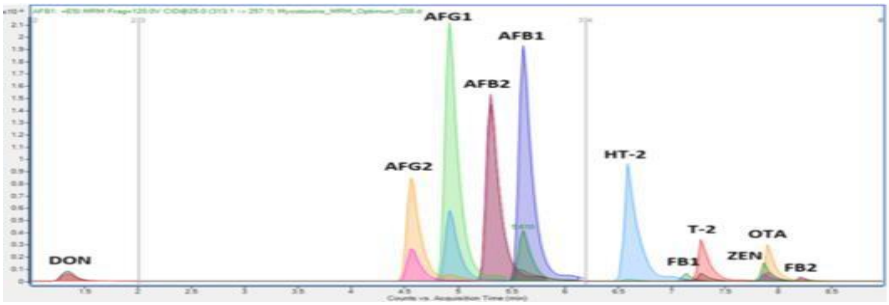

Figure 2. *MRM chromatogram of 11 Mycotoxins*

Following instrument optimization, linearity was tested first in pure solution and then in spiked protein isolate matrix. The calibration curves for AFB2 and AFB1 in pure solution, spiked protein isolate and spiked extracts are shown in Figure 3. Matrix effects and recovery of the extractions were calculated by comparing the signal intensity of a given mycotoxin in pure solution, spiked protein isolate, and spiked protein isolate extract.

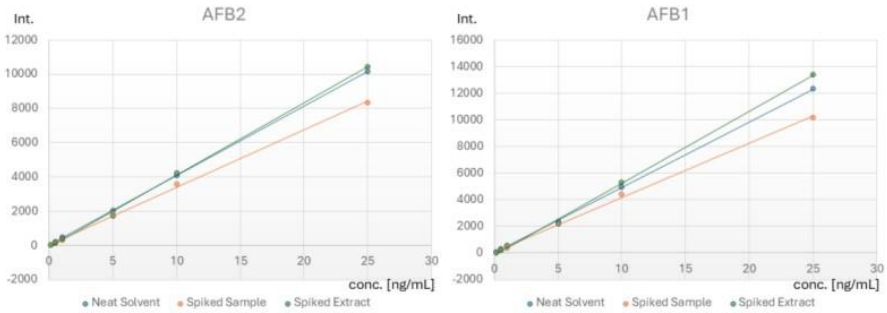

Figure 3. *Calibration curves for AFB2 and AFB1 mycotoxins*

Using the QuEChERS extraction procedure, fumonisin mycotoxins were not recovered, and therefore the method was not validated for matrix effect and extraction recovery. The other extraction methods, organic liquid extraction (SLE), solid-phase extraction (SPE), and SASLE, resulted in the recoveries of all 11 mycotoxins, with the extraction recoveries shown in Figure 4. The SASLE method was modified to include a two-step extraction, initially with an organic solvent with the addition of $MgSO_4$, followed by aqueous solvent extraction.

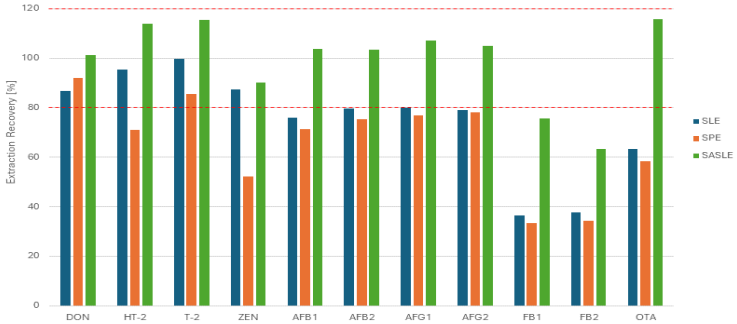

Figure 4. *Extraction recoveries of mycotoxins from protein isolate with three extraction methods*

Conclusion

This study presents a robust LC-MS approach for analyzing mycotoxins in fava bean protein isolate, identifying effective MRM transitions for accurate quantification. Among the evaluated extraction methods, a modified SASLE method demonstrated effective recovery of all 11 mycotoxins. These findings highlight the critical role of optimized extraction techniques and analytical parameters in ensuring accurate mycotoxin detection in plant-based protein sources, thereby enhancing food safety.

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OR15

A multiparametric study for the monitoring of contaminants in biological fluids

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Summary: *This work aimed to screen bovine milk samples in order to provide a profile of contaminants including heavy metals, mycotoxins and bisphenol, using mass spectrometry and Liquid Chromatography coupled to high resolution mass spectrometry.*

Keywords: *Mass Spectrometry; Contaminants; Bovine milk*

Introduction

Environmental pollution caused by anthropogenic activity is the subject of constant study and monitoring. Various classes of pollutants are known nowadays, such as persistent bioaccumulative toxic substances (PBTs), potentially toxic elements (PTEs), and antimicrobials. PBTs and PTEs are classified as “endocrine disruptors”, i.e., they own the capacity of interfering with all endocrine systems (reproductive system, immune system, ecc.) by mimicking or blocking the activity of hormones. Thanks to their abilities, these contaminants cause both acute and chronic toxicity and can bioaccumulate through the food chain, posing a risk also for human consumers. Indeed, several studies have demonstrated that the main route of exposure to these compounds for humans is the oral route, and in particular through foods, namely meat and milk.

Experimental

Metal analysis by ICP-MS

250 microliters of each milk samples were wet digested by using a mix of hydrochloric acid and nitric acid, put for 16 h at 90 °C and suddenly diluted to ICP-MS analysis.

Mycotoxin and Bisphenol analysis

Samples were subjected to a protein precipitation with methanol in ratio 1:3 v/v, after that were centrifuged at 10000 rpm for ten minutes and the supernatant was vacuum dried and resuspend in a mix of water/ methanol. Suddenly each sample was purified by solid phase extraction and analyzed by HPLC-MS/MS in MRM modality.

Results

The study of rural ecosystems in terms of One Health, i.e., under a holistic view, which also consider the presence of human consumer of the product of animal origin, assumes thus a particular importance. This project aims at the determination of the contamination levels in terms of heavy metals, mycotoxins and bisphenols contamination in cow's milk samples from different Italian dairy farms. Metals analysis was performed by inductively coupled plasma-mass spectrometry after mild digestion, while mycotoxins and bisphenol determination was conducted by high performance liquid chromatography coupled to mass spectrometry in Multiple Reaction Monitoring, taking advantage by sensitivity and specificity of this tandem mass spectrometry technique.

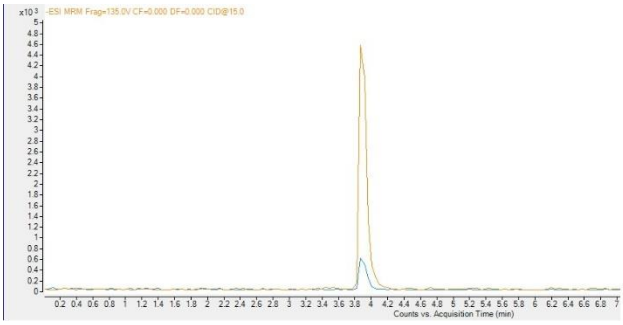

Figure 1. *Chromatogram showing MRM transitions of Bisphenol A*

Conclusions

The positive impacts expected from the research are in line with EU Green Deal policies; to provide information on the state of environmental contamination the presence of different class of contaminants in farmed animals, and their products, was investigated. Moreover sensitive, specific, and robust methods for the determination of contaminants were developed and validated, and new data for use in epidemiological studies will be available.

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OR16

Two years (2023-2024) of Perfluoroalkyl substances (PFASs) results from Italian official food control plans: what are the raising issues?

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Summary: *Since maximum levels for PFASs (PFOS, PFOA, PFNA, PFHxS and their sum) were set in food in 2023, they were included in official European monitoring plans. 224 samples belonging to different food categories were analysed in 2023-2024. The higher levels were measured in liver, fish and bivalve mollusk although very few were samples exceeding the maximum. Limits for long-chain carboxylic acids, anyway, are not jet set, and in some matrices they represent an important contribution to the total PFASs contamination.*

Keywords: *PFASs, food, official control*

Introduction

Perfluoroalkyl substances (PFASs) are a family of highly fluorinated aliphatic compounds, largely synthesized and used in many industrial applications. They turned out to be toxic, persistent, and bio-accumulative in biota and humans [1]. Diet is the most important route for PFASs exposure. In 2020 the EFSA CONTAM panel assessed the risk associated with consumption of PFAS contaminated food, looking at four substances (PFOA, PFOS, PFNA, PFHxS). A safety threshold of 4.4 ng/kg/bw was set for the cumulative Tolerable Weekly Intake (TWI) considering the four PFASs [1]. In January 2023 maximum levels (MRLs) for the four PFASs and their sum in food (eggs, fishery products and bivalve molluscs, meat and edible offal) entered in force therefore PFASs were included in European official control plans [2]. Official Laboratories (OFLs) are requested to monitor these contaminants in food and check the compliance to regulatory limits. Very few Italian OFLs have to date accredited methods following the requirements of UNI CEI EN ISO/IEC 17025:2018. Here we collected and analysed the results of the official control in food carried out during 2023-2024, trying to define the back ground contamination levels and eventual criticism raising from the two years monitoring data.

Materials and Methods

Sampling

The food samples analysed from Istituto Zooprofilattico Sperimentale dell'Umbria e della Marche (IZS-UM) were collected in 2023-2024 in different North and Central Italian regions, in the frame of official monitoring plans issued by the Italian Ministry of Health. Two hundred twenty-four samples were analysed, which included bivalve mollusc (n= 55, 20 mussels and 35 clams), marine fish (n=26), meat (n=60), offal (n=12), bovine milk (n=13), eggs (n=34) and vegetables (Brassicaceae, n=20).

Perfluoroalkyl substances analytical method

11 perfluoroalkyl carboxylic acids (PFCAs: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA) and 8 perfluoroalkane sulfonic acids (PFSAs: L-PFBS, L-PFPeS, L-PFHxS, L-PFHpS, L-PFOS, L-PFNS, L-PFDS and L-PFDoS) were analysed in LC-MS/MS as previously described in detailed [3]. Limits of quantification (LOQs) were set at 0.010 µg/kg (PFBA: 0.20 µg/kg). PFAS sum (ΣPFASs) was calculated in lower bound approach (l.b. concentrations <LOQ = 0), both as sum of nineteen PFASs (Σ19PFASs) and of the four regulated compound (Σ4PFASs).

Results and Discussion

Σ19PFASs in food ranged from <LOQ to 6.6 µg/kg (mean 0.30 µg/kg). Detailed results are reported in Table 1. PFASs were not quantified in all the milk and in most of the meat samples: in chicken all the analytes were always <LOQ and in ovine meat were measured the higher PFAS levels. The highest concentrations were found in offal of terrestrial animals (with the exception of chicken offal, always <LOQ), confirming that perfluoroalkyl substances have a greater affinity for hepatic proteins and phospholipids. However, none of the sample exceeded the MRLs set in offal for the four PFASs by Reg. 915/2023. Non-negligible were the levels in chicken eggs (mean 0.17 µg/kg), and one sample showed a concentration very close to MRLs (PFOS: 0.97 µg/kg; Σ4PFASs: 1.3 µg/kg). As for most of the organic contaminants, the higher level (Σ19PFASs) were in fish and fish product (Table 1). MRL for PFOA was exceed in one sample of cooked yellow clams from Vietnam, where 5.2 µg/kg was measured.

The contamination pattern of the different food matrices highlighted important differences (Figure 1a). PFOS (calculated as sum of linear and branched isomers) was the dominant compound in eggs, meat, and offal,

where it accounts for 48%, 70%, and 83% of the total PFAS contamination respectively. In clams (*C. gallina* e *T. semidecussatus*), PFOS and PFOA generally contribute equally to the contamination (27 and 26% of the total) followed by PFCAs C9-C14, while in mussels and marine fishes PFOS and PFTrDA (C14) were the most abundant, followed by the long chain PFCAs C11-C14. On the contrary in vegetables PFOA, the short-chain PFCAs and PFBS were the analytes mostly quantified.
Figure 1b compares the average Σ19PFASs and Σ4PFASs in the different food categories. The Σ4 regulated compounds account for a substantial portion of the overall total PFASs contamination, (between 64 and 66%) in meat, offal, eggs and clams, while in fish and mussels the Σ4 account only for 30% of the total Σ19PFASs. Therefore the maximum limits do not account for an important portion of the contamination, specifically the long-chain carboxylic acids (C9-C14), for which thresholds were not yet set. Monitoring is surely highly recommended to better understand the contribution of other not yet regulated PFAS (Commission Recommendation (EU) 2022/1431).

Table 1. *PFASs concentration (Σ19 and Σ4 regulated) in food. In bracket: number of sample (n) and frequency of detection (%)*

ΣPFAS l.b. (µg/kg)	Σ4 regulated	Σ19	Σ4 regulated	Σ19	Σ4 regulated	Σ19
	Mussels (n=20; 100%)		**Meat (Total samples n=59; 24%)**		**Offal (Total samples n=13; 77%)**	
median (min-max)	0.11 (0.012-0.22)	0.31 (0.094-0.46)	<loq (<loq-0.24)	<loq (<loq-0.27)	0.51 (<loq-3.4)	0.59 (<loq-3.6)
mean±sd	0.10±0.057	0.29±0.11	0.016±0.042	0.018±0.049	1.1±1.3	1.3±1.5
	Clams (Total samples n=35; 100%)		*Ovine meat (n=8; 63%)*		*Ovine offal (n=4; 100%)*	
median (min-max)	0.19 (0.049-6.2)	0.40 (0.086-6.6)	0.013 (<loq-0.24)	0.020 (<loq-0.27)	2.890	3.458
mean±sd	0.51±1.1	071±1.1	0.056±0.086	0.061±0.093	2.6±0.92	3.0±0.94
	C. gallina (n=25; 100%)		*Bovine meat (n=24; 25%)*		*Bovine offal (n=5; 100%)*	
median (min-max)	0.17 (0.049-0.76)	0.30 (0.086-1.0)	<loq (<loq-0.11)	<loq (loq-0.11)	0.21 (0.11-2.6)	0.27 (0.16-3.1)
mean±sd	0.22±0.15	0.38±0.21	0.014±0.033	0.014±0.033	0.71±1.1	0.86±1.3
	T. semidecussatus (n=9; 100%)		*Swine meat (n=14; 21%)*		*Chicken offal (n=3; 0%)*	
median (min-max)	0.47 (0.18-1.7)	0.68 (0.36-2.2)	<loq (<loq-0.84)	<loq (loq-0.19)	n.c.	n.c.
mean±sd	0.69±0.60	0.97±0.72	0.012±0.028	0.019±0.052		
	P. undulata (n=1)		*Chicken meat (n=13; 0%)*		*Swine offal (n=1)*	
concentration	6.197	6.583	n.c.	n.c.	0.858	0.917
	Marine fishes (n=26; 85%)		**Eggs (n=34; 38%)**		**Bovine milk (n=13; 0%)**	
median (min-max)	0.024 (<loq-0.96)	0.16 (<loq-3.3)	<loq (<loq-1.3)	<loq (<loq-2.2)	n.c.	n.c.
mean±sd	0.10±0.20	0.35±0.68	0.11±0.28	0.17±0.45		
	Vegetables (n=20; 65%)					
median (min-max)	0,029 (<loq-0211)	0,042 (<loq-0,82)		**mean±sd**	0,050±0,12	0,066±0,20

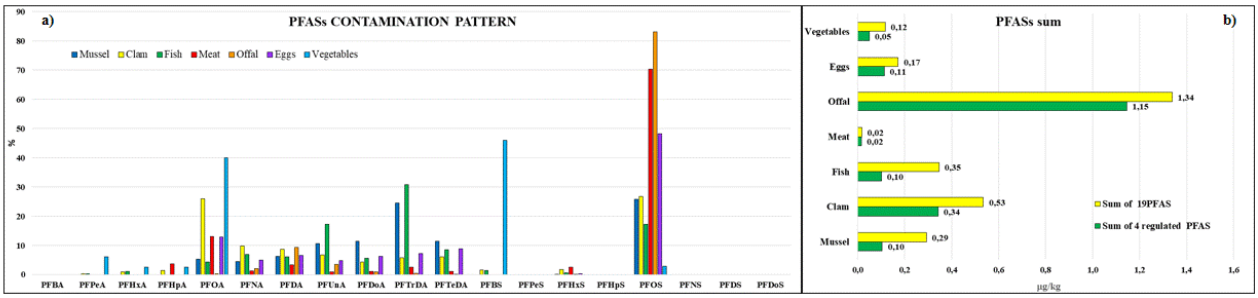

Figure 1. *a) PFAS contamination patterns (%). b) Σ 19PFASs vs Σ4PFASs (PFOS, PFOA, PFNA, PFHxS)*

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OR17

CP-MIMS: a new frontier for the real-time monitoring of hazardous chemical migration from food contact materials

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Summary: *CP-MIMS is a direct mass spectrometry technique, suitable for real-time monitoring of dynamic processes. In this research, a CP-MIMS method was developed, optimized, and validated for the analysis of Bisphenol A released from food contact materials allowing to detect and quantify its migration in real-time for the first time ever.*

Keywords: *Membrane Introduction Mass Spectrometry, real-time monitoring, food contact materials.*

Introduction

Contemporary trends in analytical chemistry are aimed not only at improving analytical performances, but also at minimizing analysis time and reducing environmental impact, with many modern techniques that embrace Green Analytical Chemistry (GAC) principles. Within them, Condensed Phase-Membrane Introduction Mass Spectrometry (CP-MIMS) is a promising technique for direct on-line analysis that does not require sample preparation and chromatographic separation [1]. It involves a semi-permeable hollow fiber membrane that is immersed directly into the sample and through which a liquid acceptor phase continuously flows, conveying the permeated compounds to the MS ion source. Since only compounds fulfilling certain physicochemical properties can permeate through the membrane, adequate selectivity can be reached even in untreated complex matrices. These aspects, together with a fast analysis time, make CP-MIMS a suitable strategy for the continuous real-time monitoring of dynamic processes. These characteristics can be valuable for the assessment of emerging and re-emerging contaminants migration from food contact materials (FCMs), whose continuous update of maximum accepted contamination levels make it a current topic. In this context, the recent reduction of the tolerable daily intake of bisphenol A (BPA) by EFSA has renewed interest in evaluating its release from FCMs, for which current specific migration limit is set at 50 µg/kg (EU Reg. 2018/213) [2]. Because of that, in this study, for the first time, a CP-MIMS method was developed and validated for the real-time monitoring and quantification of BPA released from FCMs. The CP-MIMS probe was coupled to an electrospray (ESI) ion source.

Experimental

The CP-MIMS configuration of this application is based on few components. A HPLC Dionex UltiMate 3000 SD series (Thermo Scientific) was used to deliver the acceptor phase (AP) through the probe, carrying permeated compounds to an LTQ XL (Thermo Scientific) mass spectrometer equipped with an ESI source and linear ion trap analyzer operated in negative tandem mass spectrometry mode (MS/MS). The CP-MIMS interface was a U-shaped custom-made probe consisting of a PDMS hollow fiber membrane that connects two sides of a metal capillary. One end of the capillary was connected to the AP delivery system, whereas the other end to MS detector. The optimized conditions are the following: a 25% v/v heptane in methanol mixture was used as AP, whereas the permeation was performed over a 3 cm PDMS membrane (Dow Corning) operating under 800 rpm stirring and 70°C of sample temperature. Raw chronograms were acquired using Xcalibur (Thermo Scientific) and then processed using a dedicated signal processing tool developed in Matlab (MathWorks). Analytical grade BPA and β-naphthol (internal standard) were prepared in methanol to be spiked in water, simulant A (ethanol 10% v/v) and simulant B (acetic acid 3% w/v). A set of commercial 16 food contact items were subjected to migration tests; 13 were of recent production whereas 3 were 25 years old, thus placed on the market before the entry into force of EU Regulation N. 10/2011 on FCMs [3].

Results

After many preliminary experiments aimed at defining the experimental domain, a full factorial DoE was applied to optimize experimental conditions based on multiple response variables (AP flow and composition, membrane length, sample temperature and stirring.). The experimental set was composed of 32 experiments with 3 central replicates at a BPA concentration of 100 µg/L. Optimal conditions were 70 °C and 800 rpm for

sample temperature and stirring, 25% v/v heptane in methanol acceptor phase flowing at 70 µL/min, and 3 cm of membrane length. Method validation was performed in water and food simulants, obtaining LODs in the 1-3 µg/L range, and LOQs between 2.5 and 7 µg/L. Precision was evaluated at two concentration levels for all three matrices by acquiring 10 replicates within the same day (intra-day) obtaining RSD% <9.1%, or covering two weeks (inter-day), with RSD% in the 17.9-22.8% range over 10 working days. The method linearity was proved in the LOQ-4000 µg/L range in all the matrices. Trueness was assessed at three concentration levels, ranging from approximately 80% close to the LOQ to >97% at 75 µg/L. Once validated, the method was used to test commercial FCMs in the three migration media; experiments were conducted in compliance with the OM3 conditions (70°C for 2 hours) reported in the EU Regulation N. 10/2011 acquiring the signal all time long. Whereas more recent samples did not show any increase in the BPA concentration, for 25-years old items a migration was detected in all cases, exceeding up to 35-folds the 50 µg/L specific migration limit (SML) for the baby-bottle in simulant B. For all samples, the migration was observed to a lesser extent in water and to a greater extent in simulant B. Quantitative results were confirmed by an independent method based on liquid chromatography-high resolution MS. Thanks to the CP-MIMS configuration it was possible to observe the migration of a substance from FCMs in real-time for the first time ever, thus enabling unexplored levels of material characterization for food safety. Finally, the method compliance with the green analytical chemistry principles was evaluated using the AGREE tool, giving a satisfactory result of 0.81.

Conclusions

An innovative approach based on CP-MIMS was developed, optimized and validated for the determination and on-line monitoring of BPA released by food packaging, with a complete transferability to the current testing regulations. CP-MIMS allowed to monitor the migration process in real-time for the first time ever, thus exploring unknown trends in the migration kinetic with a simultaneous greenness advancement compared to conventional approaches.

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Acknowledgements

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OR18

Optimising QuEChERS for the analysis of mussels: efficient extraction of emerging contaminants in environmental samples

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Summary: *In this study, the QuEChERS procedure was optimised to detect Emerging Contaminants (ECs) in mussels. This method was then applied to analyse environmental samples and to identify contaminants, such as ketoprofen and nicotine. This work is important not only to enhance food safety but also for environmental monitoring.*

Keywords: *emerging contaminants, QuEChERS method, seafood*

Introduction

Emerging contaminants (ECs) are chemical compounds continuously released in the environment, whose dispersion may cause significant environmental and human health damage. Despite their potential adverse effects, these compounds have yet to be internationally regulated [1]. The ECs definition encompasses a wide range of chemical species, including pesticides, pharmaceuticals and personal care products, and hormones, some of which are characterised by poor degradability and pseudo- persistence [2,3].
Filter-feeding organisms, such as bivalves, are frequently used in the literature as biomonitoring species due to their capacity to accumulate pollutants through their feeding habits. These organisms are robust sentinels, due to their characteristics: they are filter feeders with limited mobility, possess wide spatial distribution, demonstrate tolerance to diverse environmental conditions, and are easily sampled [4]. Additionally, mussels are widely consumed as food around the world. In the EU, the average annual per capita consumption of mussels is 1.28 kg. Therefore, the presence of contaminant residues in mussels and other edible marine species therefore represents a critical public health concern that necessitates rigorous monitoring through specific analyses [5].
Our study aimed to develop a method to detect extremely low concentrations of contaminants. We utilised *Adamussium colbecki*, an Antarctic bivalve filter-feeding organism known for minimal contamination, to develop and optimise this method. Subsequently, we applied the method to *Mytilus galloprovincialis* samples, which are not only used for monitoring but are also edible. Developing analytical methods to detect pollution residues in various edible aquatic species is crucial, as it combines environmental monitoring with the assessment of dietary intake risks.

Experimental

Samples of *A. colbecki*, an Antarctic bivalve filter-feeding organism, underwent a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) pre-treatment procedure optimised using the experimental design approach. The steps of the QuEChERS method were studied, by varying solvent volume, shaking mode, and clean-up phases amount. All the resulting extracts were dried and reconstituted with methanol-water (1:1) before analysis using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). After optimising the experimental procedure with Antarctic *Adamussium* samples, the refined method was applied to local mussel samples (*M. galloprovincialis*) to further assess its robustness and applicability.

Results

The procedure was designed to detect a wide range of ECs, characterised by different polarity, acidic/basic properties, and molecular mass. Obtaining analysis accuracy for all of them strictly requires a multivariate approach, also due to the several variables involved in the sample treatment strategy. A Plackett-Burman screening design was used to identify significant parameters, which resulted to be the amount of dispersive solid-phase extraction (dSPE) phase, the clean-up time, and the solvent-to-sample ratio. These parameters were subsequently optimised using a Dohelert design. The preliminary results obtained from applying the refined method to environmental samples successfully detected the presence of ECs such as ketoprofen, gemfibrozil, nicotine, ethylhexyl salicylate (EHS), ethyl hexyl methoxy cinnamate (EHMC), and octyl dimethyl p-aminobenzoate (OD-PABA) in *M. galloprovincialis* samples. Still, further analysis is required for EHMC and OD-PABA confirmation, due to the detection of traces of these contaminants in the procedural blank. Ketoprofen was detected at the LOD level; however, its presence could not be confirmed due to the absence of a qualifier ion in the mass spectrum. These results confirmed the method's effectiveness and robustness, although some results necessitate additional scrutiny to fully validate the contaminants' presence.

Conclusions

Optimising the QuEChERS method marks a significant advancement in the detection and analysis of ECs in mussel samples. Our study aims to improve the sensitivity and accuracy of detection methods for many contaminants, of utmost importance for food safety and environmental monitoring. This method has the potential to provide valuable data for regulatory and scientific efforts to safeguard public health and preserve environmental quality. By validating the technique under different environmental conditions and sample matrices, we ensured its reliability and effectiveness for detecting emerging contaminants in diverse contexts. Future research will focus on the expansion of the set of mussel samples, including those from various geographical locations and different aquatic environments, to investigate contamination in multiple areas of our territory.

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PL3

Bitter taste and mass spectrometry

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Summary: *"Sensomics", the combination of human sensory science and instrumental analysis techniques contributes knowledge to primary challenges of the food sector.*

Keywords: *taste, mass spectrometry*

The global population is growing, but the area of arable land is not. About ¾ of global agricultural land is occupied to produce animal-based foods. A transition of current nutrition patterns rich in animal-related foods towards more plant- and plant protein-based foods has been recommended for efficient use of natural resources, improved sustainability, reduced greenhouse gas production, and benefits to consumer health. Food waste must be minimized, and "side streams", by-products of food production that are not the primary product, must be utilized for food production. The food sector's primary challenge today is securing sufficient nutritious and healthy food that appeals to the senses for the future. However, including plant protein and plant side streams in food production bears both opportunities and challenges. As the hedonic value, which refers to the sensory pleasure derived from food, is the primary incentive for consumer purchase, alternatives to animal products benefit from improving, e.g., color, odor, and taste, to reach sufficient consumer acceptance. "Sensomics", the combination of human sensory science and instrumental analysis techniques, is a powerful tool that generates crucial insights into essential flavor compounds. The application of sensomics enables the detection, identification, and quantification of appealing taste properties as well as bitter or astringent off-tastes. Sensomics thus contributes to the goals mentioned above by generating knowledge that paves the way for technological measures to, e.g., remove unwanted flavors and introduce substitutes from plants for animal products into food production.

OR19

rECOBIOpack project: optimization of lipid extraction for coffee silverskin valorization

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Summary: *The rECObioPack project aims to develop a packaging material from coffee silverskin (CS). As project first step, the lab-scale supercritical CO_2 extraction of lipids from CS was optimized using design of experiment. The obtained results were then employed to perform the industrial scale supercritical extraction of these compounds.*

Keywords: *coffee silverskin, design of experiment, supercritical fluid extraction.*

Introduction

In the coffee year 2022/23, 10.092 million tons of coffee were produced generating waste at every step of the value chain.[1] During coffee roasting process, the only generated by-product is silverskin (CS), the thin tegument covering green coffee beans. CS represents the 4.2% (w/w) of the beans and, from a circular economy perspective, it can be reused in other productive processes. CS has been mainly used for fuel or for composting so far. In this context, the rECOBIOpack project (funded by PRIN 2022) aims to develop a functional food packaging material from the main components of CS: cellulose as polymeric backbone and lipids, proteins and polyphenols as additives. In this work, the extraction of lipids, carried out using CO_2 supercritical fluid extraction, was optimized using Design of Experiment (DoE). Moreover, the laboratory extraction was scaled up at ILSA S.p.A. (Arzignano, Italy) using an industrial supercritical CO_2 apparatus.

Experimental

CS deriving from a mixture of Arabica coffee beans has been kindly supplied by the Italian coffee company Illycaffè S.p.A (Trieste, Italy). The lab-scale supercritical fluid extraction of lipids from CS was performed using the biomass as flakes, considering the following industrial application. A response surface methodology, based on DoE, was employed for the optimization of the extraction.[2] The selected design was the Central Composite Face-centered (CCF), a design that supports a quadratic model (Eq.1), exploring the factors (variables having an influence on the system) at three levels (+1, 0, -1).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (1)$$

With b_i the model coefficients and X_1 and X_2 the investigated factors.
Based on previous results,[3] the selected factors were pressure (100, 300, 500 bar) and temperature (40, 60, 80°C). The extraction was performed in alternate static and dynamic cycles of 30 min and 10 min, respectively. To explore the experimental domain and to evaluate the experimental variability, a total of 11 experiments was performed. The optimized responses were extraction yield (Y%), free fatty acid content (FFA%), and percentage of unsaturated fatty acid (UNSAT%).
The fatty acid composition of the samples was evaluated using GC-MS (Bruker Scion SQ Instrument, Milan, Italy), after derivatization (both transesterification and sylilation were employed).
The significance of the resulting models was evaluated, afterwards the models were validated and the best extraction condition were selected.[2]
The fatty acid composition of the extracts obtained at the optimal conditions, were compared with the results of a conventional extraction using *n*-hexane.
Finally, the industrial extraction was performed for 3 h at 400 bar and 60°C, in dynamic conditions (flow of CO_2 26 kg h^{-1}). The biomass was extracted using two vessels (10 L) in series each one containing 0.7-0.9 kg of CS.

Results

Three DoE models were computed by multiple linear regression, however only the models obtained for Y% and FFA% resulted statistically significant. It is worth noting that the model coefficients related to pressure and to the interaction between pressure and temperature were significant for both the models but with opposite effect on each response. The extraction yields of the different DoE experiments (0.19-2.92%) permitted to highlight the influence of pressure on the CO_2 solvent power. In particular, at lower value of pressure, the low

density of CO_2 resulted in lower yields due to its poor solvating power. On the other hand, higher FFA% were obtained for the experiments in which CO_2 presents the lower value of density (100 bar and 60°C, and 100 bar and 80°C). This behaviour can be explained considering the lower solubility of lipids in these conditions, and the higher volatility of free fatty acids compared to glycerides.
Due to the opposite effect of the factors on the investigated responses, the optimal extraction conditions were selected taking into account the Y% and were set at 400 bar and 50°C. Additional experiments were performed at the *optimum* and the model was successfully validated.
Regarding the fatty acid composition, the GC-MS results showed a clear difference between sylilated and transesterified samples. The first present only the composition of free fatty acids, while the latter the total fatty acid composition [3]. In particular, the sylilated samples presented a higher amount of long chain fatty acids (Figure 1).

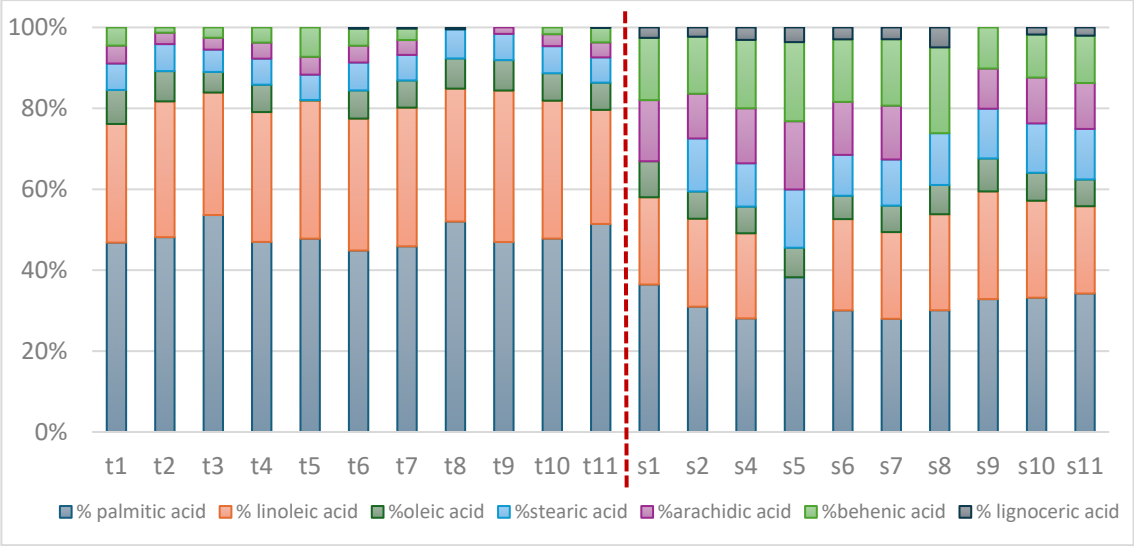

Figure 1. *Results of GC-MS analysis of fatty acid in the CS lipidic extracts obtained in the DoE experiments (1-11) using transesterification (t) or sylilation (s).*

Compared to the conventional *n*-hexane extraction, supercritical CO_2 extraction using the best extraction conditions did not show significant differences in terms of Y%, FFA% and characteristic fatty acids.
The industrial extraction has been successfully carried out providing higher yields compared to the lab-scale, likely due to the higher (and more efficient) CO_2-biomass ratio.

Conclusions

The specificity of supercritical CO_2 relies on its physical properties, which can be modulated by changing pressure and/or temperature. The use of DoE highlighted the influence of these parameters on lipid extraction and allowed to find the optimal conditions in order to maximum the yields. The application at industrial scale of supercritical CO_2 has provided the lipidic extract and delipidized CS, necessary to the subsequent steps of the rECOBIOpack project, under the perspective of a zero-waste valorisation approach.

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OR20

Characterization of alkylpyrazines by HS-SPME-GC-MS on roasted coffee from different botanical species and *terroir*

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Summary: *In the present work, several samples of green Arabica and Robusta beans thermally treated under different conditions were characterized by HS-SPME-GC-MS with special attention to alkylpyrazines. The effects of geographical origin and post-harvest treatment were also studied. Although the highest amounts of these heterocyclic compounds were found in several Robusta coffee samples, alkylpyrazines content cannot be consider as an appropriate marker to discriminate Robusta from Arabica coffee.*

Keywords: *roasted coffee, alkylpyrazines, Coffea arabica, Coffea canephora*

Introduction

The coffee aroma is remarkably important for both quality and big success of this popular drink, and it has been the subject of many studies aimed at disclosing its composition and sensory perception. Most aroma compounds such as alkylpyrazines are formed during the roasting process because of the Maillard reaction between sugar and aminoacids. Alkylpyrazines can also arise from the pyrolysis of serine and threonine and from thermal treatment of glycerol and aminoacids. Alkylpyrazines such as 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine were reported to contribute to the earthy-roasty character of roasted coffee [1]. Czerny et al. [2] identified unsaturated alkylpyrazines, such as 2-ethenyl-3,5-dimethylpyrazine, as further potent earthy-smelling compounds in roasted coffee. In addition to the relevant role played by alkypyrazines in coffee aroma, these coffee compounds have been suggested as possible contributors to the putative beneficial health effects of coffee. In facts alkylpyrazines have been found to modulate platelet aggregation, a critical process in CVD and thrombosis, at least in part by modulating phosphodiesterases activity and cAMP homeostasis in humans [3]. Several studies have highlighted that alkylpyrazines can play a role in characterizing the flavor of roasted *Coffea canephora* (Robusta) brews and contribute to causing the difference in aroma compared to roasted *C. arabica* (Arabica). Caporaso et al. [4] reported on the analysis of volatile compounds by SPME-GC-MS for individual roasted coffee beans of the two botanical species. In this study, alkylpyrazines were found to be generally higher in Robusta, e.g. 2-methyl-pyrazine, 2,6-dimethylpyrazine, 2,5-dimethylpyrazine, pyrazine, ethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine and 3-ethyl-2,5-dimethylpyrazine. Many other studies have suggested alkylpyrazines as possible markers (together with sulfur-containing compounds and phenols) to discriminate roasted Robusta coffee from Arabica. Given the role played by free amino acids and amino acid residues formed by the heat degradation of proteins in generating nitrogen-containing compounds through the Maillard reaction, it is not surprising that Robusta coffee, generally richer in alkylpyrazine precursors, could be characterized by a higher amount of these compounds. However, in addition to some studies reporting a higher content of alkylpyrazines in Arabica, several important factors, such as post-harvest treatments, geographical origin, and roasting degree, can affect the content of alkylpyrazines in roasted coffee. These factors have not yet been thoroughly investigated to fully understand the potential role of alkylpyrazines content as a marker of roasted Robusta coffee. In the present work, several samples of Arabica and Robusta thermally treated under different conditions have been characterized by HS-SPME-GC-MS with special attention to alkylpyrazines. The effects of geographical origin and post-harvest treatment have also been studied.

Experimental

2 g of Arabica and Robusta green coffee bean samples (commercial lots) from different geographical origins and post-harvest processing were inserted into a 20 ml vial and roasted in a thermostatic dry bath (Galli, Italy) at 210°C for different times in the range 5-38 minutes, then closed with a screw cap. 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 to 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 um 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. Among several aroma compounds, 11 different alkylpyrazines were identified (resorting to both standards and library) and semi-quantified by peak area integration.

Results

During the roasting process the alkylpyrazines are generated already in the first 5 minutes, then they increased after 10-15 minutes and remain constant in the range of 15-32 minutes. They tend to decrease in darker samples, as already reported by Baggenstoss et al. [5], who found a fast increase in the beginning of roasting process and a decrease for darker degrees of roast for 2,3,5-trimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine.

In general, the highest observed amounts of total alkylpyrazines have been found in Robusta samples, however, not every examined Robusta coffee beans set after roasting, exhibit more alkylpyrazines than Arabica samples. In particular, some Robusta samples have been found to contain lower alkylpyrazines content than that of the examined Arabica samples. As far as the geographical origin is concerned, in our experiments, alkylpyrazines were generally higher in samples from Brazil than from India. Washed (wet-processed) coffees versus natural (dry-processed) coffees were also compared. This comparison showed that the alkylpyrazine profile is different according to the post-harvest process, and more abundant in natural dry-processed coffees.

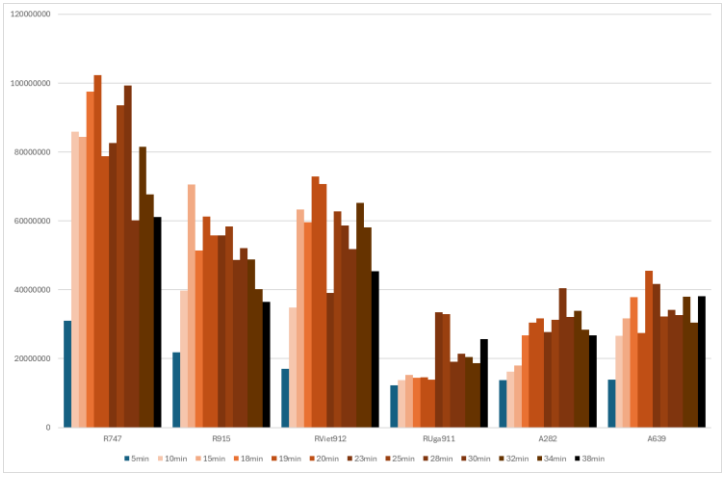

***Figure 1**. Sum of the areas of 11 alkylpyrazines in Robusta (R) and Arabica (A) coffee samples in the range of 5-38 minutes of roasting. A selection of investigated samples.*

Conclusions

Several samples of Arabica and Robusta green coffee beans have been thermally treated and characterized by HS-SPME-GC-MS with special attention to alkylpyrazines. Since the development of alkylpyrazines occurs at the beginning of the thermal process, the obtained results are not affected by the roasting degree.

Highest amounts of these heterocyclic compounds have been found in Robusta coffee samples, however, a systematical higher content of these compounds in all examined Robusta coffee samples has not been found. The present study strongly suggests that the alkylpyrazines content as possible marker of Robusta coffee cannot be of practical use. Data showed also that the post-harvest treatment as well as the geographical origin play a role in the development of alkylpyrazines after the roasting process and these aspects cannot be neglected for comparison purposes.

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OR21

Hazelnut products traceability through combined isotope ratio mass spectrometry and multi-elemental analysis

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Summary: *Raw and processed hazelnut commodities are often the subject of fraudulent geographical declarations. The present study aims to demonstrate the effective assessment of the provenience of Italian hazelnuts by analyzing relative isotopic ratios of carbon and oxygen, while including also multi-elemental analysis features*

Keywords: *EA-IRMS, geographical origin, hazelnuts*

Introduction

Raw and processed hazelnut commodities are often the subject of fraudulent geographical declarations. Italian products are the main target of these illegal activities as their quality is high and certified, and the prices are bigger.
Isotope ratio mass spectrometry (IRMS) could play a key role in origin discrimination.
The present study aims to assess the provenience of Italian hazelnuts, by analysing relative isotopic ratios of carbon and oxygen, while including also multi-elemental analysis features.

Results

Method development was performed by evaluating samples' repeatability, reproducibility, and robustness. The results are reproducible and robust, having acceptable standard deviations.
One-way ANOVA demonstrates the significant statistical difference between Italian and non-Italian samples. Furthermore, a data fusion approach, with inductively coupled plasma–optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS), permitted to build multivariate statistical models to confirm the differences of geographical provenience.
A design of experiment (DoE) was created to sample correctly, considering factors such as variety, processing, and peel percentage.

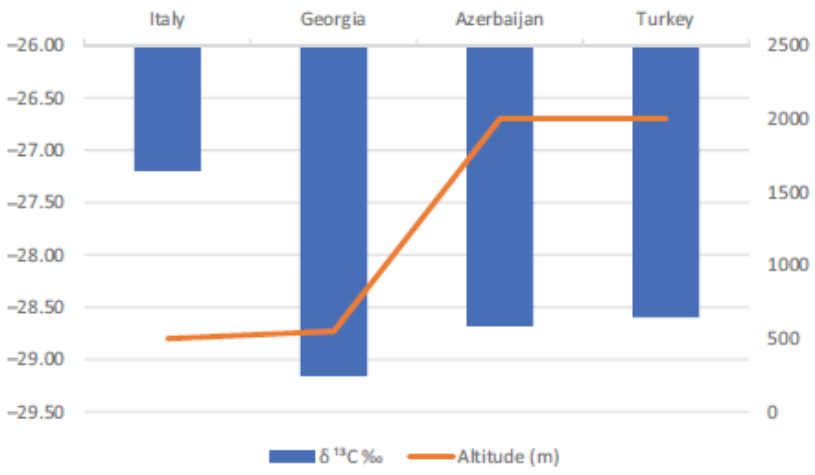

Figure 1. *Mean δ 13C-altitude values histogram plot of the 2021 campaign hazelnut sample set*

Conclusion

96 hazelnut lots, from Italy, Turkey, Georgia, and Azerbaijan, were analysed for the geographical assessment: this strategy demonstrates promising potentialities, as food isotopic abundances reflect ground and climate-related features, typical of precise locations.

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OR22

Assessment of Pomodoro Riccio metabolomic profile through a multimethodological approach for food safety

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Summary: *The present study is aimed at a full chemical characterization of an old tomato cultivar, Pomodoro Riccio, and improve food safety and genuineness through a multi-methodological approach based on untargeted, high-resolution mass spectrometry (HRMS) and NMR, and targeted, HPLC-MS, methods to maximize the coverage of several classes of metabolites.*

Keywords: *tomato; metabolomics; food safety.*

Introduction

Tomatoes and tomato-based food are worldwide diffused foodstuffs, whose nutritional importance is related to their contents of many antioxidant compounds, like carotenoids, polyphenols, and vitamins. [1-3] However, tomatoes are a food category extremely exposed to safety risks related to the presence of chemical residuals, like pesticides [4], and microbial contaminants, including bacteria and fungi. [1,5-6] In this context, the present study, carried out in the frame of the ONFOODS consortium (Research and Innovation Network on Food and Nutrition Sustainability, Safety and Security – Working ON Foods) [7] stemming from the National Recovery and Resilience Plan (NRRP), is aimed at the metabolomic profile and possible identification of new and (re)-emerging hazards of an old tomato fruit ecotype, i.e., "Pomodoro Riccio". This is a cultivar particularly suited for growing on clay soils, with a low demand for water, that could ultimately mean a lower intake of contaminants. In fact, it has been proven that water is the primary source of heavy metals and pesticide residues, [4] thus, this old cultivar has the potential to enhance the quality of tomatoes that emerges as a matter of priority for customer's safety.

Experimental

Pomodoro Riccio samples (fresh tomatoes, tomato paste and waste) harvested in two different years (2022 and 2023) were provided by "La Sbecciatrice" company (Caserta, Campania, https://www.lasbecciatrice.it) and were subjected to a pre-treatment protocol, including freeze-drying, homogenization, and storage at -80°C, and then to Bligh-Dyer (BD) extraction that allows to extract both water- soluble and fat-soluble metabolites. [3] Untargeted MS characterization was achieved thanks to the high resolution of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) allowing, by a fast single measurement, the identification of several classes of metabolites present in the matrix.
After appropriate dilution, the solutions were directly injected in electrospray ionization (ESI) source. High resolution ESI-MS analyses have been carried out by using a 9.4 T Bruker hybrid SolariX XR FT-ICR mass spectrometer, both in positive and negative ionization mode. Spectra were acquired in the *m/z* range 100-1000 mass range and each sample was analysed in three replicates. The unsurpassed accuracy of FT-ICR measurements gives a univocal molecular formula, which has been assigned to several metabolites with an uncertainty of less than 1 ppm.
Furthermore, complementary methodologies such as untargeted Nuclear Magnetic Resonance (NMR) spectroscopy and targeted High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) were employed to obtain the chemical profile as co-exhaustive as possible in order to maximize the metabolomic coverage.

Results

The hydroalcoholic and organic BD extracts have been subjected to the multimethodological analytical protocol comprising untargeted (NMR, ESI FT-ICR MS) and targeted (HPLC-DAD, HPLC-MS) methodologies. Untargeted analyses produced over 1000 metabolites in both the hydroalcoholic and organic fraction, including primary metabolites, such as amino acids, carbohydrates, organic acids, and secondary metabolites, such as terpenes and alkaloids.

A qualitative visualization of molecular classes was achieved by van Krevelen graphs and histograms of relative frequency.
Untargeted ^{1}H NMR-based metabolomics revealed the presence of primary metabolites, such as amino acids, carbohydrates, organic acids.
The targeted approach based on HPLC-MS/MS methods has allowed to determine fat-soluble vitamins, carotenoids and polyphenols. In particular, chlorogenic acid, rutin, caffeic acid, phloridzin, 4-*o*-caffeoylquinic acid, naringenin, were identified and quantified.

Conclusions
Overall, the high sensitivity and mass accuracy achieved with ESI FT-ICR MS enables elemental formulas of many metabolites and harmful compounds present in trace amounts, like pesticides, agrochemical derivatives and metals, to be determined. Overall, a broad metabolomic profile has been gathered throughout the combined application of both targeted and untargeted methodologies to monitor the quality and traceability of Pomodoro Riccio tomatoes.

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OR23

All-ion fragmentation and high-resolution mass spectrometry as key tools for the untargeted profiling of glucosinolates in Brassica microgreens

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Summary: *All-ion fragmentation (AIF) at high-resolution mass spectrometry allows the monitoring of glucosinolate (GLS) diagnostic product ions without any need for precursor ion isolation. In this work, combined AIF and full-MS scan experiments were exploited to develop an untargeted analytical approach for the identification of GLS signals in complex chromatographic profiles.*

Keywords: *All-ion fragmentation, high-resolution mass spectrometry, glucosinolates*

Introduction

Glucosinolates (GLS) are plant secondary metabolites typically produced by Brassica vegetables. All GLS share a common structural motif, consisting of a ***β***-thioglucopyranose moiety whose S atom is linked to the C atom of a sulfated oxime [1]. As shown in Figure 1, the same C atom binds a distinctive R-side group for each GLS.

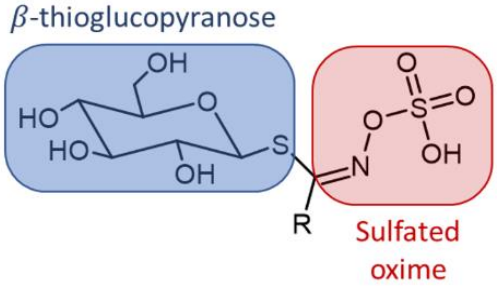

Figure 1. *Molecular structure of glucosinolates*

The unstable aglycones of GLS, generated enzymatically, are crucial for plant defence and serve as precursors to compounds beneficial for human health [2]. Recently, Brassica microgreens have gained attention as excellent sources of GLS [3]. They offer the added benefit of being consumed as raw, thus preventing the thermal degradation of GLS during food processing (*e.g.*, boiling or steaming).
In this communication, we describe the application of a new analytical approach based on reversed-phase liquid chromatography coupled to electrospray ionization Fourier-transform mass spectrometry (RPLC-ESI(-)-FTMS), in which the all-ion fragmentation (AIF) operation mode was exploited to enhance the untargeted characterization of GLS occurring in the polar extracts of four Brassica microgreen crops, namely garden cress, rapeseed, kale, and broccoli raab. The sulphate radical anion $[SO_4]^{\bullet -}$ (*m/z* 95.9523) was recognized as the best qualifying product ion from higher-energy collisional dissociation (HCD)-FTMS^2 spectra of GLS as deprotonate molecules, $[M-H]^-$. The corresponding extracted ion current (EIC) chromatograms by RPLC-ESI(-)-AIF-FTMS were used as a guide to determine the position of GLS signals in the RPLC-ESI(-)-FTMS total ion chromatograms (TIC). The identification of GLS was supported by tandem MS spectra of the corresponding $[M-H]^-$ precursor ions.

Experimental

Based on an existing protocol [1], the extraction of GLS was performed on lyophilized microgreens using a heated (75 °C) methanol/water 70:30 (v/v) mixture as the extraction medium. A further purification step aiming at protein precipitation and polar lipid removal was introduced.
The RPLC-ESI(-)-MS analysis of GLS extracts was achieved using two LC-MS platforms implementing a Q-Exactive (Thermo Fisher) high-resolution quadrupole-Orbitrap and a Velos Pro (Thermo Fisher) low-resolution, multi-stage linear ion trap mass spectrometer, respectively.
RPLC separations were performed using a multi-step binary elution gradient, based on water and acetonitrile, both containing 0.1% (v/v) formic acid. The separations were performed with a C18 Ascentis Express column

(15 cm length, 2.1 mm internal diameter) packed with core-shell 2.6 µm particles (Supelco). The mobile phase flow rate was set up to 200 µL/min.

Results

As shown in Figure 2A the RPLC-ESI(-)-Full-FTMS TIC chromatograms of a kale microgreen exhibited a large number of signals. However, the alignment of these peaks with those detected in the AIF extracted ion current (EIC) chromatogram at *m/z* 95.9523 (see Figure 2B) allowed the identification of signals pertaining to putative GLS species. To increase the confidence level in GLS identification [4], the following steps were taken: (1) the ESI(-)-FTMS mass spectrum was averaged under each detected peak and subtracted from the background spectra averaged on both sides of the same peak; (2) isotope patterns were verified for signals potentially related to GLS species; (3) accurate m/z values of monoisotopic (M+0) peaks were used to retrieve information about the corresponding molecular formulas, achieving the fourth level in terms of identification confidence. The results were compared with the molecular formulas of known glucosinolates in the METLIN database (https://metlin.scripps.edu/). The putative structures were tentatively corroborated up to confidence levels 2 and 3 by combining the retention time and the interpretation of targeted HCD-FTMS/MS and CID-MS^n (n = 2, 3) spectra. Finally, molecular structures were confirmed (confidence level 1) with suitable analytical standards, if available.

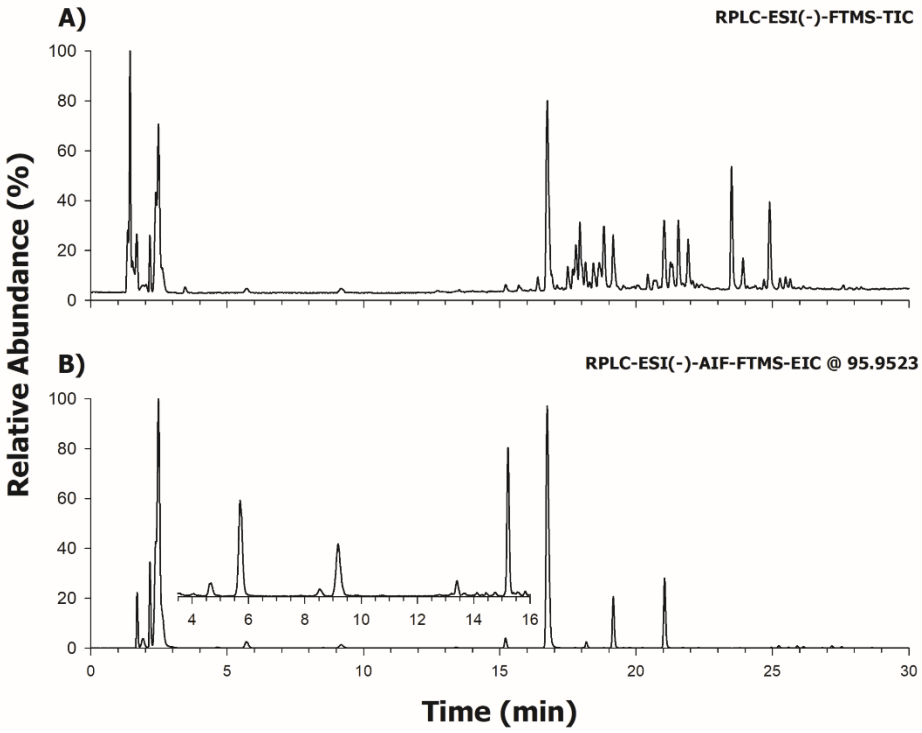

Figure 2. *(A) Total ion current chromatogram resulting from RPLC-ESI(-)-FTMS of a kale microgreen extract. (B) Extracted ion current (EIC) chromatogram obtained by RPLC-ESI(-)-AIF-FTMS for the GLS product ion corresponding to sulphate radical anion (m/z 95.9523)*

Conclusions

A new approach was proposed for an extended untargeted screening of GLS in plant samples, based on the AIF-FTMS operation mode. As a result, up to 27 different GLS were identified with a confidence level equal to or higher than 3 in four Brassica microgreen crops.

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OR24

Antioxidant reactivity of anthocyanins in red cabbage with an AAPH-incubating method using liquid chromatography coupled with high-resolution tandem mass spectrometry

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Summary: *This study investigates the antioxidant activity and reactivity of natural antioxidants from red cabbage. The screening of the anthocyanins present in the red cabbage extract was conducted using HPLC coupled with DAD and HRMS/MS. Then, an AAPH-incubating method was developed to assess the antioxidant reactivity of anthocyanins.*

Keywords: *Antioxidant activity, high-resolution mass spectrometry, AAPH*

Introduction

Recent studies have highlighted the potential of natural antioxidants to replace synthetic additives in the food industry [1]. However, the antioxidant mechanisms and their links to molecular structures are not fully understood. Current methods like ORAC, DPPH, and FRAP assays are concentration-based and lack efficienct data on their reactivity in neutralizing radicals. Additionally, these assays measure the capacity but overlook the reaction kinetics and interactions, thereby failing to identify the most reactive antioxidants. A possible method to assess antioxidant activity of natural extracts is based on 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), a radical initiator able to release ROO$^{\bullet}$ at 37°C and pH 7.4 using high-performance liquid chromatography (HPLC) coupled with diode array detector (DAD) and high-resolution tandem mass spectrometry (HRMS/MS) [4]. This approach could allow to monitor changes in anthocyanin consumption rates, which can then be correlated to the molecular structure of each compound.

Experimental

Red cabbage extract was analyzed with Dionex Ultimate 3000 UHPLC device (Thermo Fisher Scientific, Waltham, MA, USA) coupled to a Q Exactive™ Orbitrap high resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The same instrument was used to monitor the reaction between red cabbage extract and AAPH. The separation was carried out by using a Kinetex Biphenyl 2.6 µm column (100 × 2.1 mm) at a flow rate of 0.3 mL/min at 40.0°C. The gradient was set with a mixture of solvents A (Milli-Q water with 2% of formic acid) and B (methanol with 2% of formic acid). For full MS analysis, the electrospray source was operated in a positive ionization mode with a capillary voltage of 3.5 kV at a temperature of 350°C. The scan range was set at 100–1500 m/z with an acquisition rate of 1 microscan per second and a resolution of 70,000, AGC target at 1e6 and maximum injection time of 50 ms. The parameters of the data dependent MS2 acquisition were as follows: ddMS2 AGC target 5e5, maximum injection time 50 ms, resolution 17,500, loop count 5, isolation window 4.0 m/z, isolation offset 1 m/z and normalized collision energy 15%. Anthocyanins present in red cabbage extract were tentatively identified through appropriate molecular formulas, using the extracted ion chromatogram, and confirmed by the fragmentation spectra with accepted mass error <5 ppm. The reaction with AAPH was conducted using phosphate buffer solution (PBS) 0.01 M pH 7.4 and adding the red cabbage extract, cyanidin which was used as internal standard, and AAPH 20 mM.

Results

13 anthocyanins were putatively identified in the red cabbage extract with the most predominant being cyanidin 3-diglucoside 5-glucoside and other being acylated cyanidins derived from cyanidin 3- diglucoside 5-glucoside. After the identification of anthocyanins present in the extract, the reaction with peroxyl radicals (ROO•) released from the initiator 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was monitored under physiological conditions at 37°C and pH 7.4.

Table 1. *Peak number, accurate and exact mass, accurate mass of the fragment ions, mass error found and retention time of the synthetic cathinones and their hypothesized metabolites, elemental composition and name of the anthocyanins in HPLC-DAD-ESI-HRMS/MS.*

Peak	accurate mass (m/z)	exact mass (m/z)	Add.	ddMS2	Formula	Proposed Name
1	773.2129	773.2135	M+	611.1604, 449.1075, 287.0544	C33H41O21+	cyanidin 3-diglucoside 5-glucoside
2	611.1613	611.1607	M+	449.1069, 355.0692, 287.0541	C27H31O16+	cyanidin 3,5-diglucoside
3	979.2685	979.2714	M+	817.2162, 449.1066, 287.0540	C44H51O25+	cyanidin 3-(sinapoyl-diglucoside) 5-glucoside
4	1141.3213	1141.3242	M+	979.2688, 449.1071, 287.0543	C50H61O30+	cyanidin 3-(sinapoyl-triglucoside) 5-glucoside
5	1141.3213	1141.3242	M+	979.2688, 449.1071, 287.0543	C50H61O30+	cyanidin 3-(sinapoyl-triglucoside) 5-glucoside
6	287.0545	287.055	M+		C15H11O6+	cyanidin (std)
7	919.2497	919.2503	M+	757.1959, 449.1069, 287.0542	C42H47O23+	cyanidin-3-(p-coumaroyl-diglucoside)-5-glucoside
8	949.2602	949.2608	M+	787.2067, 449.1071, 287.0543	C43H49O24+	cyanidin 3-(sinapoyl-xylosyl-glucosyl) 5-glucoside
9	979.2707	979.2714	M+	817.2162, 449.1066, 287.0540	C44H51O25+	cyanidin 3-(sinapoyl-diglucoside) 5-glucoside
10	817.218	817.2186	M+	655.1652, 449.1074, 287.0544	C38H41O20+	alatanin c
11	1125.3063	1125.3082	M+	963.2530, 449.1069, 287.0542	C53H57O27+	cyanidin 3-(coumaroyl-sinapoylsophoroside) 5-glucoside
12	1155.3182	1155.3187	M+	993.2634, 449.1073, 287.0544	C54H59O28+	cyanidin 3-(feruloyl-sinapoylsophoroside) 5-glucoside
13	1185.3289	1185.3293	M+	1023.2740, 449.1067, 287.0540	C55H61O29+	cyanidin 3-(disinapoylsophoroside) 5-glucoside

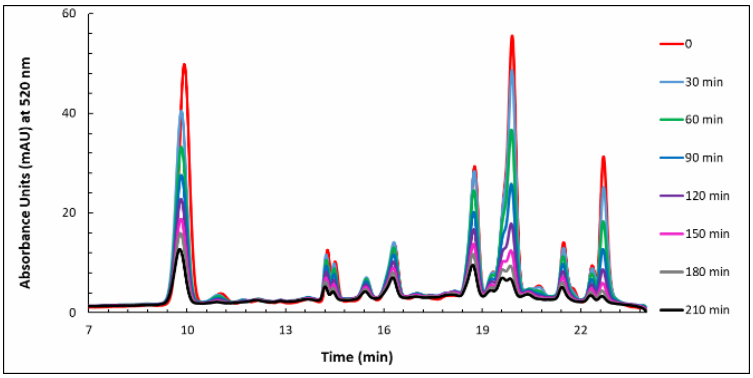

Figure 1. *Progressive decrease of peak areas of anthocyanins in the red cabbage extract after incubation with AAPH*

During the reaction a different consumption rate among anthocyanins was observed. The relative reduction in peak areas for each anthocyanin indicates specific efficiencies in neutralizing peroxyl radicals, likely reflecting their unique molecular structures. Alatanin C, a cyanidin derivative with two glucose units and sinapic acid at the C3 position, exhibited the highest relative reduction and antioxidant efficiency.

Conclusions

An efficient method for the screening of anthocyanins in red cabbage was developed using high-resolution mass spectrometry and for the first time the reaction between anthocyanins and AAPH was conducted identifying the most reactive anthocyanins present in the extract. This study can thus enhance the understanding of anthocyanin's antioxidant reactivity, revealing their capacity to neutralize peroxyl radicals and their potential as natural alternatives to synthetic additives.

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OR25

Optimization of HPLC-HRMS method for the detection of antioxidant Maillard reaction products and study of reaction conditions for their production as food preservatives

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Summary: *This study developed an HPLC-HRMS method to detect and identify antioxidant Maillard reaction products (MRPs). Optimal pH, reaction time, and amino acid-sugar combinations were determined, leading to a library of potential antioxidant MRPs. Key findings provide a foundation for developing natural antioxidant food preservatives from common food compounds.*

Keywords: *Maillard reaction; antioxidants; HPLC-HRMS*

Introduction
The Maillard reaction (MR) involves chemical interactions between reducing sugars and amino acids or proteins during heating, resulting in Maillard reaction products (MRPs) that contribute to food flavors, aromas, and color (Nooshkam et al., 2019; Salter et al., 1989; Sun et al., 2022). Some MRPs also exhibit antioxidant activity, which can offer health benefits and enhance food preservation (Michalska et al., 2008; Nooshkam et al., 2020; Wang et al., 2011). This has led to interest in using MR to produce natural antioxidant food preservatives from common food compounds and wastes.
Natural food preservatives are gaining attention due to health concerns about synthetic additives, the clean label trend, and the demand for sustainable food practices (Novais et al., 2022).

Aim and Experimental
This study aimed to develop a method for detecting and identifying potential antioxidant MRPs using High-Pressure Liquid Chromatography (HPLC) coupled with High- Resolution Mass Spectrometry (HRMS). By optimizing the chromatographic method, molecules produced during MR were separated and detected. Known antioxidant MRPs were used as reference signals for method development (Kanzler et al., 2016; Yanagimoto et al., 2002).
Additionally, the study explored the influence of pH, reaction time, and different sugars and amino acids on the production and composition of antioxidant MRPs. An untargeted HPLC-HRMS analysis of sugar and amino acid mixtures at pH 7 identified potential antioxidant MRPs, which were monitored using a radical initiator that reduced their signals. These potential antioxidant MRPs were then tracked in MR samples under varying conditions to comprehensively evaluate how each variable affects their production.

Results
The main results of this study include the definition of analytical condition to detect and identify the antioxidant MRPs, the definition of the best pH and amino acids and sugars combination to obtain each antioxidant MRPs, and the creation of a library of m/z signals corresponding to potential unknown antioxidant MRPs.
Incubating the MR samples with a radical initiation, the signals decreasing was monitored, and a library composed of 50 m/z signals was built and used to evaluate the best condition for MR to obtain the higher antioxidant potential. As expected, at neutral and basic pH the reaction resulted faster than with acidic pH, and the production of antioxidant MRPs is comparable at pH 7 and 8. Therefore, pH 7 was selected as the best for the purpose of this study. Moreover, 20 amino acids and 6 sugars, the most common in food industry, were combined and the best combinations to produce each known antioxidant MRP and potential antioxidant MRP was defined. In *table 1* the known studied antioxidant MRPs and the best combination to obtain them are reported.

Table 1. *Antioxidant compounds produced by MR and reported in literature, with exact mass, theoretical m/z, retention time and best amino acid and sugar combination for their production. *Compounds verified with analytical standards.*

Compound	Theoretical *m/z*	Retention time	Best AA x sugar
Maltol*	127.03897	13.27	Lysine x maltose
Isomaltol*	127.03897	10.98	Tryptophan x arabinose
2-acetylpyrrole*	110.06004	18.79	Methionine x fructose
Sotolon*	129.05462	14.56	Lysine x galactose
Norfuraneol*	115.03897	9.25	Aspartic acid x arabinose
Furaneol	129.05462	12.62	Lysine x galactose
DDMP	145.04954	10.50	Threonine x maltose
2-pyrrole-carboxaldehyde	96.04439	4.00	Gutamine x arabinose
1-methyl-2-pyrrole carboxaldehyde	110.06004	6.00	Thronine x maltose
2-acetyl-1-methylpyrrole	124.07569	9.30	Threonine x arabinose

Conclusions

In conclusion, this study has developed an analytical method based on HRMS and elucidated the significant influence of some key variables in MR like pH, time and reagent composition, on the antioxidant compounds production. The way for more precise and comprehensive analyses of antioxidant MRPs has been paved, and the next crucial step in this research would be to identify the specific MRPs that exhibit the most interesting antioxidant activities. This will involve isolating MRPs and characterizing their structures and mechanisms of action. Such efforts will not only enhance our understanding of the Maillard reaction's contribution to food chemistry but also open new avenues for the development of functional foods that valorise these natural antioxidants.

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OR26

Characteristics and critical issues of packaging intended for dairy products

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Summary: *This study aims to evaluate the characteristics of the packaging intended for milk and dairy products, focusing on critical aspects of different types of packaging that can be evaluated with different analytical techniques.*

Keywords: *food packaging, bisphenols and styrene migration, shelf life.*

Introduction

Milk and dairy products are packaged using various materials such as plastic, metal, glass, and cardboard. In the past, milk was primarily packaged in glass. Despite its recyclability, this material proved impractical because of its fragility and bulkiness. As a result, the need arose to develop alternatives that could offer the same hygienic and preservation qualities as glass, but with easier handling and management. [1]
Among composite packaging materials, beverage cartons, like those produced by TetraPak®, are among the most well-known. These cartons are typically composed of paper, which makes up 75% of the material, polyethylene, a plastic that accounts for 20%, and a thin layer of aluminum, which constitutes 5%. The aluminum is included in cartons intended for products with a long shelf life at room temperature, while it is absent in cartons used for fresh milk, which must be stored at low temperatures. Metal, including aluminum, is used to make containers, foils, and closures. Thanks to its strength, metal packaging can be processed at high speeds with minimal product loss, increasing overall efficiency and reducing CO2 emissions. Metal is unbreakable, reducing the risk of loss or breakage during transport, storage, and sale. In the dairy sector, metal is used in specific applications, such as cans for condensed and powdered milk, and aluminum foil for butter. [2]
Plastic, available in various types like PET, PE, HDPE, PS, and PP, offers many advantages, including flexibility, lightness, strength, and ease of sterilization. This material is highly resistant to moisture, heat, and cold, allowing for easy storage and distribution of perishable products. Some types of plastic can be recycled, reused, or composted. HDPE bottles are particularly suitable for storing pasteurized milk, while colored PET is used to protect milk from light, preventing lipid oxidation and taste alterations. Polystyrene (PS) is commonly used for packaging yogurt and milk-based desserts, due to its ability to form thin and lightweight containers. [3]
Ultimately, there is no single ideal material for packaging milk and dairy products. The choice of material depends on various factors, such as the type of product, processing and storage conditions, and final use. The main goals in packaging development are to increase shelf life, improve material design and sustainability, and expand the use of recycled materials, all while ensuring the food safety of the product. [4]

Experimental

We analysed cans with epoxy-resin coatings to evaluate the migration levels of bisphenols. To avoid overestimation deriving from the use of simulants like ethanol, we chose to analyse the actual food: in this case condensed milk. The extraction was performed with QuEChERS method, the extracts were analysed with UHPLC-HRMS (QTof 4600 AB Sciex).

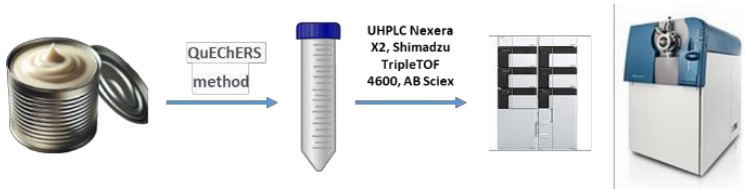

Figure 1. *Protocol of analyses for food packaging samples for bisphenols quantification*

We analysed PS samples with HS GC-MS/MS to determine the content of residual styrene monomer, then we performed specific migration tests both with simulants and foods. We chose four packaging samples: PS ice-cream cups (sample A), PS coffee cups (sample B), PS beverage cups (sample C) and EPS food trays (sample D).

For migration tests with simulants we selected three different simulants (AcOH 3%, EtOH 50% and EtOH 95%), we chose the condition of contact (2h at 70°C) and we analyzed the migration solutions obtained with HS GC-MS/MS.

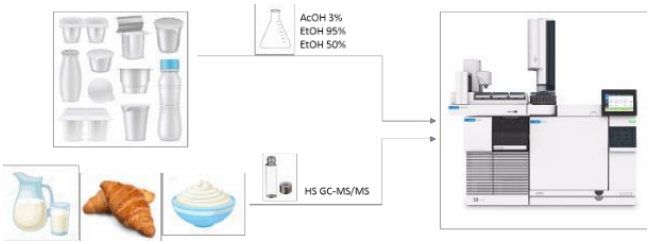

Figure 2. *Protocol of analyses for food packaging samples for styrene determination*

For migration test with food we selected representative categories of food that are usually packed in PS plastic: milk, yoghurt and bread. All the analyses were performed with Agilent 8890 GC-MS coupled with Agilent 7000E TQ.
We also conducted shelf life studies to evaluate the differences between the different types of packaging, both from a microbiological point of view and from the aspect of the aromatic profile.

Results and discussion
As reported in *Figure 3*, we observed that the migration of BPA increases as the percentage of fat in the food increases. This can be attributed to the lipophilic nature of bisphenols.

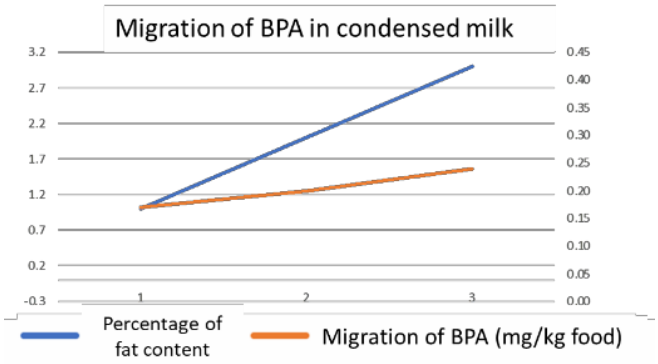

Figure 3. *Migration levels of Bisphenol A*

We analysed polystyrene samples to determine the residual content of styrene monomer, obtaining results ranging from 50 mg/kg to 120 mg/kg.
The results obtained from the migration tests, both with food simulants and actual food, are summarized in Table 1.

Table 1. *Styrene migration levels (µg/kg food)*

Food or simulant	Sample A	Sample B	Sample C	Sample D
AcOH 3%	ND	ND	ND	ND
EtOH 50%	488.5	718.7	728.7	7803.6
EtOH 95%	12973.5	8289.8	10027.2	44912.8
Bread	ND	ND	ND	ND
Yogurth	33.5	23.1	29.9	204.0
Milk	822.0	252.2	148.4	288.2

We observed higher concentration with food simulants, with the highest value occurring with 95% ethanol. We hypothesized that the food simulants overestimate the real migration values, so we performed migration test with actual food, obtaining values ten times lower. The migration in milk was higher, which aligns with other

studies finding that migration is greater with fatty foods and those with a higher surface-to-volume ratio. For this reason, milk could be chosen as the worst-case simulant to perform routine migration analyses on food contact materials for assessing styrene migration.
The shelf-life studies did not show significant differences between the different packaging analysed.

Conclusion
This study assessed various classes of compounds that could potentially pose a risk to milk and dairy products packaged in plastic, metal, and cardboard. The key finding is that the most concerning issues arise from chemicals that may migrate from the packaging, rather than from microbiological factors or the aromatic profile.

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OR27

Compounds isolated from licorice root (*Glycyrrhiza glabra L.*) as natural antioxidants in prevention of lipid oxidation

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Summary: *In this research, four major fractions were isolated from licorice root extract using semi-preparative HPLC system. The main compounds were identified using 1H NMR and HPLC coupled to coulometric array detector and HRMS. The fractions with stronger antioxidant properties were used to inhibit lipid oxidation of a vegetable oil.*

Keywords: *antioxidants, high resolution mass spectrometry, lipid oxidation*

Introduction

In recent years, licorice (Glycyrrhiza glabra L.) root has garnered significant attention for its distinct sweetening and flavoring attributes and a potential source of valuable bioactive compounds with strong antioxidant, anti-inflammatory, health-promoting, and antimicrobial properties.

Previous published research studies have focused on the determination of the main chemical compounds of licorice root extracts, metabolic profiling, and identifying specific bioactive compounds, such as glabridin and glycyrrhizin, responsible for their health-promoting properties [1, 2].

Despite the variety of research studies on licorice root extracts, there remains a notable gap in knowledge regarding the antioxidant properties of specific antioxidant compounds from these extracts. Moreover, to the best of our knowledge, no practical application of licorice antioxidants in a food system, such as, vegetable oils has not been extensively undertaken.

Experimental

Dried plant material (10 g) was extracted with 100 mL of solvent (water: ethanol=1:1, v/v) facilitated by ultrasounds (STEEL®, digital ultrasonic generator, Italy) at 60% of amplitude for 40 min at 40℃ (345 W, 37 kHz).

The screening of antioxidant compounds was accomplished using HPLC with triple detector system [3]. For identification of the bioactive compounds, the analysis was performed using Ultimate 3000 UHPLC system (ThermoFisher Scientific, Walthman, MA, USA) coupled with a QExactive Orbitrap (ThermoFisher Scientific, Walthman, MA, USA) mass spectrometer. The separation of analyte was performed using a SunFire C18 5µm 3.0x100 mm column (Waters, Italy). For the full-MS analysis, the ESI source operated in both positive and negative ionization modes, with a capillary voltage of 4 kV, temperature of 320 °C, scan range 75-1000 m/z, resolution of 70,000 and AGC target of 55. In the data-dependent settings the resolution was set at 17,500 and the AGC target at 15 with a NCE of 30.

Further analysis for screening and selection of the main antioxidant compounds in the extract was done using an Agilent 1260 Infinity HPLC system coupled to a diode array detector (DAD) and a coulometric array detector (CAD) poised at increasing potentials from +50 mV to + 800 mV. The DAD was set to two selected wavelengths of 265, 280 and 320 nm with a function of recording spectrum in the range from 200 to 600 nm.

Results

The chemical composition of the licorice root extract was analyzed using liquid chromatography coupled to three detector system composed by CAD, DAD and MS (Figure 1). The analysis revealed a complex mixture of compounds detected at 265, 280 and 320 nm (DAD).

Four main fractions were isolated using preparative LC system. Each fraction was analyzed by the high-resolution mass spectrometry and 1H NMR spectroscopy to identify 14 major antioxidants.

Major metabolites in the first fraction were p-hydroxybenzylmalonic acid (1), syringic acid (2), protocatechuic acid (3), licroagroside (4) and vicenin (5). The main compounds in the fraction 2 were liquiritigenin-7-O-apyosylglucoside (6) and liquiritin apioside (7). Fraction 3 contained isoliquiritin apioside (8), licuraside (9) and licorice glycosides (licorice glycoside B, 10). The principal compounds in the fraction 4 were glycyrrhizin (11), glabridin (12), glabrone (13) and glabrol (14).

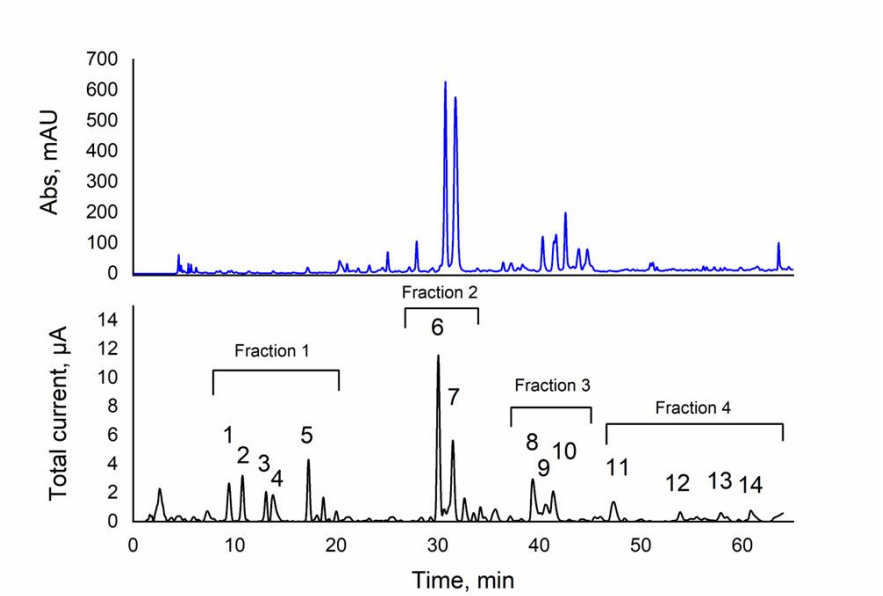

Figure 1. *HPLC-DAD chromatogram of licorice extract measured at 265 nm (blue), and the accumulated sum of current obtained from the 16 CAD channels (black). Main compounds were identified by high-resolution mass spectrometry*

As the next step, the antioxidant activity of the fractions was evaluated using a novel DPPH• stopped-flow kinetic assay [4]. Prior to analysis, the phenol content of the fractions was standardized to 60 µM using the Folin-Ciocalteu method. Two out of the four fractions (3 and 4) demonstrated significantly higher radical scavenging kinetics and antioxidant activity. Finally, the fractions with stronger antioxidant properties, isolated from licorice root, were used to inhibit lipid oxidation in a vegetable oil rich in polyunsaturated fatty acids. Among all fractions the fraction 4 showed the highest reactivity against lipid oxidation.

Conclusions

The chemical composition of licorice root extract was analyzed, and four main fractions were isolated and identified through HRMS and 1H NMR spectroscopy, leading to the identification of 14 major antioxidants. Subsequent antioxidant activity testing with a novel DPPH• stopped-flow kinetic assay indicated that fractions 3 and 4 possessed significantly higher radical scavenging kinetics and antioxidant activity. These fractions, particularly fraction 4, which contained glycyrrhizin, glabridin, glabrone, and glabrol, effectively inhibited lipid oxidation in a vegetable oil rich in polyunsaturated fatty acids. Fraction 4 demonstrated the highest reactivity against lipid oxidation, underscoring its potential as a potent antioxidant source.

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OR28

The impact of industrial peeling on the lipidome of canned cherry tomatoes (*Solanum lycopersicum* v. cerasiforme): a HILIC-ESI-FTMS study

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Summary: *Lipid extracts from both raw ripe and canned peeled cherry tomatoes were analyzed using hydrophilic interaction liquid chromatography coupled with electrospray Fourier-transform mass spectrometry (HILIC-ESI-FTMS). This study aimed to identify differences in the lipid profiles caused by storage, cooking, and other stressors that might activate enzymes responsible for glycerophospholipid degradation.*

Keywords: *Tomatoes, industrial processing, HILIC-ESI-FTMS*

Introduction

After the colonization of the Americas, tomatoes were introduced to Europe around 1540, thriving in the Mediterranean climate.[1] Today, tomatoes are a cornerstone of the Mediterranean diet.[2] According to the Food and Agriculture Organization of the United Nations, tomatoes are the second most cultivated vegetable, with over 180 million tons grown on 5 million hectares.[3] Beyond being enjoyed fresh, tomatoes are available in various processed forms, such as sauces, peeled tomatoes, juices, and ketchup, all of which contribute significantly to the intake of antioxidants.[2]

The health benefits of regularly consuming tomatoes are primarily attributed to their rich content of lycopene, β-carotene, vitamins C and E, and various polyphenols. These compounds are believed to help prevent cancer and cardiovascular diseases and play a crucial role in slowing cellular aging.[2] While many studies have focused on these beneficial compounds, the characterization of tomato glycerophospholipids has received less attention. However, lipidomics – the comprehensive study of lipids – can offer valuable insights into the storage and quality of tomatoes throughout their supply chain.[2]

Experimental

Lipid extraction was carried out through the Bligh and Dyer method [5] on cherry tomato (*Solanum lycopersicum* v. cerasiforme) samples, which were subsequently grinded in isopropanol to hinder the activity of phospholipases.[6]

Deuterated standards were added in proper amounts during the extraction to compare their retention times with the ones observed in the real specimens investigated by HILIC-ESI-FTMS. Among the main glycerophospholipid (GPL) classes, phosphatidylethanolamines (PEs), phosphatidylinositols (PIs), phosphatidylglycerols (PGs), and phosphatidylcholines (PCs) were identified. Additionally, the possible presence of their lyso-forms was revealed by extracted ion current chromatograms in all ion fragmentation (AIF) mode focused on the diagnostic ions of each lipid class.

Major GPL species were identified through both accurate *m/z* ratios provided by negative ion mode high-resolution mass spectra (ESI(-)-FTMS) related to the chromatographic peaks and ensuing targeted tandem MS examination.

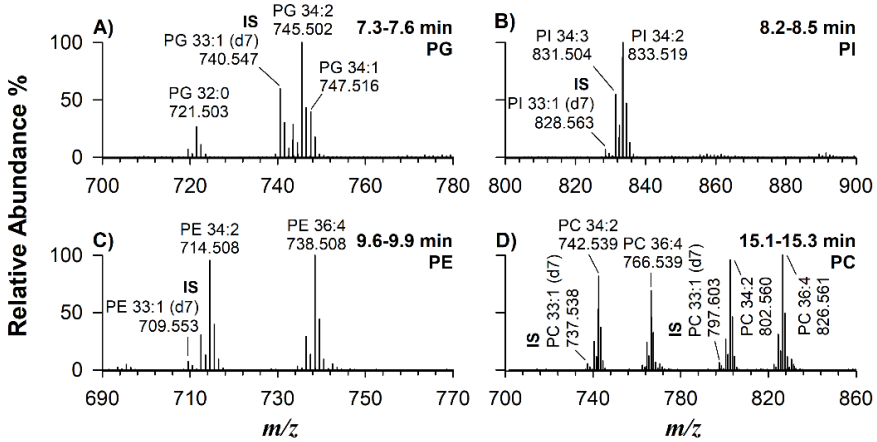

Figure 1. *ESI(-)-FTMS spectra acquired at the retention time of PGs (A), PIs (B), PEs (C), and PCs (D) identified in ripe cherry tomato extracts. Deuterated standards are labelled as IS.*

Results

Both ripe tomato and canned peeled cherry tomato extracts were analysed using HILIC-ESI(–)-MS. The chromatographic profiles revealed an increase in lyso-forms and a decrease in intact forms in the canned samples compared to the ripe tomatoes. The quantitative data are presented in Figure 2.

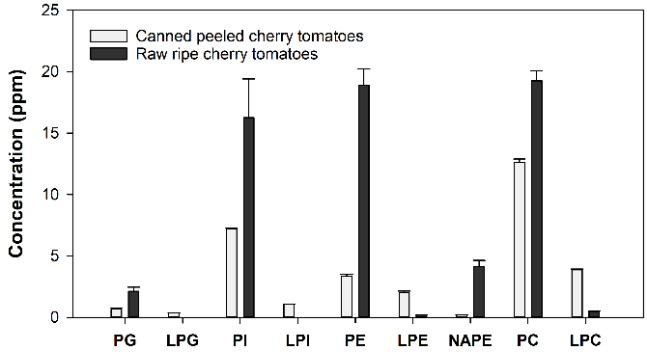

Figure 2. *Comparison of the main di- and monoacyl-PL classes in canned peeled (black) and raw ripe cherry tomatoes (white). Error bars are shown as half-width standard deviation.*

Lyso-forms are known as aging by-products triggered by the enzymatic action of phospholipase, specifically phospholipases A_1 (PLA_1) and A_2 (PLA_2), which hydrolyse acyl residues at the *sn*-1 and *sn*-2 positions, respectively.[7] In canned peeled samples, these enzymes are likely activated during industrial processing, which helps extend shelf life. Full-MS spectra showed common acyl residues among LPGs, LPIs, LPEs, and LPCs, with fatty acids FA 18:2, FA 18:1, and FA 16:0 being the most prevalent. Conversely, ripe samples had higher concentrations of lyso derivatives of N-acyl-PE_S (L-NAPEs). The industrial processes for cherry tomatoes are likely harsh enough to degrade the enzymes involved in forming L-NAPEs.

Conclusions

In this study, the characterization of GPLs occurring in cherry tomatoes was carried out by HILIC-ESI-MS. Quantitative analyses compared ripe tomatoes to canned peeled ones, revealing an increase in monoacyl-GPLs in the latter, while levels of L-NAPEs significantly decreased. These changes are likely due to industrial processing, which activates phospholipases leading to the formation of by-products and exposes the tomatoes to thermal stressors that degrade L-NAPEs.

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PL4

Benchtop volatilomics and machine learning – principles, applications, opportunities

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Summary: *This presentation will demonstrate principles and examples of benchtop "volatilomics" approaches in food and fermentation processes.*

Keywords: *Volatilomics, machine learning, foodomics*

The term “volatilomics“ addresses the comprehension of the volatilome, which depicts an important, but yet scarcely understood part of the metabolome – in systems biology, but also in fields, such as food analysis. Here, such approaches are often also called “foodomic” strategies and meanwhile are an integral part in food authentication and quality control. Volatilomics are an elegant way of correlating volatile organic compounds (VOCs) via the gas phase from the matrix with specific properties of that product: authenticity, quality or provenance are just a few examples. This is in particular relevant, as a major part of the aroma-relevant compounds in foods, e.g. in roasted coffee, citrus oils or saffron belong to the VOC fraction, which can be analyzed without sample contact by using the headspace over the sample.
A major challenge is the complexity of the enormous amount of different substances found, which often are not relevant as individual species, but rather their total “fingerprint”, resulting of all amenable substances. This high-dimensional spectral information cannot be interpreted without applying powerful machine learning algorithms or chemometrics, a strategy which is generally referred to as "omics" approaches. These data are combined with modern machine learning techniques to extract the maximum possible information from products to improve quality and confirm authenticity.
VOCs are also an important source of information in fermentation processes. As integral part of modern biotechnology, they feature an enormously complex gas phase, which is so far not part of existing, molecule-specific monitoring strategies.
Typically, the required techniques are laboratory-based and not useable at the so-called “point-of-need”, which limits their use. This presentation will demonstrate principles and examples of benchtop "volatilomics" approaches in food [1] and fermentation [2] processes, consequently named as “fermentomics”, that in the future could be used directly at the location where they are needed.

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OR29

Exploring monacolins in red yeast rice: monitoring, quantification and cytotoxicity assessment

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Summary: *Red yeast rice (RYR) is a traditional Chinese medicine known for its lipid-lowering monacolins, particularly monacolin K (MK), similar to lovastatin. EFSA reevaluated RYR due to variability in monacolins and lack of characterization data. This study explores monacolins content, composition, and cytotoxicity in commercial RYR supplements.*

Keywords: *red yeast rice, phytochemical characterization, cytotoxicity*

Introduction

Red yeast rice (RYR) is a traditional Chinese medicine and a food supplement popular in East Asian countries used to treat indigestion, diarrhea, blood circulation stasis, limb weakness, however it is particularly known for its lipid lowering capability [1]. Specifically, RYR food supplements are highly valued for stabilizing normal blood LDL-cholesterol levels due to a class of compounds called monacolins. The most abundant among them is monacolin K (MK), that shows the same chemical structure of Lovastatin, an already approved drug by Food and Drug Administration (FDA) since 1987 despite reported adverse effects [2]. Taking into account this aspect, European Food Safety Authority (EFSA) reevaluated the use of RYR not only for the variability of monacolins composition in commercialized food supplements but also for the lack of characterization data regarding their whole components [3]. Thus, the aim of this work was to explore the monacolins content in RYR commercialized food supplements in terms of monitoring their composition, quantification and cytotoxicity assessment.

Experimental

Phytochemical analysis of a representative number of food supplements (twenty-seven) was performed using LC coupled with UV detector and high-resolution mass spectrometer in order to detect and quantify monacolins present in the samples and identify their relative ratios (single monacolin/MK+MKA). Moreover, a comprehensive metabolic profiling was provided for RYR food supplements, considering not only monacolins but also secondary metabolites by applying an untargeted approach to understand the complexity of several RYRs. Lastly, the cytotoxicity of a cohort of food supplements containing RYR was evaluated in comparison with single monacolins and statins in an *in vitro* model of human hepatocyte cells in order to shed light on eventual toxicological events.

Results

The analytical characterization of RYR food supplements provided information about the monacolins profile within twenty-seven samples. Successively, the investigation on the other secondary metabolites, was performed through MS untargeted analysis, followed by a bioinformatic elaboration. The analysis allowed to obtain a characterization as most complete as possible that can greatly contribute to the phytochemical investigation of RYR food supplements. The cytotoxic results evidenced a difference in the effects of single monacolins compared to the RYR samples tested and statins. No toxic effects were observed for MK, acidic monacolin K (MKA) and RYR characterized by low content in dehydromonacolin K (DMK) at all the concentrations tested, while a more significant toxic effect was demonstrated for DMK for higher dose treatments. As far as is concerning statins, simvastatin resulted to be more cytotoxic than atorvastatin.

Conclusion

The analyzed samples resulted to be clustered in different groups based on their relative ratios of monacolins. The untargeted analysis allowed to evidence the presence of many classes of compounds occurring in different relative percentages in several RYRs. A different cytotoxicity between RYR extracts and statins vs MK was observed. Specifically, the results evidenced that high levels of DMK seemed to be correlated to an increased cellular mortality.

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OR30

A method to measure the qualitative level of durum wheat and milk raw material by high resolution mass spectrometry

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Summary: *Raw materials for food manufacturing are often considered similar in the whole agricultural world. Their quality is always identified by standard guidelines (i.e., nutritional values) without considering their flavor and taste. Untargeted mass spectrometry based metabolomic approach is a useful tool to determine the quality level of raw material samples.*

Keywords: *HR-MS; metabolomics; food products*

Introduction

The agro-food market is determined by the Commodities Exchange that usually sets, for each raw material, a similar price around the world. This is a disadvantage for small farms that have better quality but paid at the same price as the big distribution. In fact, often countries and regional areas claim their own diversity and a different, better quality of raw material.
Food quality always refers to the nutritional values or food process technologies and is poorly connected to other characteristics such as aroma, flavor and mouthfeel. While the wine field is interested in studying these aspects and the molecules related to them, there are still a small number of studies regarding other food raw materials [1].
Milk is classified in the same way despite it being produced by cows under different feeding regimen. In the same way, durum wheat is considered all the same without taking in consideration the yield product.
The metabolic profile of a raw material can be considered as an analytical signature (fingerprint) of a food product and thus can help to distinguish different production practices, reflecting the impact of both endogenous and exogenous factors as well as food properties.
In this work, we investigated the molecular complexity of milk obtained from cows fed under different regimens and durum wheat with different yield by high-resolution mass spectrometry with the aim to find a correlation between metabolic *fingerprint* and the quality of raw materials.

Experimental

Milk samples were taken from different dairy farms located in Basilicata region (Italy) during spring 2022 and 2023, and classified in Extensive Farming Milk (EFM, grazing) and Intensive Farming Milk (IFM, barn indoor) according to the feeding regimen. Durum wheat from two harvesting years (2022 and 2023) was collected from farmers in Basilicata and Sicily region and divided in two groups based on Low Yield Wheat, LYW and High Yield Wheat, HYW, based on their yield per hectare. Sample extracts were prepared following the procedure reported by Zhang et al [2].
The mass spectrometry analysis on the sample extracts were carried out by Zamboni group at the ETH Zurich using on a platform consisting of an Agilent Series 1100 LC pump coupled to a Gerstel MPS2 autosampler and an Agilent 6520 Series Quadrupole Time-of-flight mass spectrometer (Agilent, Santa Clara, CA) equipped with an electrospray source operated in negative and positive ionization mode [3]. Data were collected and analyzed by using SLAW Approach [4]. For the multivariate statistical elaboration of metabolomics-based data MetaboAnalyst [5] program was used. After data normalization, both unsupervised and supervised multivariate statistics were carried out based on principal component analysis (PCA). For the Van-Krevelen diagram, data were processed by using an in-house R-script [6,7].

Results

Untargeted mass spectrometry based metabolomic approach was used to compare milk collected from cows under different feeding regimen and durum wheat from different yield production. MS spectra showed a huge number of signals revealing the wide diversity of metabolites occurring in the samples. Overall, a total of 1492 molecular features were putatively annotated (Level 1) across milk samples and 1626 molecular features across durum wheat samples according to the Human Metabolome Database (HMDB). Unsupervised principal component analysis (PCA) revealed a different distribution between EFM and IFM systems. A similar

distribution was observed for durum wheat samples, where LYW are wilder (more spread) compared to HYW. By checking compound annotations, we observed that the number of discriminant compounds differs between EFM and IFM as well as LYW and HYW. As reported in Figure 1, we can observe that EFM and LYW samples follow the same trend as annotated discriminant compounds mainly belong to lipids and polyphenols. Van Krevelen diagrams are also used as a visualization tool to highlight the different distributions on the molecular maps.
Despite the different origin of raw materials (animal and plants), EFM system and LYW system behave in the same way, confirming our hypothesis that these extensive farming milk and durum wheat from low yield have a higher quality as their composition is more diversified.

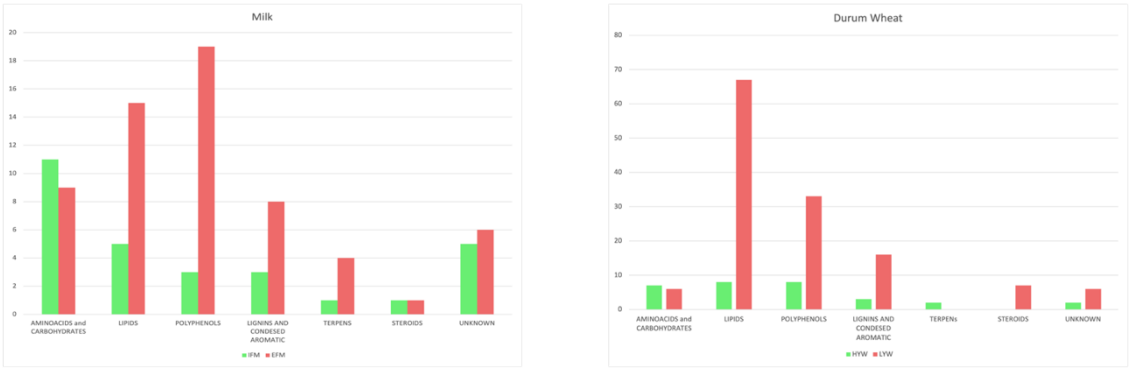

Figure 1. *Histograms of different organic compound classes identified in milk and Durum Wheat*

Conclusions

High-resolution mass spectrometry has been shown to be a valuable tool in assessing the complexity of agri-food products.
Unsupervised principal component analysis and Van Krevelen diagrams revealed important differences in the raw material metabolomic fingerprints, showing how important variability could account for their different quality.

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OR31

Empowering veterinary clinical diagnosis in industrial poultry production by ambient mass spectrometry

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Summary: *Untargeted methods, by ambient mass spectrometry and chemometric tools, has made a dramatic impact on food analysis. In a similar vein, this study attempted the translation of this experience to the standardization of farmed poultry meat.*

Keywords: *DART-HRMS, veterinary diagnostic, ambient mass spectrometry*

Introduction

Chicken feed ingredients undergo changes according to fluctuations of market prices, therefore, costs of feed ingredients, leading to high feed costs, can determine the replacement of some components of the ration. Unfortunately, in some cases, a rough check of these nutrient macro-categories is insufficient to avoid intestinal performance issues, which can lead to dysregulated energy metabolism or variations of the intestinal absorption capabilities with consequences on the final products. Rapid interventions must be implemented to reduce the losses and preserve production and the subsequent economic returns. Direct analysis in real time high resolution mass spectrometry (DART-HRMS) was used in an untargeted manner to analyze fresh chicken tissues (abdominal fat, leg skin, liver, and breast) of pigmented and non-pigmented chicken to investigate the metabolic causes of lack of pigmentation in final chicken meat products of an organic poultry farm.

Experimental

The study was conducted using the carcasses of 5 pigmented and 3 nonpigmented 60-d-old, sexed, male, slow growing Hubbard broilers that had been bred and raised on one farm in Italy. Amounts (1 g) of each homogenized tissues (pigmented and non-pigmented abdominal fat, leg skin, liver, and breast) were extracted using 10 mL ethyl acetate, vortexed for 30 s and sonicated for 15 min. The obtained sample extract was centrifuged for 5 min at 12,000 × g and 1 mL of the supernatant was transferred into a new plastic tube. Untargeted metabolomic analysis of the extracts was carried out using a DART SVP 100 source coupled to an Exactive Plus Orbitrap Mass Spectrometer. The resolution was set to 70,000 full width at half maximum and the mass range was 75 to 1,125 Da in positive ion mode. The extracts were analyzed in triplicate Statistical analysis was applied to the DART-HRMS data to retrieve the molecular features that characterize the pigmented and non-pigmented chicken products.

Results

Higher abundance of oxidized lipids, high abundance of oxidized bile derivatives, and lower levels of lipophilic vitamins, such as tocopherol isomers (Vitamin E) and retinol (Vitamin A), were captured in non-pigmented than in pigmented chicken tissues (Table 1). Note that the antioxidant effect of carotenoids requires the presence of vitamin E in tissues. Moreover, the bioaccessibility of natural pigments is facilitated by their solubilization into micelles riches in saturated fatty acids and therefore, a diet rich in oxidizable polyunsaturated fatty acids (due to the replacement of sunflower oil with soy oil due to sunflower shortage due to Ukraine war) limited the solubilization of natural pigments in micelles and their subsequent absorption in the intestinal lumen. In this study, the DART-HRMS system performed well in retrieving valuable chemical information that explained the differences between the two groups of chicken in absorption of the lipophilic xanthophylls and the subsequent lack of proper pigmentation in affected chickens. However, this study provided data that allowed the farm management to: i) increase the amount of administered Vitamin E to compensate for the oxidation of lipids observed in non-pigmented final chicken meat products, ii) evaluate the possibility of restoring the use of sunflower oil in the feed and iii) evaluate the use of anti-oxidant emulsifiers in the feed.

Table 1. *The most discriminatory compounds, teased out by volcano plot, in pigmented and nonpigmented tissues. The observed m/z, the theoretical m/z, the ion assignment, the elemental formula, error (Dppm), fold change (FC), adjusted p-value (p-value adj) are listed. Only the metabolites with p.adjusted < 0.05 are reported.*

Tissue	*m/z* obs. by DART-HRMS	Theoretical *m/z*	Ion Assignment	Predicted molecular formula	Δppm	Annotation	p-value adj	FC
Abdominal fat	170.0669	170.0672	$[M+NH_4]^+$	$C_5H_4N_4O_2$	-1.76	xanthine	0.0038	0.2249
Abdominal fat	300.2891	300.2897	$[M+NH_4]^+$	$C_{18}H_{34}O_2$	-1.99	oleic acid	0.0056	2.3575
Abdominal fat	302.3048	302.3054	$[M+NH_4]^+$	$C_{18}H_{36}O_2$	-1.98	stearic acid	0.0056	2.1147
Abdominal fat	322.2736	322.2741	$[M+NH_4]^+$	$C_{20}H_{32}O_2$	-1.55	arachidonic acid	0.0051	2.1015
Abdominal fat	417.3719	417.3727	$[M+H]^+$	$C_{28}H_{48}O_2$	-1.92	γ-/β-tocopherol	0.0038	4.3545
Abdominal fat	431.3877	431.3884	$[M+H]^+$	$C_{29}H_{50}O_2$	-1.62	α-tocopherol	0.0038	17.5860
Skin	285.1689	285.1697	$[M+H]^+$	$C_{18}H_{33}NO_5$	-2.81	oxoundecanoyl carnitine	0.0037	0.2038
Skin	343.2470	343.2479	$[M+H]^+$	$C_{22}H_{43}NO_5$	-2.62	hydroxypentadecanoyl carnitine	0.0037	0.2151
Skin	351.2524	351.2536	$[M+H-H_2O]^+$	$C_{21}H_{36}O_5$	-3.41	MG(18:3)-OH	0.0037	0.2189
Skin	371.2782	371.2792	$[M+H]^+$	$C_{21}H_{38}O_2$	-2.69	MG(18:1)-O	0.0037	0.1687
Liver	269.2258	269.2270	$[M+H-H_2O]^+$	$C_{10}H_{14}O_3$	-4.4	retinol (Vitamin A)	0.0111	3.9917
Liver	285.0750	285.0757	$[M+H]^+$	$C_{16}H_{12}O_5$	-2.46	dehydrated cholecalciferol (Vitamin D3 derivate)	0.0274	0.2011
Liver	295.1114	295.1117	$[M+H-H_2O]^+$	$C_{14}H_{20}N_2O_4S$	-1.01	tyrosyl methionine/methionyltyrosine	0.0156	4.8117
Liver	464.3370	464.3370	$[M+NH_4]^+$	$C_{27}H_{45}NO_5$	0	3-hydroxyarachidonoylcarnitine	0.0152	0.1389
Liver	424.3059	424.3057	$[M+NH_4]^+$	$C_{24}H_{38}O_5$	0.49	dihydroxy bile acid (dihydroxy-12-oxocholanoic acid)	0.0183	0.0698
Liver	427.3022	427.3001	$[M+H-H_2O]^+$	$C_{31}H_{40}O_2$	4.9	menaquinone (Vitamin K2)	0.0220	20.571
Liver	427.3234	427.3218	$[M+H-2H_2O]^+$	$C_{28}H_{46}O_5$	3.7	tetrahydroxy bile acid (dolicosterone)	0.0111	0.0058
Liver	431.3868	431.3884	$[M+H]^+$	$C_{29}H_{50}O_2$	-3.7	α-tocopherol (Vitamin E)	0.0093	6.2415
Liver	781.5763	781.5753	$[M+H-2H_2O]^+$	$C_{45}H_{85}O_{10}P$	1.28	PA (42:2)-OH	0.0153	0.0145
Leg muscle	417.3730	417.3727	$[M+H]^+$	$C_{28}H_{48}O_2$	2.08	γ- tocopherol	0.0187	12.8660
Leg muscle	429.3725	429.3727	$[M+H]^+$	$C_{29}H_{48}O_2$	-0.47	hydroxysitocalciferol(hydroxy Vitamin D5)	0.0187	4.1043
Leg muscle	433.3669	433.3676	$[M+H]^+$	$C_{28}H_{48}O_3$	-1.61	hydroxy-γ- tocopherol	0.0340	4.3316
Leg muscle	447.3828	447.3833	$[M+H]^+$	$C_{29}H_{50}O_3$	-1.12	hydroxy-α-tocopherol	0.0187	4.7460
Leg muscle	633.5084	633.5089	$[M+H]^+$	$C_{39}H_{68}O_6$	-0.78	DG(36:4)-OH	0.0047	0.0450
Leg muscle	635.5260	635.5245	$[M+H]^+$	$C_{39}H_{70}O_6$	2.36	DG(36:3)-OH	0.0047	0.0581

Conclusions

The results suggest this ambient mass spectrometry method could be useful in providing near real-time feedback to aid in veterinary decision-making in poultry farming for the production of highly standardized chicken meat products.

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OR32

Novel food production: towards a sustainable approach within a circular economy framework

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Summary: *This research explores the use of marine algae as sustainable dietary supplementation for house crickets (Acheta domesticus) mass rearing to optimize the nutritional composition of crickets, with focus on the fatty acid composition. The determination of the fatty acid composition was performed by gas chromatography-mass spectrometry.*

Keywords: *novel food, Acheta domesticus, algae, sustainable*

Introduction

By 2050, the world's population is expected to reach over 9 billion, leading to an increase of 70% in food demand (WHO, 2019). Nowadays, traditional food production systems are raising concerns regarding their impact on the environment but also on human health. Insects have emerged as promising alternative protein sources, being highly nutritious, as well as more sustainable compared to conventional animal farming [1]. Among the edible insects, *Acheta domesticus* (house cricket, AD), recently authorized by the European Commission as novel food (EU 2022/188), is one of the most consumed worldwide, due to its high nutritional value, rich in protein, lipids and other essential nutrients such as minerals and vitamins [2]. However, its notably high lipid presence, primarily characterized by a significant percentage of saturated fatty acids (SFAs) presents health concerns. Currently, insect producers have shown interest in using more environmentally friendly substrates for cricket's rearing, such as marine algae (micro and macroalgae). Algal biomass is considered a promising sustainable resource with high productivity and a low environmental impact [3], rich in nutrients, including carbohydrates, minerals, vitamins, high-quality proteins, and lipids [4]. To the best of our knowledge, only [5] investigated the use of the red alga *Palmata palmata* as feed ingredient for AD, so far. This study aims to evaluate the use macroalgae, in particular the red alga *Palmaria palmata* and the brown alga *Ascophyllum nodosum*, as ingredients of AD diet, to obtain crickets with a low-fat content rich in high-quality fatty acids (FAs), such as monounsaturated (MUFAs) and polyunsaturated (PUFAs) ones, that could be used as ingredient in the so-called "functional foods". Moreover, we investigate the potential of cricket's frass as a nutrient source for cultivating *Chlorella vulgaris* microalgae, and the use of this macroalga in house crickets' production, in a circular economy perspective.

Experimental

The study consisted of two separate feeding trials for AD. In trial 1, the red alga *Palmaria palmata* (PP) was added to the standard diet at three different percentages: 5 % (PP5), 10 % (PP10) and 20 % (PP20). In trial 2, the brown alga *Ascophyllum nodosum* (AN) was added to the standard diet in the percentage of 20 % (AN20) and 40 % (AN40). Algae biomasses were purchased from the company Ocean Harvest (Ireland). In both trials, the control diet (Ctrl), consisted of a sole standard substrate made of 100 % plant-based ingredients, provided by Nutrinsect Srl (Montecassiano, MC (Italy)). The feeding experiments with seaweed-enriched diets started at 20 days post-hatching (dph) in trial 1, and 14 dph in trial 2, ending after 7 and 15 days in trial 1 and trial 2, respectively.

Lipid extraction and fatty acids analysis. Prior to analysis, algae, diets and crickets' samples were homogenized and freeze-dried until constant weight. Lipid extraction was carried out with Microwave Assisted Extraction (MARS-X, 1500 W, CEM, Mathews, NC, USA), and the mass of extracted lipid was determined gravimetrically. FA were determined using a gas chromatograph (Agilent-6890) coupled to an Agilent-5973N 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

Trial 1. AD fed diets with the highest inclusion of PP (20 %) showed a significant ($p<0.05$) lower lipid content (13 g/100 g DM), compared to the other groups. PP10 and PP20 diets led in AD to a significant increase

(p<0.05) of SFAs content, and a significant decrease (p<0.05) of MUFAs, n-6 and n-9 content with respect to AD fed the Ctrl diet, while PUFAs was not affected by dietary treatment. The health promoting 20:5n-3 (EPA) was not detected in the AD fed the Ctrl diet, but it increased significantly (p<0.05) in AD with increasing the level of PP in the diets, up to 0.055± 0.003 g/100 g FAs in AD fed PP20 diet. The health promoting 22:6n-3 (DHA) showed the highest percentage in AD fed PP20 diet.
Trial 2. The total lipid content of AD decreased significantly (p<0.05) with the increasing inclusion of AN in the diet (-33 % and -49 % for AN20 and AN40 groups, respectively, compared to the Ctrl group), reflecting the trend observed in the diets themselves. AD fed AN-enriched diets showed a significant decrease of SFAs (p<0.05), and a significant increase of unsaturated FAs (due to a higher MUFAs and PUFAs content) varying from ~61 g/100 g FAs in the Ctrl group to ~67 g/100 g FAs in the AN40 group (+10 %). EPA (not detected in the AD fed the Ctrl diet), increased in AD with increasing the levels of AN in the diets, up to 0.51±0.05 g/100 g FAs in AD fed AN40. DHA showed a ~ten-fold increase in AN40 compared to the other groups.
The microalgae *Chlorella vulgaris*, showed a good capacity to grow on frass-enriched medium (dilution 4 g/L), and its FA composition did not vary significantly with respect to the microalgae grown on the standard medium. AD fed with *Chlorella vulgaris* added to drink water showed a good cricket's performance, whereas analyses of FA profile are in progress.

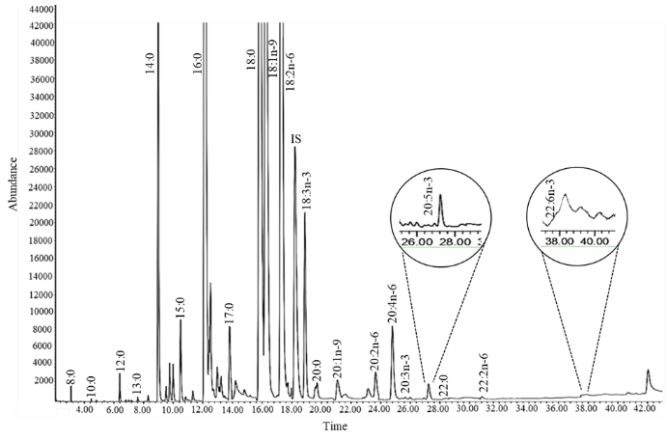

Figure 1. *Example of chromatogram of the FAs composition of Acheta domesticus fed AN40 diet.*

Conclusions
The inclusion of PP and AN resulted in insects with a low-lipid content. The inclusion of the red alga PP in the diet affected the FAs composition of AD to a lesser extent, likely due to the shorter period of administration of seaweed-enriched diet to AD (7 days) with respect to the period considered in trial 2 (15 days). However, growing AD on PP and AN-enriched diets introduced EPA and DHA. The results of this research achieved the following objectives: 1) production of a healthy low-fat AD, rich in high-quality fatty acids, through the use of sustainable ingredients with a low environmental impact, 2) effective management and valorization of waste products; 3) reduction of microalgae production costs, and 4) creation of a circular and sustainable production chain.

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OR33

Nontarget Screening workflow (NTS) for the analysis of Per and Polyfluoroalkyl Substances (PFAS) in animal products using QTof

Andrea Perissi

Waters Corporation

Summary: *The study [1] demonstrates the advantages using Xevo G3 QTof for identification and quantification of PFAS:*

- Nontarget screening workflow using retention time and mass defect

- PFAS identification and quantification in fish at low ng/Kg concentrations meeting EURL POPS Guidance

- Nontarget screening for PFAS compounds where no standards are available

Keywords: *PFAS, HRMS, NTS*

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OR34

Automazione nell'analisi dei pesticidi in GC-MS/MS tramite µ-SPE online

Antonello Laricchiuta

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Il metodo di estrazione QuEChERS seguito da una purificazione manuale dispersiva in fase solida (dSPE) è a oggi la metodologia più comune applicata nell'analisi dei residui di pesticidi in matrici alimentari e non solo. Il metodo è semplice e veloce ma la dSPE non è sempre sufficiente nel rimuovere efficacemente la matrice interferente che può portare anche a contaminazione del sistema.
L'estrazione in micro fase solida (µSPE) online rappresenta una valida alternativa per l'automazione dell'approccio dSPE. L'utilizzo della µSPE apporta miglioramenti sostanziali in termini di clean-up del campione in quanto c'è un contatto migliore tra campione ed adsorbente e si riesce a controllare con elevata accuratezza il flusso di eluizione nella cartuccia. La riduzione del carico della matrice nell'estratto finale migliora la robustezza dell'analisi GC-MS, prolunga la durata della colonna e del rivestimento aumentando gli intervalli di manutenzione, e soprattutto, elimina le interferenze che possono influire negativamente sull'identificazione e quantificazione dei pesticidi.
L'approccio µSPE può essere completamente automatizzato grazie all'utilizzo del Triplus RSH Smart e quindi l'analista deve solo trasferire l'estratto grezzo nelle vial e posizionarle nel rack dell'autocampionatore. La procedura di clean-up e successiva iniezione vengono eseguite dal braccio robotico dell'autocampionatore, con conseguente miglioramento della ripetibilità e riduzione sostanziale del rischio degli errori umani.
Nel presente lavoro viene presentato il workflow della uSPE disponibile per Thermo Scientific™ Triplus RSH SMART Autosampler ed alcuni risultati che si possono ottenere con l'accoppiamento ad un sistema Thermo Scientific™ TRACE™ 1610 Series Gas Chromatograph - Thermo Scientific™ TSQ™ 9610 Triple Quadrupole GC-MS con sorgente AEI (Advanced Electron Ionization).

OR35

Exploring nitrosamine formation in meat: impact of cooking, digestion, and preservation

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Summary: *Scientific studies have shown that nitrosamines, formed from the reaction between nitrites and amines, are carcinogenic. This research developed a method to quantify 15 nitrosamines in meat using HPLC-HQOMS. Results indicate that raw meat contains nitrosamines, cooking reduces their levels, and digestion increases them, especially with added nitrites. Combining meat with spinach surprisingly inhibited nitrosamine formation.*

Keywords: *nitrosamines, meet, Orbitrap*

Introduction

The carcinogenicity of nitrosamines and their presence in many food products, including meat, has been demonstrated in several scientific studies [1,2]. They are derived from the reaction between nitrite or nitrous oxides and nitrosable substrates like secondary amines. Most of the nitrosyl ions in meat products come from nitrite, usually used as a food additive. Nitrite in acidic conditions is converted to nitrous acid. Due to the instability of nitrous acid, it is easily decomposed into nitros anhydride (N_2O_3) that further reacted with amines to generate nitrosamine [2].

Experimental

The objectives of this research were to develop a new method for the determination and quantification of 15 nitrosamines, evaluating both APCI and HESI ionization coupled with liquid chromatography and high-resolution mass spectrometry (HPLC-HQOMS) and SPE-online. The developed method was then used to investigate the formation of nitrosamines in commercially available meat, as well as during the cooking and digestion processes.

In this regard, four samples of meat treated with different preservatives (no preservatives, mixture of nitrates and nitrites, ascorbic acid, and ascorbic acid with nitrites) were analysed. The samples were then processed in different ways (raw, cooked, cooked combined with spinach, and digested) to assess the real consumption situation in the daily diet and the further natural development during digestion.

Results

The method allowed the quantification of 15 nitrosamines using both HESI and APCI because not all these compounds were well ionizable with a single source (Table 1).

The analysis revealed that the formation of nitrosamines already occurs in raw meat. Both cooking and digestion alter the composition of nitrosamines present by degrading some and favouring the formation of others. Cooking has a more destructive effect on the total quantity of nitrosamines, reducing their concentration. Digestion, on the other hand, leads to an overall increase in nitrosamine concentrations if nitrites or nitrates are present. The combination of cooking and digestion has a synergistic effect, significantly increasing the quantity of nitrosamines present in the meat. Finally, when combined with spinach, they have an inhibitory effect on the formation, contrary to expectations, significantly reducing the total content (Figure 1).

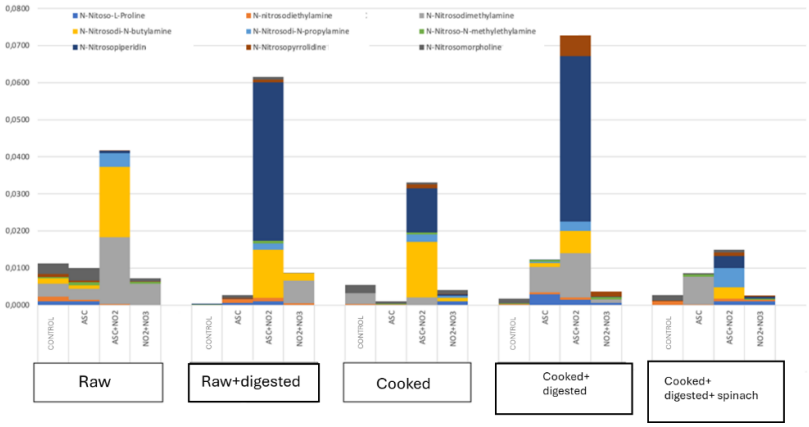

Figure 1. *Nitrosamine production during different processes*

Table 1. *Validation parameters of the analytical method*

Nitrosamine	RT (min)	$[M+H]^+$ (*m/z*)	Ions (m/z)	LOD (ug/kg) APCI	LOD (ug/kg) HESI	Recovery % APCI	Recovery % HESI
N-Nitrosodimethylamine	4,30	75,0553	-	1	1,5	17,27	0,00
N-Nitrososarcosine	4,65	119,0451	92,0499	1	25	0,00	0,00
N-Nitrosonornicotine	4,90	178,0975	120,0682	2	0,5	87,26	61,81
N-Nitrosomorpholine	5,25	117,0659	87,068	2	0,2	0,00	74,54
N-Nitoso-L-Proline	5,27	145,0608	121,966	0,5	5	30,91	0,00
N-Nitroso-N-methylethylamine	5,46	89,0709	61,0402	0,1	2	48,18	50,00
N-Nitrosopyrrolidine	5,50	101,0709	55,0548	0,1	0,3	36,36	49,09
N-nitrosodiethylamine	7,43	103,0866	75,0557	0,1	5	30,91	12,73
4-(N-Nitrosomethylamino-1-(3-pyridyl)-1-butanone	7,45	208,1081	122,06	3	0,5	85,45	14,54
N-Nitrosopiperidine	8,00	115,0866	69,0703	0,2	2	27,27	37,26
N-Nitrosodi-N-propylamine	10,62	131,1179	89,0711	0,5	1	17,27	26,36
N-Nitrosodi-N-butylamine	12,69	159,1492	103,0866	1	3	10,00	9,09
N-nitrosodiphenylamine	13,43	199,0866	169,0882	-	0,5	0,00	5,45
3-Nitroso-4thiazolidinecarboxylic Ac.	6,57	161,0026	71,025	1	5	30,91	38,18
N-Nitroso-2-methylthiazolidine-4-carboxylic Ac.	7,99	175,0183	71,025	1	5	154,53	24,54

Conclusions

The developed method allowed for the quantification of 15 nitrosamines using two different sources. The online SPE enabled sample concentration and lowered the LOQ. The study conducted on various types of commercially available meat confirmed the potential formation of nitrosamines even in meats preserved with ascorbic acid, although in lower quantities. The combination of ascorbic acid and nitrites, however, significantly increased their development. Among the various treatments of meat, digestion is the most concerning factor, yet it is unavoidable in meat consumption.

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OR36

Radical neutralization reaction using pre-column reactor and high resolution mass spectrometry

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Summary: *High resolution mass spectrometry can identify antioxidant compounds but does not provide information about their radical scavenging functionality. By coupling a reactor to HPLC-MS, the reaction between officinal herbal extracts and radicals can be monitored, allowing the determination of the reactivity of each compound and the characterization of the reaction products*

Keywords: *reactive oxygen species (ROS), pre-column reactor, antioxidants, officinal herbal extracts*

Introduction

This study presents the development of an innovative pre-column reactor integrated with high-resolution mass spectrometry (HRMS) to evaluate the antioxidant properties of plant extracts. Traditional antioxidant assessment methods, such as DPPH, ORAC, and FRAP, provide concentration information but overlook reaction dynamics. Furthermore, although advanced methods like LC-MS offer excellent identification capacity, they lack insights into the functional properties of antioxidants. Our novel pre-column reactor approach overcomes these limitations, enabling real-time investigation of radical neutralization rates and detailed characterization of antioxidant behavior in complex matrices. Results on sage (*Salvia officinalis*) and oregano (*Origanum vulgare*) show the excellent potential of combining the high resolution and linear response of the QExactive Orbitrap analyzer with the kinetic information achievable from a pre-column reactor design.

Experimental

Samples consisted of sage (*Salvia officinalis*) and oregano (*Origanum vulgare*). Plant materials were finely ground (<250 µm), extracted with ultrasound (60 W, 30°C, 30 min), and freeze-dried. For analysis, 250 mg of each extract was solubilized in 50% methanol and filtered (0.22 µm PTFE). The extracts were mixed in a pre-column reactor set at 37°C and pH 7.4, under stirring, with 2,2'-azobis(2- amidinopropane) dihydrochloride (AAPH), which generates peroxyl radicals (ROO•). At equal intervals, samples were injected and analyzed in a QExactive Orbitrap MS detector for continuous monitoring. A gradient elution was performed on a Kinetex® Biphenyl column (100 × 2.1 mm, 2.6 µm) and mobile phases consisting of MilliQ water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B). The gradient profile started at 10% B, increased to 90% B over 20 min, was held for 5 min, and then returned to the initial conditions. The flow rate was set at 0.3 mL/min and 30°C. Both positive and negative ionization modes were performed using an ESI source set to 4 kV and 320°C. The full-MS scan range was 100-1500 *m/z* with a resolution of 140,000. Data were analyzed using Xcalibur 3.1 and Compound Discoverer 3.1 software (ThermoFisher Scientific, USA). The method enabled the quantification of radical scavenging activity and provided insights into the kinetic profiles of reactive species. Further details on the calibration curves, standards, and data analysis methods can be found elsewhere [1].

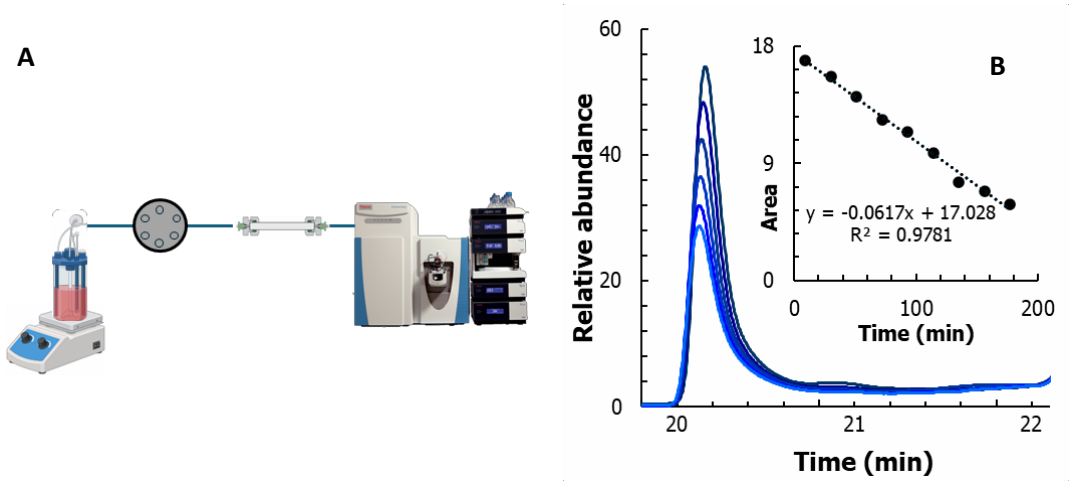

Figure 1. *A - Scheme of the pre-column reactor coupled to HPLC-ESI-Qexactive- MS/MS. B - Decrease in the intensity of rosmarinic acid during its reaction and fitting of experimental data*

Results

Significant differences were observed in the phenolic profiles of sage and oregano. Key antioxidants, such as rosmarinic acid and quercetin derivatives, identified in both extracts, showed a peak intensity loss of more than 95%, indicating their high efficiency as radical scavengers. Carnosic acid, the predominant peak in sage extract, exhibited a 75% loss during the reaction, making it a medium-reacting antioxidant. The pre- column reactor provided an unprecedented capacity to screen highly reactive analytes, including rosmarinic acid, salvianolic acid and quercetin derivatives. The HRMS offered outstanding identification power due to its accuracy and sensitivity. The acquired dd-MS2 spectra enabled a robust putative identification of all significant peaks. Furthermore, the detection of reaction intermediates and products at 26.4 and 27.2 min revealed distinct kinetics of antioxidant activity and the potential development of undesired or even harmful products. As an example, Figure 2A highlights the peak at RT of 26.6 min, which was observed after 840 min of reaction, and it was identified as a product (m/z 556.2776) with its fragmentation pattern reported in Figure 2B. In summary, sage exhibited higher reactivity than oregano in neutralizing radicals, with specific reaction rates quantified and compared in real- time. This method allows both identification and characterization of antioxidant reactivity. Although it does not provide yet a comprehensive measure of total reactivity, the proposed approach provides objective advantages over traditional chromatographic methods, and specific antioxidant assays, such as the DPPH, ORAC, and FRAP [2].

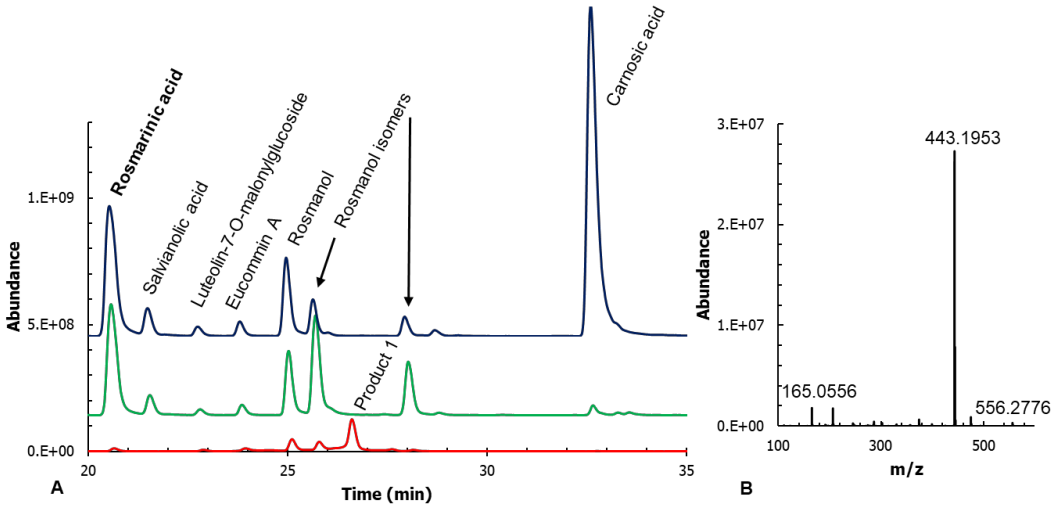

Figure 2. *A - XIC of the concentration reduction of most significant peaks for sage extract after 0, 135 and 840 min. B – fragmentation spectrum of rosmarinic acid*

Conclusions

The integration of a pre-column reactor with HRMS marks a significant advancement in studying plant-based antioxidants. This method provides a comprehensive understanding of the kinetics of antioxidant reactions and their in real- time efficacy, offering valuable insights for applications in food preservation and health-related industries. By combining the high resolution of mass spectrometry with a pre-column reactor, it is possible to determine the presence of active compounds and elucidate their functionality, even within complex biological matrices. This provides unique information for developing safer and more efficient products.

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OR37

Assessing chemical drivers of acidity in *arabica* coffee using a flavoromics approach

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Summary: *Coffee's acidity is a key product attribute influencing quality and consumer preference, described either as "brightness" or "sourness". The study aims to provide insights into coffee acidity. From an analytical standpoint, few studies have been reported to comprehensively profile coffee acidity. In this work, flavoromics analysis are used to identify non-volatile compounds that influence coffee's acidity.*

Keywords: *Flavoromics, Coffee, Acidity, Arabica coffee*

Introduction

Coffee is among the most consumed beverages in the world, with an estimated market of 200 USD billion. The quality and sensory attributes of coffee and specialty coffee, particularly acidity, play a crucial role in consumer preference. Acidity in coffee is often described as "brightness" when favorable and "sour" when unfavorable. It significantly contributes to the liveliness and fresh-fruit character of the coffee, which is immediately perceived upon tasting [1,2]. The primary compounds responsible for acidity in coffee are organic acids: chlorogenic, quinic, citric, and malic acids, among others. These acids are formed during the maturation of green coffee beans, undergo changes during post-harvest processing, and are further modified by roasting and brewing methods [3,4].

Understanding the chemical drivers of coffee acidity requires a comprehensive approach that integrates sensory evaluations with chemical profiling. Recent advancements in flavoromics have enabled the identification of key compounds, that influence flavor perception through both targeted and untargeted chemical analyses [5,6]. To date, there has been limited published literature on the application of untargeted omics methods, such as flavoromics, to further understanding of coffee acidity and its potential impacts on the final quality. This study aims to identify non-volatile compounds that contribute to the coffee's perceived acidity and impact the overall flavor experience, by comparing different geographical origins of *arabica* coffee.

Experimental

Fourteen *Arabica* coffee samples were selected from diverse geographical origins (Brazil, Colombia, Costa Rica, Ethiopia, Honduras, Nicaragua, Peru, Sumatra, Tanzania and Uganda). Sensory evaluation was conducted by seven Q-grader panelists who assessed the acidity intensity of the coffee samples using a 15-point hedonic scale. Evaluations were conducted with nose clips to prevent the impact of olfactory perception on acidity evaluation. ANOVA and Fisher LSD post-hoc analysis ($\alpha = 0.05$) were used to analyze sensory data. Additionally, new analytical procedures were developed and validated for analyzing acidity in coffee beverages. The analysis was conducted using UPLC-MS/QToF (Agilent 1290 Infinity II LC system coupled to an Agilent 6545 Q-ToF Mass Spectrometer with Electrospray Ionization source) for non- targeted chemical profiling. After the data were normalized by Pareto scaling, they were analyzed by SIMCA-P+ version 16 (Umetrics, Umea, Sweden) using unsupervised principal component analysis (PCA) and supervised orthogonal partial least squares analysis (OPLS) to correlate chemical profiles and sensory data validating the impact of specific compounds on coffee's acidity perception (Figure 1).

Results and conclusions

Preliminary results showed that this flavoromics approach successfully bridges the gap between sensory evaluations and chemical analyses, elucidating the contributions of specific non-volatile compounds to the overall coffee sensory experience.

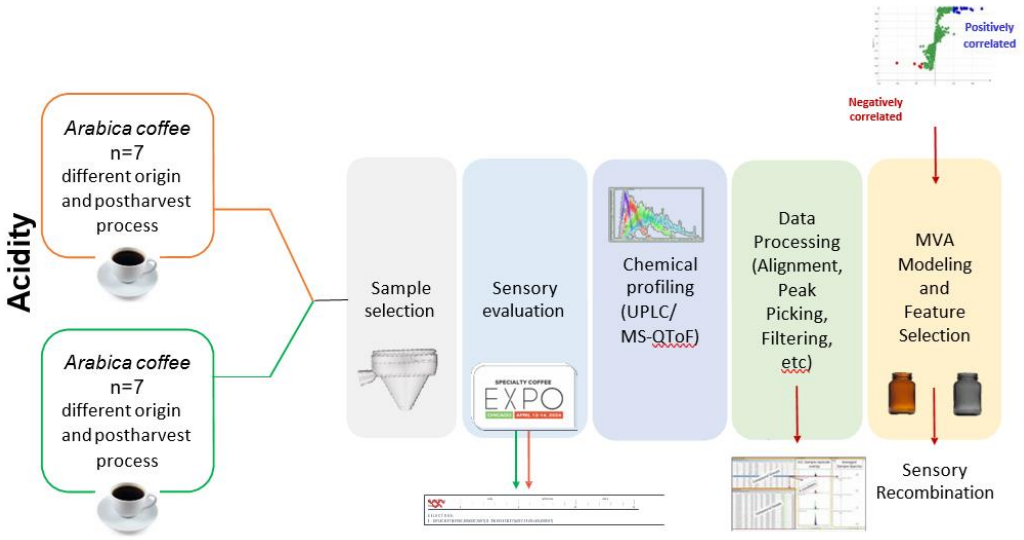

Figure 1. *Workflow highlighting the steps followed in this study for the untargeted flavoromics and chemometrics analyses.*

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OR38

Unveiling diversity in amino acid stable isotope profiles for classifying Italian rice varieties, refining types and cultivation methods

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Keywords: *isotope ratio mass spectrometry, food authentication, cereals*

Introduction

Approximately 50% of rice (Oryza sativa L.) in the European Union is produced in Italy, predominantly in the Northern regions following river Po. Highly valued Italian rice cultivars belong to the japonica variety and include Carnaroli, which is elastic, resistant and ideal for risotto dishes, Arborio, with wide grains and the ability to maintain large amounts of starch when cooking, and Baldo, which has an elongated grain and is the richest variety in minerals (Riso Delta Po PGI, 2020). Even though these possess different qualitative properties, their morphological differences are minimal [1],thus rendering them targets of fraudulent activities [2]. Therefore, it is important to develop methods that can distinguish between the different rice varieties.
The public perception of organic foods as healthier, as well as the transition towards organic rice agriculture in the Italian rice sector [3], makes the authentication of these products highly relevant. Moreover, the availability of market choice between white and brown rice can have further implications on the identification of authenticity markers. Brown rice includes the endosperm, embryo and bran and is nutritionally superior to white rice [4].
Studies employing isotope ratio mass spectrometry (IRMS), have examined the individual effects of refining type, variety and cultivation on the bulk stable isotope values of different food products [4,5]. However, Compound-specific (CS) IRMS methods can prove more effective than bulk in the discrimination of organic and conventional food products, providing information on individual components (e.g. amino acids, fatty acids, nitrate) by the addition of a separation step prior to the isotope analysis [6]. In this work, we applied bulk and CS amino acids analysis by Elemental Analyser (EA)- IRMS and Gas Chromatography - Combustion (GC - C) - IRMS, respectively, with the aim to obtain the stable isotope profile of different Italian rice varieties (Carnaroli, Arborio, Baldo, S. Andrea, Rosa Marchetti), refining types (brown/ white) and cultivations (organic/ conventional). The findings of this research aid in the identification of robust stable isotope markers for the organic and, potentially, varietal authentication of cereals.

Materials & Methods

A total of thirty eight rice samples of different cultivation systems (organic/ conventional), types (brown/ white) and varieties (Carnaroli, Arborio, Baldo, Rosa Marchetti and S. Andrea) were collected from Northern Italian provinces (Lombardy and Piedmont) in 2022. The values of bulk and 9 amino-acid $\delta^{15}N$ and $\delta^{13}C$ were analysed by EA and GC-C-IRMS. The results were evaluated by MANOVA, followed by an LDA classification and a decision tree model.

Results & Discussion

Brown rice was found to exhibit significantly lower $\delta^{13}C$val and $\delta^{13}C$leu values, but higher $\delta^{13}C$gly and $\delta^{13}C$phe values overall in the conventional samples ($p<0.05$), while no significant differences were observed between the brown and white samples of organic cultivation ($p>0.05$). Interestingly, bulk $\delta^{13}C$ values were not found to be significantly different between brown and white rice in neither organic nor conventional rice samples. Statistically significant separation ($p<0.05$) was achieved between the brown organic and brown conventional samples based on the $\delta^{15}N$ values of ala, val, ile, leu, gly, pro, asx, glx and phe. Notably, these were significantly higher than the difference between the bulk values (+0.4 ‰).
The significantly lower $\delta^{15}N$ values noted for conventional brown rice compared to conventional white rice can be attributed to the prominent effect of the synthetic fertilizer in the nitrogen isotopic composition of the rice grain, especially in the outer layer, which is retained to a greater extent in the case of the former rather than the latter. On the other hand, organic brown and white rice exhibited more similar ranges, since the $\delta^{15}N$ profile of organic fertilizers is closer to the natural background levels, resulting in some degree of homogeneity throughout the grain.
The LDA model successfully separated the conventional from the organic Carnaroli rice, as well as the conventional Arborio from both the conventional and the organic Carnaroli samples. Generic rice was also clearly differentiated from all other classes. Lastly, $\delta^{13}C$AAs and $\delta^{15}N$AAs contributed significantly more to the

LDs compared to the bulk values.
Decision tree analysis differentiated the Generic rice from all other classes based solely on its δ^{15}Nleu value, which was lower than 2.5 ‰, with a probability of 1. Additionally, the δ^{13}Cbulk value proved to be a key differentiator between classes, with a value higher than or equal to -26 ‰ indicating Arborio_Conv, and a value between -27 and -26‰ indicating Carnaroli_Org, with a probability of 1, when the δ^{15}Nleu value was higher than or equal to 2.5 ‰.

Conclusions
This study highlights the added value of the extractable information from compound-specific IRMS analysis, compared to bulk analysis, being able to achieve separation both between premium rice varieties and cultivation methods.

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Acknowledgements
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ABSTRACTS

Poster communications

P1

Honey, I Found the Metals! The paradigm of heavy metals in honey. A Comparative Study of European and Argentine Honey using a validated ICP-MS method

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Summary: *The aim of this study was to evaluate and compare the metal profile of European and Argentine honeys using the analytical technique ICP-MS. Forty metals were assessed in samples from different geographical regions of both. The data obtained from this study provide valuable information on the metal composition of honey.*

Keywords: *ICP-MS; Metal profile; Honey*

Introduction

Honey, a sweet substance primarily produced by honeybees, is derived from nectar collected by the bees. Through a unique process that starts in their honey sacs, bees transform nectar into honey within the hive. While nectar is approximately 80% water, Honey contains only about 20%, as bees reduce the water content to create a viscous self-preserving product. Honey nutritionally rich due to its sugar content, mineral salts, and antioxidants. Traditionally, honey has been used for its healing, antibiotic and cough-treating properties. Honey is a Newtonian fluid, meaning its viscosity remains constant regardless of deformation speed. This characteristic is now exploited for the production of medical devices for the treatment of various mild pathologies [1].

The global honey market is substantial, with the top honey exporters in 2022 being China, New Zealand, Argentina, and India, while the main importers were the USA, Germany, Japan, and France [2]. Italy ranked 23rd as an exporter and 6th as an importer, primarily sourcing honey from Hungary and then Romania, with increasing imports from Argentina in 2022 [2]. Global market dynamics, weather conditions, trade relations, and import policies, necessitate divers import channels! Consequently, we started a comparative research project to study the characteristics of Argentine honey in relation to European honey.

This work focused on comparing the inorganic element profiles, as monitoring elements is crucial for nutritional and food safety. But where do the metals present in honey come from? Metals in honey predominantly originate from soil, entering plans through roots and subsequently being transferred to honey by bees. Environmental contamination also contributes to metal content in honey [3], making it a biological indicator of pollution.

We analysed fourteen European honey samples and six of Argentine multifloral honey samples, monitoring forty elements using an inductively coupled plasma mass spectrometer (ICP-MS) validated method. This highly responsive analytical technique allows for precise determination of numerous elements, even at extremely low concentrations, providing a stable framework for multi-elemental assessment. The validation of the method involved the evaluation of various certified matrices chosen in order to cover as many elements as possible. By examining the elemental composition of honey, this research aimed to explore the potential of using elemental signatures to understand the metallomic profile of honey samples and classify traits, such as geographical origin and cultivation type.

Experimental

Sample preparation. Each honey sample (1 g) was subjected to acidic digestion using a microwave digestion system (Preekem M3) with 5 mL of concentrated nitric acid (67-70%) in a digestion vessel. The digested sample was then diluted to a final weight of 50 g with ultrapure water and filtered through a 0.2 µm nylon filter for analysis.

Instrumental parameters. An ICP-MS system (iCAP Qc ICP-MS, Thermo Scientific) equipped with Kinetic Energy Discrimination (KED) mode was used to analyse the samples. Pure helium (He) was used as collision gas during analysis. Data acquisition was performed using Qtegra software (Version 2.10.4345.236). The ICP-MS setup included a Thermoflex 2500 chiller (Thermo Scientific), a quartz spray chamber, a PFA nebulizer, and a demountable quartz torch with a 2.5 mm inner diameter (i.d.) quartz injector. Both nickel sampler and skimmer cones were utilized. An ASX-560 autosampler (Teledyne-Cetac Technologies) introduced the prepared samples for analysis, and the instrument featured an integrated ESI prepFAST auto-dilution system.

Analytical conditions. Calibration for each element was achieved using linear calibration curves, with element identification relying on the established isotope patterns. A comprehensive quality control program was implemented, including the linearity of calibration curves, continuing calibration blank (CCB), continuing

calibration verification (CCV), recovery of the quality control standard (QCS), and internal standard recovery. The requirement for the linear correlation coefficient was set at ≥ 0.990. The average percentage recovery for internal standard (^{72}Ge, ^{103}Rh, and ^{115}In), CCV and QCS needed to fall within ±25%. Standard reference material (SRM) Rice Flour 1568b, Peach leaf 1547 and Tomato leaf 1573a were used to assess recovery.

Results

The development and validation of a multi-elemental ICP-MS analytical method provided a robust framework for analysing the elemental composition of multiflora honey samples from Europe and Argentina. The method's validation was supported by the use of two reference standard materials with certified elemental content, ensuring recovery, repeatability and stability. Forty elements (Figure 1) were analysed to obtain unique elemental signatures of the twenty multiflora honey samples from Europe and Argentine.

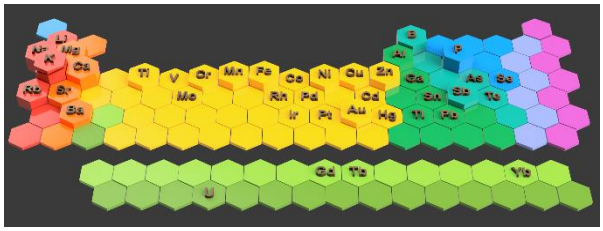

Figure 1. *Elements analysed in the honey samples. the heights of the elements are proportional to their concentration in a logarithmic scale.*

Among the forty elements analysed, only seventeen were detected in the samples: *Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, Rb, Sr, Ti, Zn*. The remaining elements were below the method's LoQ.
Principal component analysis (PCA) and hierarchical cluster analysis (HCA) revealed distinct groupings based on geographical origin and cultivation type, whether organic or conventional, highlighting the potential for these statistical models to differentiate honey samples.
Potassium (*K*) emerged as the most abundant element (Figure 1), with concentrations ranging from 0.03% to 0.2%, followed by *Ca, P, Mg, Na* and *B,* with levels between 5-150 mg/kg. The other elements had concentrations between 3 mg/kg and 0.05 mg/kg. Notably, Argentine honeys exhibited higher average element concentration compared to European honeys.
These findings align with existing literature, which typically reports around twenty elements in honey samples. This study stands out by evaluating a broader range of forty elements, providing a more comprehensive understanding of the mineral composition of honey.

Conclusion

This study's comprehensive analysis of forty elements in honey samples represents a significant advancement in honey characterization.
In conclusion, this work not only expands the scope of elemental analysis in honey but also demonstrates the potential for using elemental signatures to evaluate honey quality. By establishing clear connections between elemental profiles, geographical origins, and cultivation methods, this research contributes to the growing body of knowledge on honey's nutritional attributes, providing valuable insights for the honey industry.

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P2

Inorganic elements of different honeys from Algeria: safety and health effects

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Summary: *Different botanical honeys from different regions of Algeria were analyzed. The objectives were to assess the level of contamination of these honeys, to differentiate them in terms of geographical and botanical origin, and to evaluate the potential toxicological risk to humans from honey consumption. The results showed that some honeys exceeded the EU regulatory limits and that a small amount of Algerian honey can be safely consumed*

Keywords: *Food safety; honey; inorganic contamination*

Introduction
Honey is the sugary substance produced by bees (*Apis mellifera L.*) from the nectar of flowers or secretions of living parts of plants or found on them.[1,2] The concentration of inorganic elements in honey can vary between 0.1-0.2%, percentages influenced by different factors: the environment in which the plant grows, the botanical and geographical origin, etc.[3] However, honey is one of the most susceptible matrices to inorganic contamination, for example lead (Pb) and cadmium (Cd).[4]

Experimental
In this research, the As, Be, Ca, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Sn, Ti, and Zn content in 54 honeys from North and South Algeria and from different botanical origins by ICP-MS and DMA-80 was evaluated. In addition, Algerian honeys were discriminated since their geographical and botanical origin. Finally, the potential toxicological risk to humans from honey consumption was assessed.

Results
The mineral element profile was influenced by geographical origins. Honeys from North Algeria had the highest Ca content (mean concentration: 100.52±7.29 mg/kg) than those from South Algeria (mean concentration: 83.44±12.86 mg/kg). For magnesium and sodium, the trend was opposite.
Most of the honeys analyzed exceeded the Codex Alimentarius and EU Regulation 915/2023 limits for Mg, Fe, Zn, Cd, and Pb.
Statistical analysis and PCA pointed out that honey samples could be discriminated more effectively in relation to the geographical origin than the botanical.
Consumption of the honeys studied resulted in very low average intakes or exposures of the elements compared to the respective reference value. Furthermore, for the non-carcinogenic risk assessment, the Hazard Quotient (HQ) did not exceed the threshold of 1 for each potentially toxic element. Consequently, the non-carcinogenic health effects of consuming these Algerian honeys are negligible.

Conclusion
Honey is one of the matrices most prone to contamination. As there are numerous regulations in Europe, unlike in non-EU countries, continuous monitoring is essential to reduce inorganic contamination of this matrix and thus minimise risks to consumers. This would help to harmonise food safety standards worldwide.

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P3

Black tea infusions: mineral element transfer, safety, and health effects

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Summary: *Different black teas brands were analyzed. The study has four objectives: i) to assess the level of mineral elements of the dry materials and the corresponding infusions; ii) to calculate the percentage of transfer from the dry material to the infusion; iii) to differentiate them in terms of geographical origin and tea type iv) to evaluate the potential toxicological risk to humans from tea consumption. The results showed that these teas can be safely consumed.*

Keywords: *Food safety; black teas; inorganic elements.*

Introduction

Tea, one of the world's most popular non-alcoholic beverages for its smooth taste and potential health benefits, is made from the leaves of *Camellia sinensis*. Tea infusions are made from tea leaves using boiling drinking water. The health benefits of tea, including disease prevention, blood purification, immune disorders, and brain rehabilitation, are mainly due to its content of polyphenols (especially catechins), polysaccharides and theanine1,2. Tea provides several macro- and trace elements, such as B, Ca, Co, Cu, Fe, K, Mg, Mn, Na, Se, Sr, and Zn. In addition, the content of toxic and potentially toxic elements such as Al, As, Cd, Cr, Ni and Pb is also of particular importance2,3. In general, the different accumulation of mineral elements in tea leaves is related to the production area (physicochemical properties of the soil, specific environmental and agricultural conditions of the tea garden soils) and to the types of tea (age of the leaves and different stages of the production process)4. In addition, the amounts of each element transferred from the tea leaves to the corresponding infusions can vary depending on the solubility of the element and the infusion time.

Experimental

In this study, 210 samples of five brands of black teas (cultivated in China, Argentina, and India) were purchased from local markets in Messina (Italy) in 2022. The analyses were first carried out on the leaf samples and then on the corresponding infusion prepared at different temperatures and times. In all samples, the B, Na, Mg, Al, K, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Cd, Ba, Hg, and Pb content was determined by ICP-MS and DMA-80. The percentage of transfer from the dry material to the drink obtained from the infusion of the same was calculated. Finally, was evaluated if the intake of these teas can positively or negatively affect the consumer health.

Results

The results showed that the highest average content of elements analysed in tea leaves was of K with 1479.6 mg/kg. Al, Mg, Mn, Na, Ba, Fe, B and Zn ranged from about 3.5 to 150 µg/kg, while other elements were below 1 µg/kg. Se and Hg were always below their limit of quantification. Of note were the highest levels of Pb, As and Cd in Chinese black tea, Cu and Sr in Chinese decaffeinated black tea, and Cr in Argentinian decaffeinated black tea.

Brewing time had a significant effect on the percentage extraction of the almost all the elements analysed, especially when the infusions were made with tea bags as they were. After 8 minutes of infusion, K, Al, Mg, Na, Ba, B, Zn, Ni, Co and As show transfer rate values exceeding approximately 60%, while Fe, Cu, Cr, Pb, Sr and Cd remained mainly in the tea leaves (transfer rate value not exceeding 16%). The transfer rate of K, Na and Pb was significantly lower in infusions with bags than in infusions without bags.

Regarding essential mineral elements, the tea infusions cover the Recommended Daily Allowance (RDA) to a small extent. Furthermore, the consumption of one cup of tea (250ml) results in minimal exposure to toxic and potentially toxic elements. The HQ values were all less than 1.

Conclusion

Tea is one of the world's most popular non-alcoholic beverages. Therefore, continuous monitoring is important to ensure an adequate level of consumer safety. In this study, brewing time had a significant effect on the percentage extraction of the almost all the elements analysed. In addition, according to the results, the intake of essential elements was low and, in terms of toxic and potentially toxic elements, the consumption of these tea infusions would not pose a significant health risk to consumers.

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P4

Inorganic elements in Italian and Moroccan carobs (*Ceratonia siliqua L.*)

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Summary: *In this study different carob samples from Italy and Morocco were analyzed. The objective was to evaluate the levels of mineral elements by ICP-MS, differentiate them in terms of geographic origin, and assess the potential toxicological risk to humans from daily consumption of carob. The results showed that some carob from Morocco has a high amount of inorganic elements and that the daily consumed of carob must be controlled in order to be safely assumed.*

Keywords: *inorganic contamination, carob fruit, food safety*

Introduction

Carob is a legume, that grows wild from a long-lived evergreen tree named *Ceratonia siliqua*. Thanks to its nutritional value, carob is used as a health ingredient in many foods. The concentration of inorganic elements in carob can vary depending on several factors such as genetic variability, environmental conditions, varieties, cultivation practices, geographical origin, harvesting period and many factors related to soil type (temperature, drought, irrigation, fertilization, and salinity). Among the largest producers of carob beans worldwide there are Italy and Morocco. In Italy, Sicily is the largest producer, with a high production in the provinces of Ragusa and Siracusa, which also extends to a small extent to the provinces of Messina, Reggio Calabria and Cosenza. In Morocco, on the other hand, high carob production is concentrated on the northern edge of the Atlas Mountains, the Rif Mountains and some valleys of the south-western Anti-Atlas, mainly represented by natural trees domesticated in agroforestry systems. The two main components of carob are the pod (90%) and the seeds (10%). It is mainly the flour made from the carob seeds that has led to the gradual rediscovery of this fruit as it is widely used in the food industry. In general, carob contains 48-56% sugars, mainly sucrose, glucose, fructose, and maltose in varying proportions, 3-4% protein, 0.2-0.6% low fat, and high fiber (up to 39.8%) and polyphenol (about 20%) content [1,3]. These components give carob its health benefits. In fact, carob may have several medical applications in this respect: 1) it reduces and controls diarrhea; 2) it reduces cholesterol levels due to its high fiber content; 3) it has long been used to control weight, and is known as a tonic for weight loss because it stimulates metabolism and improves digestion; 4) it has also been used in the management of type 2 diabetes and can reduce insulin [2,4]. That said, knowledge of the concentrations of mineral elements in carob can serve not only to improve human health but also to prevent a potential toxicological risk from the consumption of carob beans, if the latter exceeds the maximum daily limit.

Experimental

In this research, 90 samples of carobs (40 Italian and 50 Moroccan) were taken into consideration by assessing the content of Li, Be, Na, Mg, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Cd, Sb, Pb, Sn and Hg by means of ICP-MS and DMA-80. The samples analyzed come from different geographical origins of Italy, Italy (Messina, Syracuse, Cosenza and Reggio Calabria) and Morocco (Al-Hoceimà, Fahs-Anira, Agadir- Ida-Ou Tanane, Tangier-Assilah and Rehamna). In addition, the potential toxicological risk to humans from carob consumption was assessed.

Results

The order of abundance of the average macro-nutrient concentration in the carob samples was K, Ca, Mg and Na. More precisely, the values of the carob samples of the respective elements are reported according to the area of origin: K (9566.34±30.03 - 10108.78±18.08 mg/kg, in Syracuse), Ca (range: 3747.28±59.70 - 3850.97±52.42 mg/kg, in Al-Hoseyma), Na (range: 677.29±4.32 - 679.44±6.19 mg/kg in Al-Hoseyma) and Mg (range: 1378.69±12.15 - 1481.92±31.11 mg/kg in Reggio Calabria). However, this trend depended on the sample and the area of origin. In general, the carob samples analyzed contained a considerable amount of Zn. In particular, the Italian samples were characterized by a higher contribution of zinc than the Moroccan counterpart. Thus, the levels of this element, whose contribution in the analyzed samples ranged from 1.66±0.09 to 9.33±0.11 mg/kg, were also closely related to the geographical origin. In reference to European Regulation 2023/915, we have a maximum limit for lead and cadmium of 0.10 and 0.020 mg/kg respectively. Consequently, only some of the Moroccan carobs analyzed exceeded these limits. For the most of toxic and potentially toxic elements, the results are not worrying.

Even though carobs are only eaten sporadically nowadays, they are an exceptional fruit, rich in healthy nutrients and able to provide the body with multiple benefits. For this reason, given that the average weight of a single carob is around 10 g, an average daily consumption of 10 g/day has been chosen to calculate the absorption rate of mineral elements. The uptake percentage was estimated considering an adult of 70 Kg.
To calculate the uptake percentage for macro- and essential microelements, the reference RDAs for Ca, Cr, Cu, Co, Fe, K, Na, Mg, Mn, and Zn, reported by the European Food Safety Authority (EFSA, 2005) and the European Commission (European Communities Commission, 2008), were used.
For the toxic and potentially toxic elements, the reference TDI and BMDL01 for As, Hg, Ni, and Pb, and the reference TWI for Cd and Al were used. With reference to this data reported by EFSA, this study showed that carob is a good source of macronutrients. Regarding essential trace elements, the absorption of chromium in the carob sample from Al-Hoseyma is 63.8%. For the other essential trace elements (Cu, Ca, Fe, Mn, Mg, Zn and K), the percentages were 20% or less. Therefore, even if daily limits are not exceeded, continuous monitoring of mineral elements is of fundamental importance for the health of consumers.
For most toxic and potentially toxic elements the results are not worrying. However, special attention should be paid to mercury absorption which, although the daily intake is below the maximum limit, reached 36.5% in the Fahs-Anita sample, respectively.

Conclusion

Carob is a matrix with multiple health effects, but it can be subject to contamination depending on where it originates. As there are many sources of contamination in Morocco, from Cd-rich rocks to industries such as lead mines, so continuous monitoring is necessary to reduce inorganic contamination of this matrix and thus minimize risks to consumers.

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P5

Analysis of ultrashort- and short-chain Per- and Polyfluoroalkyl Substances (PFAS) in vegetables: method development and preliminary validation study

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Summary: *Per- and polyfluoroalkylated substances (PFAS) are widespread environmental contaminants. A method for the determination of nine ultrashort- and short-chain PFAS (C1-C6) at ng/kg levels in vegetables was developed applying LC-Q-Orbitrap. From preliminary experiments, good performances were obtained.*

Keywords: *Ultrashort- and Short-Chain PFAS, vegetables, LC-Q-Orbitrap*

Introduction

Per- and polyfluoroalkyl substances (PFAS) include a large group of synthetic chemicals with a long history of use in industrial and consumer products. Regulatory and public health agencies have recognized that exposure to high levels of some PFAS may cause adverse effects for human health.
Therefore, in 2020, the risks related to some PFAS were reassessed by the European Food Safety Authority (EFSA) [1]; while, two years ago, the EU Commission issued new demanding guidelines for the regulation and analysis of PFAS in food (EU 2022/2388; EU 2022/1428) [2-3]. The potential adverse effects on animal and human health have been studied and four long chain PFAS regulated in food of animal origin fixing maximum limits (EU 2023/915) [4]. To date, the interest of the scientific community is moving towards expanding the range of foods to include fruits and vegetables. In these matrices ultrashort- and short-chain PFAS, usually referred from C1 to C6 compounds, are considered to be more bioaccumulative than long chain PFAS. In this study, a specific LC-HRMS method for the determination of ultrashort- and short-chain PFAS, including four perfluorinated carboxylic acids (PFCAs) and five perfluorinated sulfonic acids (PFSAs) in vegetables, has been developed and validated.

Experimental

Nine ultrashort- and short-chain PFAS were quantified by isotopic dilution with the addition of five labelled compounds as internal standards (table 1). Two grams of each vegetable sample were extracted and purified following the protocol published by Moretti et al. [5] with minor modifications. The quantification was performed by liquid-chromatography coupled to Q-Orbitrap analyser (LC-Q Exactive, Thermoscientific) using ESI negative ionization mode and full scan/SIM acquisition. The mobile phases were water and MeOH, both containing 5 mM of ammonium acetate. The analytes separation was achieved on a LC column Atlantis PREMIER BEH C18 AX (50 x 2.1mm, 1.7µm, Waters). The method performances were assessed carrying out a preliminary validation study. Different types of vegetables, such as cauliflower, zucchini and salad, were spiked at four concentrations: 50, 100, 250 and 500 ng/kg. For each level, four replicates on three different days were analysed.

Table 1. *List of analytes and related labelled compounds*

Class	Acronimus	Analytes	Labelled compounds	Class	Acronimus	Analytes	Labelled compounds
PFCAs	PFPrA	Pentafluoropropionic acid	[13C4]PFBA	PFSAs	TFS	Trifluoromethansulphonic acid	[13C3]L-PFBS
	PFBA	Perfluoro-n-butanoic acid	[13C4]PFBA		L-PFPrS	Heptafluoro-1-propansulfonic acid	[13C3]L-PFBS
	PFPeA	Perfluoro-n-pentanoic acid	[13C5]PFPeA		L-PFBS	Potassium perfluoro-1-butanesulfonate	[13C3]L-PFBS
	PFHxA	Perfluoro-n-hexanoic acid	[13C5]PFHxA		L-PFPeS	Sodium perfluoro-1-pentanesulfonate	[13C3]L-PFHxS
					L-PFHxS	Sodium perfluoro-1-hexanesulfonate	[13C3]L-PFHxS

Results

The method validation was carried out according to the EURL Guidance Document on analytical parameters for the determination of PFAS in food and feed [6]. The chromatographic conditions proved to be optimal (injection volume: 3 µL) and, as shown in Figure 1, the separation between peaks was suitable with a 10 minutes chromatographic run. In order to obtain a significant improvement of instrumental signal, in-source collision-induced dissociation (IS-CID) has been set to 5 eV to promote the loss of CO_2 of PFCAs.
Precision (repeatability and intra-lab reproducibility) was estimated at each level by ANOVA with coefficients of variation (%) below 15 and 20, respectively. Trueness was in the range 80-105% for all analytes thanks to

the application of isotopic dilution. Recovery factors were generally above 80%. Limits of quantification (LOQs) were fixed at the first validation level (50 ng/kg). In each batch, procedural blanks were also analyzed to monitor background contamination and only two analytes were found at concentrations below the LOQs, i.e. PFBA and PFPrA.

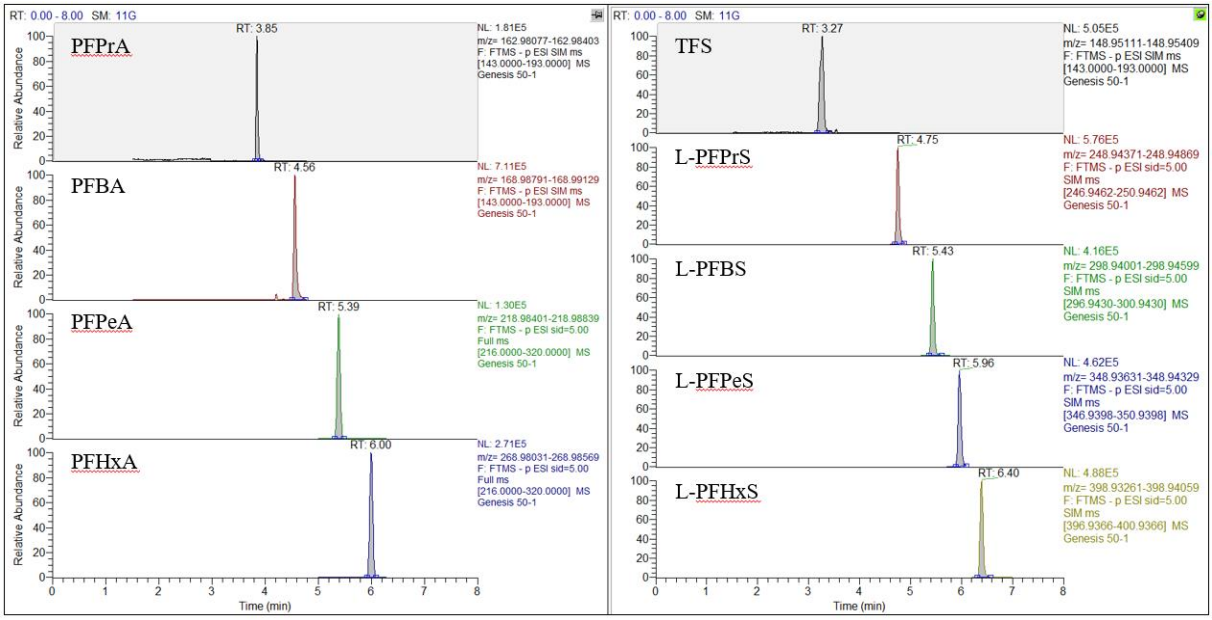

Figure 1. *LC-Q-Orbitrap chromatograms of a zucchini fortified at 50 ng/kg. At the left side PFCAs, at the right PFSAs.*

Conclusions

The developed method accurately quantifies nine PFAS in vegetables starting from 50 ng/kg. The procedure can be very useful for analyzing matrices in which ultrashort- and short-chain PFAS contamination could play a crucial role.

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P6

One method with dual detection for the analysis of PBDEs, HBCDs and eBFRs: levels in fish from the Adriatic Sea

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Summary: *An efficient procedure for simultaneous analysis of PBDEs, HBCDs and eBFRs in food was set up, validated and used for the assessment of BFRs in fish collected in central Adriatic Sea. The lower levels were founded in sole while in cod and red mullet comparable concentration of both PBDEs and HBCDs were quantified. Among eBFRs, PBT was the compound most frequently measured in all the selected species.*

Keywords: *PBDEs, HBCDs, eBFRs*

Introduction

Brominated flame retardants (BFRs) are used in a wide variety of commercial and industrial products, to retard or prevent the possible ignition of fire. Polybromodiphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) are the most widely used BFRs, both included in the Stockholm Convention elimination list due to their endocrine-disrupting effects [1-2]. The use of other flame-retardants, classified as "emerging" BFRs (eBFRs), is increasing, and traces of these compounds have been detected in environmental and food matrices [3]. Recommendation EU/118/2014 requests Member States to monitor different BFRs classes in food, including PBDEs, HBCDs and eBFRs, to assess background contamination levels and estimate human exposure through diet, although no maximum limits were yet defined in food. The aim of the present study was to set up and validate an efficient procedure for the simultaneous analysis of PBDEs, HBCDs and eBFRs in food. The method was used for the assessment of BFRs in fish samples collected in the Adriatic Sea.

Materials and Methods

Sample preparation and analysis

9 PBDEs (28, 47, 49, 99, 100, 153, 154, 183, 209), 3 HBCD isomers (α, β and γ) and 9 eBFRs (pTBX, PBBz, PBT, PBEB, HBBz, EHTBB, HCDBCO, BTBPE and DBDPE) were analyzed in isotopic dilution including a single sample preparation followed by a dual detection in GC-MS/MS (PBDEs and eBFRs) and LC-MS/MS (HBCDs). The analytical method for PBDEs and HBCDs analysis was already thoroughly described [4]. The same conditions were applied with good results to the analysis of eBFRs. Limits of quantification (LOQs) were estimated at 0.005 µg/kg for PBDEs and 0.010 µg/kg for HBCDs and eBFRs (0.015 µg/kg BDE-47, 0.020 µg/kg BTBPE and 0.100 µg/kg DBDPE and BDE-209).

Method validation

The PBDEs/HBCDs method was fully validated in all food categories, as already described [4]. The suitability of the protocol for eBFRs in muscle was demonstrated through replicated analysis of chicken muscle at two spiking level, in inter-laboratory reproducibility conditions (Tab.1). Precision (repeatability and intra-lab reproducibility) was estimated at each level by ANOVA. Trueness was measured in terms of corrected recoveries (isotopic dilution) and internal labelled standards recoveries.

Sampling

The validated method was applied to the analyses of 3 species of fishes selected among the ones widespread in central Adriatic Sea: red mullet (*Mullus barbatus*, n=4), European hake (*Merluccius merluccius*, n=8) and sole *(Solea solea*, n=6)

Results and Discussion

The sample preparation procedure was developed starting from the PBDEs/HBCDs method [4], making it applicable to eBFRs analysis, and tweaks made to be able to analyse simultaneously all the three BFR classes. Labelled Internal Standards (IS) for each class of compounds were added at the beginning of the procedure, to minimize both the loss of the analytes due to analytical process and the matrix effect. Syringe standards (BDE-77- and -138-$^{13}C_{12}$ and β-HBCD-$^{13}C_{12}$2) were also introduced to correct for inter-injection fluctuations and to assess the labelled congener's recoveries (Table 1). Method LOQs were set at the lowest analytes concentration tested in spiked blank samples giving a RSD_R compliant with the Horwitz's theoretical values (Tab.1). Intra-laboratory reproducibility and calibration curve contributions were taken into account in the estimation of the method relative extended uncertainty (U%). The validation data were compliant to the EURL Guidance Document on the Determination of Organobromine Contaminants, for most of the analytes [5]:

trueness ±30 %, within-laboratory reproducibility ≤ 20 %, LOQ 0.010 µg/kg (except for BDE-47 and -209, BTBPE and DBDPE), IS recoveries 30 – 140 % (Tab.1). Moreover an accurate procedure to control the background contamination was implemented [4]. The validated method was applied to the analysis of red mullet, European hake and sole collected in Adriatic Sea (Table 2). PBDEs were not quantified in any of the sole, while comparable levels were found in red mullet and European hake: PBDEs sum ranged from <LOQ - 0.24 µg/kg (median: 0.057 µg/kg) and <LOQ – 0.11 µg/kg (median: 0.053 µg/kg) respectively. BDE-47 was dominant, followed by -99 and -100. As regard HBCDs, only the α-isomer was measured. The higher levels were found in red mullet (median: 0.036 µg/kg; <LOQ – 0.13 µg/kg) and European hake (median: 0.029 µg/kg; 0.010 – 0.050 µg/kg), while in sole it was quantified in only one sample at 0.028 µg/kg. Among eBFRs, PBT was the compound most frequently quantified. It was measured in five specimen of European hake (maximum value 0.083 µg/kg), in two of red mullet (max: 0.063 µg/kg) and in only one sole at 0.041 µg/kg. In European hake and red mullet also low levels of PBBz were found, with a maximum concentration of 0.032 and 0.018 µg/kg respectively. The other eBFR compounds were mostly <LOQs (Tab.2). The presented preliminary data are among the first published on BFRs in marine fish from Central Adriatic Sea.

Table 1. *Validation results: number of replicates (n), relative standard deviations in repeatability (RSD_r) and intra-lab reproducibility conditions (RSD_R), relative expanded uncertainty (U_{rel}), apparent recoveries (Rapp) and internal standard recoveries ($IS_{R \pm SD}$)*

		LOQ	Spiking level	n	RSD_r	RSD_R	U_{rel}	R_{app}	IS R ± SD			LOQ	Spiking level	n	RSD_r	RSD_R	U_{rel}	R_{app}	IS R ± SD
		µg kg^{-1}					%					µg kg^{-1}					%		
eBFR	pTBX	0.010	0.010	15	6.3	6.3	22	96	81 ± 25	HBCD	α–	0.010	0.010	15	11	11	43	85	57 ± 21
			0.050	15	3.7	3.9	18	93			β–			15	14	13		86	63 ± 28
	PBBz	0.010	0.010	15	5.3	6.9	18	96	81 ± 25		γ–			15	13	14		98	63 ± 28
			0.050	15	3.7	3.5	12	104		PBDE	28	0.005	0.010	15	5.1	9.3	59	96	79 ± 8.6
	PBT	0.010	0.010	14	7.5	9.6	48	81	81 ± 25		49			15	3.3	14		82	88 ± 7.5
			0.050	15	6.1	6.0	44	80			47			9	7.6	7.7		83	88 ± 7.5
	PBEB	0.010	0.010	15	4.9	6.6	42	85	81 ± 25		100			15	7.9	17		95	91 ± 9.1
			0.050	15	4.9	4.6	40	81			99			15	14.0	16		97	93 ± 9
	HBBz	0.010	0.010	13	9.0	10	41	107	104 ± 7		154			15	10.2	12		96	85 ± 13
			0.050	13	11	13	36	101			153			15	9.8	14		93	88 ± 10
	EHTBB	0.010	0.010	15	7.0	6.3	26	113	32 ± 7		183			14	7.8	11		82	86 ± 9
			0.050	15	5.3	5.7	23	109			209	0.100	0.100	13	7.6	11		153	88 ± 13
	HCDBCO	0.010	0.010	12	4.9	4.8	36	119	104 ± 7										
			0.050	14	11	13	35	104											
	BTBPE	0.020	0.020	14	3.6	5.6	20	86	97 ± 10										
			0.100	13	3.3	3.6	16	93											
	DBDPE	0.100	0.100	8	14	12	43	101	83 ± 16										
			0.500	11	14	14	36	102											

Table 2. *BFRs concentration (median, minimum and maximum values) in fish from Adriatic Sea*

	M. barbatus (n=4) median (min-max)	M. merluccius (n=8) median (min-max)	S.solea (n=6) median (min-max)		M. barbatus (n=4) median (min-max)	M. merluccius (n=8) median (min-max)	S.solea (n=6) median (min-max)
	PBDE (µg/kg)				eBFR (µg/kg)		
28	n.c. (<0.005)	n.c. (<0.005)	n.c. (<0.005)	pTBX	n.c. (<0.010)	n.c. (<0.010)	n.c. (<0.010)
49	<0.005 (<0.005-0.010)	0.006 (<0.005-0.015)	n.c. (<0.005)	PBBz	<0.010 (<0.010-0.032)	<0.010 (<0.010-0.018)	n.c. (<0.010)
47	0.028 (<0.015-0.13)	0.038 (<0.015-0.072)	n.c. (<0.015)	PBT	<0.010 (<0.010-0.063)	0.024 (<0.010-0.083)	<0.010 (<0.010-0.041)
100	0.007 (<0.005-0.027)	0.009 (<0.005-0.022)	n.c. (<0.005)	PBEB	n.c. (<0.010)	<0.010 (<0.010-0.013)	n.c. (<0.010)
99	0.008 (<0.005-0.038)	<0.005 (<0.005-0.005)	n.c. (<0.005)	HBBz	n.c. (<0.010)	n.c. (<0.010)	n.c. (<0.010)
154	0.011 (<0.005-0.028)	n.c. (<0.005)	n.c. (<0.005)	EHTBB	n.c. (<0.010)	n.c. (<0.010)	n.c. (<0.010)
153	<0.005 (<0.005-0.008)	n.c. (<0.005)	n.c. (<0.005)	HCDBCO	n.c. (<0.010)	n.c. (<0.010)	n.c. (<0.010)
183	n.c. (<0.005)	n.c. (<0.005)	n.c. (<0.005)	BTBPE	n.c. (<0.020)	n.c. (<0.020)	n.c. (<0.020)
209	n.c. (<0.100)	n.c. (<0.100)	n.c. (<0.100)	DBDPE	n.c. (<0.100)	n.c. (<0.100)	n.c. (<0.100)
PBDE sum l.b.	0.057 (0.0-0.24)	0.053 (0.0-0.11)	0.0				
	HBCD (µg/kg)						
α	0.036 (<0.010-0.13)	0.029 (0.010-0.050)	<0.010 (<0.010-0.028)	γ	n.c. (<0.010)	n.c. (<0.010)	n.c. (<0.010)
β	n.c. (<0.010)	n.c. (<0.010)	n.c. (<0.010)	HBCD sum l.b.	0.036 (<0.010-0.13)	0.029 (0.010-0.050)	<0.010 (<0.010-0.028)

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P7

Contaminanti ambientali nei prodotti ittici del Mediterraneo. qualità dell'ambiente marino in relazione alla salute umana

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Premessa

La sicurezza degli alimenti è regolamentata da un sistema normativo in continua evoluzione, finalizzato a rendere controllabili gli eventi indesiderabili lungo tutta la filiera produttiva, dalla produzione primaria, alla trasformazione, fino al momento del consumo. La legge definisce limiti sulla presenza negli alimenti di contaminanti chimici sempre più restrittivi e le autorità che si occupano della salute pubblica mettono in allerta gli operatori del settore verso sostanze emergenti che è necessario conoscere. Negli ultimi decenni la presenza dei contaminanti ambientali negli alimenti è diventata una priorità per la salute pubblica, rendendo sempre più evidente il legame tra ambiente e salute.

Sostanze come pesticidi (clorpirifos, e altri organofosfati) o metalli tossici (arsenico, metilmercurio, piombo, cadmio) che finiscono nella catena alimentare sono tossici per il cervello e per il microbiota intestinale che è direttamente coinvolto nella patogenesi delle malattie neurodegenerative. L'inquinamento degli oceani e i cambiamenti climatici fanno crescere la preoccupazione riguardo lo stato generale dell'ambiente marino e le conseguenze per la salubrità del pesce e per la salute dei consumatori. Il consumo di pesci ricchi di omega-3 sebbene sia noto supportare la salute del cervello per l'elevato contenuto di acidi grassi sani noti, può al contempo favorire l'introduzione attraverso la dieta di contaminanti organici ed inorganici contenuti nei tessuti degli stessi. La sicurezza degli alimenti ittici, dunque, rappresenta un interesse prioritario della popolazione e coinvolge in modo trasversale e con differenti ruoli le istituzioni, i consumatori, i pescatori ed il mondo scientifico.

Scopo

Non essendo ancora riusciti a definire un legame quantitativo tra i livelli di contaminanti nell'ambiente marino e i livelli nelle diverse specie di organismi, c'è necessità di maggiore studio e ricerca sul trasferimento dei contaminanti dall'ambiente marino al pescato. In Italia, specialmente nel ramo dell'ittiocoltura, da diversi anni sono pianificate attività di controllo ufficiale che consentono di raccogliere dati per eventuali revisioni dei limiti massimi fissati (ex Reg. 1881/2006, oggi Reg. 915/2023) per sostanze quali ad esempio metalli pesanti, diossine e PCB, e, indirettamente, per verificare l'efficacia delle misure preventive messe in atto da parte degli operatori del settore. Nel caso, invece, di contaminanti persistenti per i quali non risulta ancora definito un limite massimo, i dati di contaminazione, soprattutto nel pescato, sono scarsi o incompleti. È evidente, quindi, la necessità da parte delle autorità competenti di attuare una politica efficacie di gestione del rischio, che poggi sulla disponibilità di dati analitici affidabili, adeguati alle necessità del decisore.

Il progetto *CAP-fish* si inserisce in questo contesto con la finalità di supportare e implementare i regolamenti nazionali ed internazionali, che definiscono la sicurezza degli alimenti ittici, attraverso lo studio della presenza nel pescato di alcune classi di contaminanti prioritari (le diossine (PCDD/F), i policlorobifenili (PCB), gli elementi metallici tossici) e altri non ancora inclusi nei programmi di controllo degli alimenti (i ritardanti di fiamma bromurati (BFR), i composti perfluoro-organici (PFAS) e la conseguente esposizione della popolazione generale attraverso la dieta.

Le istituzioni coinvolte nel Progetto, oltre ad aver maturato una lunga esperienza in tema di ricerca di microinquinanti ambientali persistenti nei prodotti ittici (con la presenza del Laboratorio Nazionale di Riferimento per i contaminanti organici persistenti alogenati di IZSAM e il Centro Nazionale per la Rete dei Laboratori dell' ISPRA che monitora, per conto del MiTE, la presenza di contaminanti nel pescato ai sensi della Strategia Marina), di analisi del rischio alimentare (con la presenza del Centro di Referenza Nazionale per l'epidemiologia veterinaria, la programmazione, l'informazione e l'analisi del rischio) di patogenesi di malattie neurodegenerative (con la presenza del IRCCS Fondazione S. Lucia), dispongono di strumentazioni analitiche di ultima generazione (spettrometri di massa ad alta risoluzione quali LC-(HR)MS/MS, HRGC-HRMS per i composti organici e, spettrometria di massa con plasma ad accoppiamento induttivo (ICP-MS) interfacciati con cromatografi liquidi e gassosi per i metalli tossici) e metodi validati e/o accreditati secondo rigorosi criteri stabiliti da organismi internazionali e già ampiamente utilizzati per le analisi dei campioni conferiti nell'ambito del controllo ufficiale.

Aree territoriali interessate e trasferibilità degli interventi

Sono prese in considerazione le 10 specie ittiche che singolarmente, per quantità di pescato, hanno superato il 2% rispetto al totale pescato in Italia nell'anno 2019. Il piano di campionamento prevede la raccolta di 136 campioni per ciascuna delle 10 specie considerate per un totale complessivo di 1360 campioni da distribuire nel periodo 2024/2026. Questa numerosità campionaria rappresenta un buon compromesso sia da un punto di vista economico che statistico garantendo, con un intervallo di confidenza del 95%, un errore nella stima della deviazione standard del campione pari al 25% ed un errore nella stima della media (in unità di deviazioni standard) pari a 0,17. Per ogni specie presa in esame, la quantità di pescato è stata ripartita in percentuale per regione e per specie ittica. Ai fini della ripartizione dei campionamenti, è stato considerato il numero minimo di Regioni che, sommate, contribuiscono ad una quantità di pescato non inferiore all'80% per quella determinata specie ittica.

Infine, a beneficio del SSN, saranno disponibili i dati sulla contaminazione delle specie ittiche oggetto di studio e gli scenari relativi all'esposizione umana a contaminanti ambientali. Questo potrebbe sostenere la predisposizione di azioni di sviluppo e implementazione alle normative europee e nazionali sulla sicurezza degli alimenti ittici. Inoltre, un processo di comunicazione, efficace ed efficiente, avrà una ricaduta in termini di credibilità e fiducia nella pubblica amministrazione e consentirà di accrescere la consapevolezza del rischio in tutta la popolazione.

P8

Long term study (2012-2021) of marine biotoxins in mussels from NW Adriatic Sea (Italy). Is rainfall a suitable forecasting tool?

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Summary: *This work reports ten years (2012-2021) monitoring of OA and YTXs levels trends in mussels farmed along the Marche Region coasts (Italy), trying to correlate them with rainfall as forecasting tool for toxic events.*

Keywords: *mussels, Okadaic acid, Yessotoxin, rainfall*

Introduction

Several marine microalgae synthesize secondary metabolites, polyether compounds, named Lipophilic Toxins (LTs). They include 3 sub-groups: Okadaic acid (OA) and Dinophysistoxins (DTX1, DTX2 and DTX3) (OAs), Yessotoxins (YTXs), Azaspiracids (AZAs) and have diarrhetic effects (diarrhetic shellfish poisoning - DSP) on humans. Filter-feeding bivalve molluscs can accumulate large amounts of LTs becoming a severe threat to consumers' health and shellfish farm industry**.** OAs, YTXs and AZAs are regulated with maximum limits (ML) of 160 µg OA eq. kg^{-1}, 3.75mg YTX eq. kg^{-1} and 160 µg AZA eq. kg^{-1} respectively [1].The northwestern (NW) part of the Adriatic Sea (Italy), the most eutrophic area of the whole Mediterranean Sea, is highly exploited for mussel farming but also subjected to recurrent blooms of toxic phytoplankton. LTs profile of NW Adriatic mussels has changed several times over the past 30 years. In the early 90's OAs were reported as predominants [2] while, successively, YTXs replaced them as main contaminants [3]. In the Marche Region there are about 25 long-line mussel farms all along the coastline. Shellfish production areas are regularly controlled by the competent authority monitoring LT levels in mollusks and toxic microalgae, as requested by regulation (EU) 627/2019 [4]. They are highly effective in terms of consumer protection but entails important economic losses for the producers, when ML are exceeded and production areas closed. The inland rainfall strongly influence river input of nutrients in the marine ecosystem, a major driving factor in the microalgae reproduction and species selection influencing LT accumulation in mussels.
This work collected and organized ten years (2012-2021) of OA and YTXs levels in mussels from Marche coast and inland rainfall data from 160 rain gauges distributed over the entire Region. The aim of the work is to investigate the possible correlation between inland rainfall and LTs in mussels. The results could help in assessing rainfall suitability as forecasting tool of LT contamination.

Experimental

Mussel samples (*Mytilus galloprovincialis*) were harvested from breeding sites biweekly in the period of interest (2012-2021).
Sample preparation, extraction and analysis were performed following the EU official method [5]. The weekly mean levels of the cumulative daily rainfall, in the ten years of the study, recorded by 160 rain gauges distributed over the main Marche Region river basin, were obtained from the Civil Protection Functional Center on the online SIRMP system (http://app.protezionecivile.marche.it)

Results

YTXs were the predominant LTs found in mussels collected along the Marche Region coast during the first three years of the study (2012-beginning of 2015) with a year by year increasing trend in terms of number and percentage of contaminated samples, levels (maximum 6.87 mg kg^{-1} in 2014 and mean contamination of 0.51 mg kg^{-1} in 2014) and percentage of non-compliant samples (2013: 0,9 % of the samples > ML).
From 2015 onwards, YTX levels in mussels dropped significantly becoming almost always not measurable during the last two years (2020-2021). November, December and January were the months most affected by LTs contamination in each year, in terms of number of non-compliant samples and maximum levels reached, although the highest toxins levels were measured in mussels collected between 2017 and 2019 from March until July.

During 2012-2013 OA was rarely quantifiable in mussels (only in 4% of the samples during 2013). In 2014, the number of samples with OA levels >LOQ increased to 22.2% of the total samples analyzed in the year, although with only very few cases of non-compliant (1.4% of samples). 2015 was the year with the first severe OA contamination event in mussels of the Marche region: 21.4% of the samples were contaminated at measurable level and the 8.4% were non-compliant. In 2015 OA reached the highest mean and absolute levels (68.3 and 1373 µg OA eq. kg^{-1} respectively) in the ten investigated years. Since then, other OA toxic events occurred in 2017 (18% of contaminated samples and 4.4% >ML), 2020 (32.6% of contaminated samples and 1.8% >ML) and 2021 (29.5% of contaminated samples and 7.8% >ML). In 2016, 2018 and 2019 OA was either absent or very low. Overall October, November and December were the most affected months although also spring OA contamination events are quite frequent.
As regard rainfall, the weekly mean of daily levels showed a decreasing annual trend during the ten years (-1.27%/year) with a marked increase in 2013 (+24% respect to 2012) followed by a continuous decrease in the following years and a main downward shifts in 2014 (-7.5%), 2015 (-10.1%), 2017 (-5.7%), 2020 (-6.6%) and 2021 (-4.3%) which are also the years most affected by OA contamination in mussels. The decreased rainfall levels in the above mentioned years may be mainly attributed to dry summer and spring seasons. Therefore since OA mussels contamination episodes occur mainly in spring and autumn a possible correlation between the two events may be found. On the other hand, the years most affected by YTXs contamination in mussels were those (2013 and 2014) with higher mean levels of daily rainfall (3.13-3.38 mm/day) suggesting a likely positive correlation between the two variables.

Conclusions
The inland rainfall regime seems to have an important role in driving the LT contamination in mussels of Marche Region. Therefore rainfall monitoring may turn out to be a useful tool for Competent Authority to timely forecast possible toxic events.

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P9

Off-odours in recirculated aquaculture systems fish

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Summary: *Recirculated aquaculture systems represent innovative farming solutions to meet global demands for seafood, yet farmed fish often suffers from off-odour taints that make them unpalatable. This work showcases mass spectrometry-based approaches to explore the presences of off-odours in aquaculture fish to provide key insights into why and how they arise.*

Keywords: *Aquaculture; fish; off-odour*

Recent decades have experienced a depletion in the global fish stock due to intensive and large-scale ocean fishing. Land-based farming alternatives in the form of recirculating aquaculture systems (RAS) represent suitable approach to meet growing consumer demands for seafood by offering high production efficiency with low environmental impact [1]. Compared to wild-caught fish, however, RAS farmed fish has a greater potential to be impaired by the presence of off-odour compounds, including geosmin and 2-methylisoborneol (2-MIB), which are described as having an earthy character that can diminish palatability and reduce consumer demand for RAS products [2]. The presence of such off-odour taints in aquaculture feeds and fish has been postulated to arise through absorption via ingestion [3,4]. In the present work, sensory evaluations were combined with analytical mass spectrometry (gas chromatography-mass spectrometry/olfactometry and proton transfer reaction-time of flight-mass spectrometry) to explore correlations between exogenous sources of odour-active compounds in several RAS-reared fish species. Specifically, three experiments were undertaken to explore the origins and pathways of absorption and accumulation of off-odour compounds in RAS fish. Trials included a comparison between waterborne and feedborne odours in Russian sturgeon (Acipenser gueldenstaedtii) and an investigation of the influence of odours present in RAS water odours and their absorption from commercial feeds. The absorption, accumulation, and transformation of waterborne geosmin was additionally examined in Rainbow trout´s (Oncorhynchus mykiss) fillets, blood, and liver to refining compartmental models of absorption [5,6]. Finally, a dose-response feeding trial was conducted using an experimental algae-based aquaculture feed to enhance marine odours in RAS-grown Atlantic salmon (Salmo salar) and assess the potential to mitigate earthy off-odours [6]. This poster presentation will outline key outcomes of these trials and showcase the utility of mass spectrometry to explore the topic of off-odour in view of enhancing understanding of how such compounds arise in aquaculture fish [7].

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P10

Metabolite profiling of sea buckthorn fruits (*Hippophae rhamnoides* L.) through UHPLC-Q-Orbitrap-MS/MS analysis

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Summary: *Hippophae rhamnoides fruits from different Countries (Italy, Poland and Russia) were analyzed. Several extractions were carried out on dried fruits: microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and decoction (Dec). The extracts were investigated by UHPLC-Q-Orbitrap analysis to compare their chemical profiles and to evaluate the occurrence of bioactive principles.*

Keywords: *Hippophae rhamnoides fruits; extractions; UHPLC-Q-Orbitrap-MS/MS analysis*

Introduction

The fruit of *Hippophae rhamnoides* L. (Elaeagnaceae family) has been used for centuries in Europe and Asia as a food with high nutritional and medicinal values [1]. It is rich in nutrients and active components, and exhibits a wide range of health benefits, such as anti-inflammatory, antioxidant, hepatoprotective, anticancer, hypoglycaemic, hypolipidemic, neuroprotective, and antibacterial activities. Phytochemical studies revealed a wide variety of phytonutrients, including flavonoids, lignans, volatile oils, tannins, terpenoids, steroids, organic acids and alkaloids [2]. Oil from fruits and seeds is used to treat eczema, lupus erythematosus, chronic wounds, and inflammatory diseases [3]. This study was aimed at investigating the metabolite profiling of sea buckthorn fruits from different Countries using an LC-ESI/HRMS system consisting of a Thermo Ultimate RS 3000 UHPLC coupled online to a Q-Exactive hybrid quadrupole Orbitrap high-resolution mass spectrometer (UHPLC-Q-Orbitrap), fitted with a HESI II (heated electrospray ionization) probe, operating in positive ionization mode, to obtain in depth knowledge on bioactive compounds occurring in these fruits.

Experimental

H. rhamnoides fruits from different Countries (Italy, Poland and Russia) were analyzed. Different extractions were carried out on dried fruits: microwave-assisted extraction (MAE) with 100% EtOH, ultrasound-assisted extraction (UAE) with 100% EtOH and a hydroalcoholic solution at 50% EtOH, and decoction (Dec). All the extracts were investigated by liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). The HRMS/MS spectra were acquired by UHPLC-Q-Orbitrap instrument operating in positive ion mode. Xcalibur software was used for data acquisition and analysis (version 2.2) combined with Compound Discoverer software (version 3.3 SP3). Comparative analysis of fruit extracts was carried out by multivariate statistical analysis. Moreover, the total phenolic content of *H. rhamnoides* extracts was evaluated along with the antioxidant activity tested by TEAC assay.

Results

Analysis by LC-(HR)MS and LC-(HR) MS/MS defined the profile of sea buckthorn fruits. Chromatographic behaviour, accurate mass measurements, fragmentation pattern analysis, and comparison with literature data, allowed the identification of metabolites belonging to the flavonoid and phenolic acid classes (hydroxycinnamic and hydroxybenzoic acids). The three different extractions (MAE, ULT, Dec) showed minimal differences. The Russian variety showed high extraction yield only with the 100% EtOH ultrasound-assisted extraction. The Italian variety appeared to be the most complex in terms of specialized metabolites. The total phenolic content tested by Folin-Ciocalteu assay was similar for all the extracts. All the decoctions showed a good antioxidant activity in the range (2.08-2.4 mM) tested by TEAC assay, compared to the reference compound quercetin (4.16 mM). Finally, multivariate statistical analysis, applied on LC-(HR)MS data showed the marker compounds relevant for the differences of the extracts obtained from samples of different geographical area.

Conclusions

Based on the biological activities reported in literature for this plant, these data support the use of the sea buckthorn in human nutrition as a food supplement rich in antioxidant phytochemicals exerting potential beneficial effects on human health.

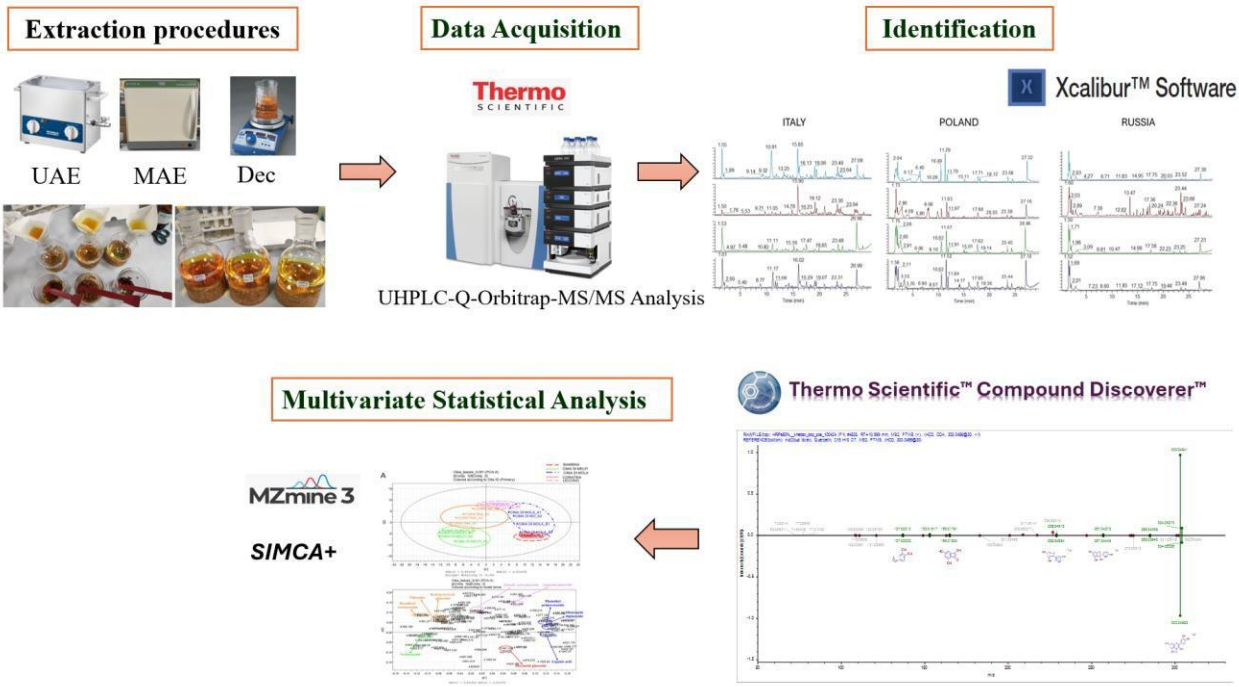

Figure 1. *Metabolite profiling workflow*

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P11

Occurrence of an emerging class of per- and polyfluoroalkyl substances (PFAS) in hen eggs

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Summary: *Occurrence of chloroperfluoropolyethercarboxylic acids (ClPFPECA), a group of emerging per- and polyfluoroalkyl substances (PFAS), has been investigated in commercial and home-produced eggs by liquid chromatography coupled to high-resolution mass spectrometry.*

Keywords: *Emerging PFAS, ClPFPECA, LC-HRMS, Hen eggs*

Introduction

Per- and polyfluoroalkyl substances (PFAS), a class of compounds bearing a carbon chain of variable length that can be partially or completely fluorinated, are recognized to be ubiquitous distributed in both environmental compartments and animal species.
The chemical properties of this group of compounds, although very important for the manufacture of a wide array of consumer goods and industrial processes [1], are the main cause of their persistence and accumulation in ecosystems, posing a serious threat to human health. In the past, in order to protect the population, American and European authorities imposed some restrictions on the use of these compounds. However, new analogues, the so-called emerging PFAS, have been synthetized and utilized to replace the banned ones. In this context, over the last decades, the emerging class of chloroperfluoropolyethercarboxylic acids (ClPFPECA) has been studied and detected by various authors [2]. ClPFPECA is a group of oligomers that differ in the number of perfluoroethoxy and perfluoropropoxy groups and in the position of the chlorine atom (Figure 1) [3].
Since eggs and related products have been identified as one of the major contributor to human PFAS dietary exposure; recently, the European Union has set maximum levels for four "legacy" PFAS in such a matrix [4]. Moreover, many studies highlighted that eggs harvested from home-producers tend to have higher levels of some environmental contaminants than commercial ones. Therefore, for the first time, we have investigated the presence of ClPFPECA in eggs collected from both types of farming.

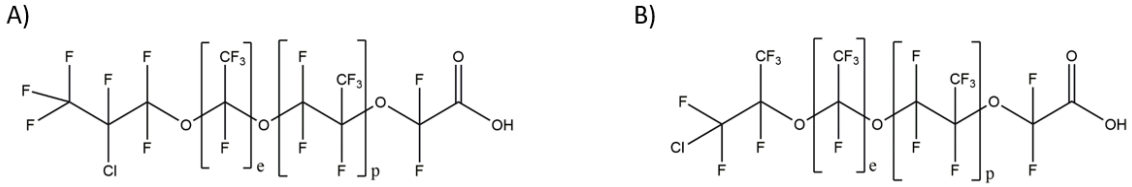

Figure 1. *Structure and composition of the ClPFPECA congener mixture: A) approximately 70%, B) approximately 30%.*

Experimental

Nineteen hen eggs were collected from both local markets (10) and home-producers (9) in the Umbria region (Central Italy) to assess the presence of six ClPFPECA congeners (Table 1). The extraction and purification were carried out following the method reported by Moretti et al [5] with slight modifications. Briefly, 2 g of sample were extracted twice with acetonitrile, the supernatant was collected, and the volume reduced by evaporation under nitrogen steam and stored at -20°C overnight. Then, the sample was purified by Strata X-AW SPE cartridge followed by dispersive SPE (Envicarb) and the residue dissolved in MeOH/ammonium Acetate 4 mM 80:20. The analysis was performed by liquid chromatography coupled to a Q-Orbitrap mass analyzer (Thermo Fisher Scientific, San Jose, CA, USA) applying ESI negative ionization mode. The analytes were quantified by external standardization due to the unavailability of the corresponding labelled compounds.

Results

None of the ClPFPECA congeners have been detected in eggs collected from local markets; whereas, in some samples harvested from home-producers, the [0,1], [0,2], and [1,1] congeners were measured. In particular, the [0,2] congener was quantified in 45% of home-produced samples, ranging from 11 ng/kg to 32 ng/kg. The [1,1] oligomer was found in two out of nine samples (at 11 ng/kg and 24 ng/kg, respectively), and the [0,1]

oligomer was identified in one sample with a concentration equal to the method quantification limit, i.e. 10 ng/kg. The other three congeners (Table 1) were never detected.

***Table 1**. List of analytes and monitored ions*

Analyte	Formula	Precursor ion (*m/z*)	Fragment ion 1 (*m/z*)	Fragment ion 2 (*m/z*)
ClPFPECA-0,1	C8HClF14O4	366.9401	200.9547	184.9843
ClPFPECA-1,1	C10HClF18O5	482.9286	200.9547	316.9421
ClPFPECA-0,2	C11HClF20O5	532.9254	200.9547	366.9401
ClPFPECA-1,2	C13HClF24O6	648.9139	200.9547	482.9286
ClPFPECA-0,3	C14HClF26O6	698.9107	200.9547	532.9254
ClPFPECA-0,4	C17HClF32O7	864.8961	366.9385	200.9531

Conclusions

First of all the results highlight that ClPFPECA were detected, even at low levels, only in home-produced eggs rather than in commercial ones. This evidence confirms the observation that long-chain carboxylated compounds, such as ClPFPECA, with large soil adsorption capacity, can accumulate in private chicken enclosure soil [6]. Second, the presence of three out of six ClPFPECA, which are the [0,1], [1,1], and [0,2] congeners, demonstrates the widespread distribution of this emerging PFAS class with the samples being collected in rural areas with small industrial activities and far away from fluorochemical plants. As already reported, these lighter oligomers (Table 1) have a wider diffusion throughout the environment compared to the others [3].

Nevertheless, it is important to underline that the maximum limits allowed for the four regulated PFAS in eggs [4] are one or two orders of magnitude higher than the ClPFPECA concentrations reported herein.

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P12

Analysis of perfluoralkyl substances (PFAS) in meat, edible offal and eggs using Xevo TQ-XS

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Summary: *Analysis of PFAS in food sources such as meat, edible offal and eggs require a sample extraction and clean up using Weak Anion Exchange (WAX) SPE. High sensitivity LC-MS/MS analysis is performed on an Acquity UPLC I-Class Plus coupled to Xevo TQ-XS [1].*

Keywords: *PFAS, food*

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P13

Development of a direct mass-spectrometry approach for the real-time monitoring of PFAS release from cookware and food contact materials

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Summary: *The rising attention dedicated to per- and polyfluorinated alkyl substances (PFAS) highlights the importance of analytical techniques allowing to determine their release from food contact materials and cookware. A Condensed Phase Membrane Introduction Mass Spectrometry approach is currently under development allowing the real-time monitoring of PFAS migration without sample preparation.*

Keywords: condensed phase membrane introduction mass spectrometry*, food safety,* per- and polyfluorinated alkyl substances

Introduction

Mass spectrometry (MS) techniques are widely used in the food safety field, thanks to their high versatility and continuous instrumental advancements. One of the most relevant topics is the determination of per- and polyfluorinated alkyl substances (PFASs) in food related samples, being a class of compounds widely used in industry due to their unique chemical attributes such as high thermal and chemical stability, high polarity, and water/lipid resistance. On the other hand, PFASs are long-term persistent compounds and prone to bio-accumulate in the environment, consequently there is a high risk of human exposure to PFASs through the food chain [1]. In 2020 EFSA set a tolerable weekly intake (TWI) of 4.4 ng/kg body weight per week for four of them [1], consequently EU Regulation 2023/915 established maximum concentration of some PFASs (at µg/kg level) in food products [2]. Food contamination can occur not only during the manufacturing process, but can also be caused by migration from food contact materials (FCMs), such as kitchenware. In this context, several anti-stick coatings used for cookware and tableware rely their water- and oil-repellent properties on PFASs, which can be potentially released into food. Currently, there is no harmonized regulation of PFASs in FCMs, however PFASs are gaining increased regulatory attention. Conventional analytical methods for the determination of PFASs are based on liquid chromatography-mass spectrometry technique (LC-MS); despite their excellent analytical performances, LC-MS methods are usually time-consuming, require significant amounts of solvents, and are not suitable for real-time analysis. A promising technique called condensed phase membrane introduction mass spectrometry (CP-MIMS) was recently introduced to fill this gap; it is a direct MS technique that eliminates the need for sample preparation and chromatographic separation. CP-MIMS uses a semi-permeable membrane as the interface between the sample and the mass spectrometer. Only compounds with suitable chemical-physical properties pass through the hollow tubular membrane, within which an 'acceptor' phase (AP) flows continuously, transporting them to the ion source for MS detection [3], whereas salts and macromolecules are excluded. CP-MIMS is suitable for direct monitoring of dynamic processes in unprocessed matrices. The present work aimed, for the first time, to expand the applicability of CP-MIMS for the determination of PFASs, characterized by high polarity, and real time monitoring of their release from FCMs.

Experimental

The CP-MIMS system used for this application consists of three parts: the pumping system for the acceptor phase, the CP-MIMS probe and the mass spectrometer. The pumping system was an HPLC Dionex UltiMate 3000 SD series (Thermo Scientific). The CP-MIMS interface was a hand-made probe created in our lab consisting of a modified Viper® Fingertight (Thermo Scientific) having the two ends connected by a PDMS hollow fibre membrane. The mass spectrometer was an LTQ XL MS (Thermo Scientific) with an electrospray (ESI) source operated in negative tandem mass spectrometry (MS/MS), acquiring the signal by product ion scan mode. A magnetic stirrer hotplate was used to control the temperature and the stirring of the sample.
Three compounds were investigated: heptafluorobutyric acid (C4-PFAS), perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), both in water and simulant B (acetic acid 3% w/v). The CP-MIMS output is a chronogram where the time dependent MS signal changes in response to a variation in analyte concentration in the sample. In particular, the signal is first collected with the membrane immersed in methanol (storage solvent); after that, the probe is immersed in the sample (donor phase) containing PFASs. After few seconds the signal increases until reaching a stable intensity (steady state) which is related to the analyte concentration. Signal is collected for some minutes and the probe is extracted, rinsed with water, and stored in methanol.

Results

Preliminary results will be presented dealing with the development of CP-MIMS methods for PFASs determination. Since CP-MIMS has not yet been applied for PFASs analysis, several preliminary experiments were necessary to define the critical experimental factors and the corresponding domain. Several parameters affect the performance of the method, as AP flow, AP composition, membrane length, sample pH and sample temperature and stirring. An experimental design approach is necessary to find the optimal experimental conditions to maximize signal intensity and stability and minimize noise. In particular, sample pH and AP composition resulted critical factors influencing the PFASs permeation across the membrane. Various AP compositions have been investigated including methanol, methanol with ammonium hydroxide, methanol/ethyl acetate, methanol/heptane, water/acetonitrile, and water/methanol. Best results were obtained with water/methanol, in line with the hydrophilic characteristics of PFAS; this hypothesis was supported by the absence of signal observed in presence of swelling agents like ethyl acetate or heptane, that are useful to boost the response for less hydrophilic compounds. Other experiments were carried out to study AP flow, sample temperature and hydrochloric acid addition to the sample, with the latter parameter strongly improving the response. Based on preliminary experiments described above it was possible to define the following experimental domain: AP flow, 40-70 µl/min; AP composition, 5-25% v/v water in methanol; membrane length, 1-3 cm; hydrochloric acid (38%) in the sample, 0-2 mL; sample temperature, 25-70°C; sample stirring, 200-800 rpm. This experimental domain will be evaluated using a full factorial design of experiments whose analysis are currently ongoing.

Conclusions

The suitability of CP-MIMS technique was proved for the analysis of C4-PFAS, PFOA, and PFOS, selected as model compounds of the PFASs. After the identification of critical experimental factors and definition of the corresponding domain, the method is currently under optimization using a full factorial design of experiments. The optimized procedure will allow the evaluation of PFASs release from FCMs into food simulant or liquid food matrices, acquiring the signal in real time during migration tests. This strategy will expand the applicability of the CP-MIMS technique and will deepen knowledge about PFASs migration from FCMs.

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P14

Ultra-sensitive PFAS analysis according to EU regulations in food and environment

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Keywords: PFAS, high throughput, triple quadrupole, regulation, robust

PFAS are highly present in the public and pose a threat to mankind and nature. Manufactured since the 1940s as water and grease repellents in consumer products, they are considered “forever chemicals”. With their persistent, bio-accumulative, toxic (PBT) properties and ubiquitous presence in the environment and organisms, there is mounting evidence that exposure to PFAS may generate adverse health effects. EPA regulations exist for the routine monitoring of certain PFAS in water and food with legal limits in sub-ppt range for the individual compound. Reaching these limits in a routine and robust manner is a challenge even for triple quadrupole systems of highest performance. Presented is a method for the routine targeted analysis by a novel, fast, robust and highly sensitive triple quadrupole which covers and exceeds the current EPA regulations for reliable and confident testing of water and food samples.
The standard PFAS list for the EPA regulation 533 was used for the evaluation of the method performance. An Elute HT UHPLC was coupled to an EVOQ DART-TQ+ (both Bruker, Bremen, Germany). The total runtime of the method incl. equilibration was 13.2 min with a gradient of 2 mM ammonium acetate in water and methanol. The columns were an Intensity Solo 2.0 100 x 2.0 mm (Bruker and a delay column 50 x 2.0 mm (Restek). The TQ was operated in both polarities with source parameters and MRM transitions optimized. The scan speed was automatically calculated per compound with a minimum number of 12 spectra per peak and a dynamic window of 0.6 min per analyte.
Mobile phase compositions were optimized, different gradients and flow rate tested, and the equilibration time at the beginning of the injection to avoid accumulation. Typical LOQ reached for all PFAS of the EPA 533 standard list were in the range of 0.1 - 0.5 ppt. Linearities had typical values for $R^2 \geq 0.999$. The system shows a high robustness with much reduced contamination and crosstalk for multiple injections. The method represents a robust, highly sensitive and fast analysis of PFAS according to and exceeding the current EU regulations.

P15

Determination of phthalates in bottled water and purifier water by SPME-GC/MS

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Summary: *Our work aimed to determine the concentrations of phthalates released from plastic bottles in natural water and from water purifiers in drinking water, through solid-phase microextraction (SPME) coupled with GC/MS. Phthalates are synthetic chemicals widely used, mainly as plasticisers, recognised as endocrine disruptors.*

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

Introduction

Food and drinks come into contact with many materials and objects during the respective phases of production, transformation, conservation, preparation and administration before their final consumption. These materials are the so-called Materials and Objects intended for Food Contact (FCM) and include packaging, plastic bottles, plastic films, paper or aluminium foil, containers used for transporting food and kitchen utensils but also all the materials that are used in food processing equipment and those in contact with drinks [1, 2].
A material intended for contact with food is safe when it does not give rise to unwanted migration phenomena or does not release potentially dangerous substances in food [3,4].
Although packaging materials contribute greatly to protecting various kinds of food, preserving their quality and extending their shelf life, the prolonged contact between the plastic container and the food often induces contamination of the latter by migration products [5]. Among the plastic components and additives likely to migrate from the container to the beverage or food content, phthalates (PAEs) have attracted considerable attention because of their potential hazard to human health [6]. PAEs are industrially obtained by double esterification of phthalic acid with different compounds, mainly alcohols lead to the formation of di-methyl phthalate (DMP), di-ethyl phthalate (DEP), di-butyl phthalate (DBP), di-ethylhexyl phthalate (DEHP), Butyl benzyl phthalate (BBP), Di-isononyl phthalate (DINP) and Di-isodecyl phthalate (DIDP) the most used in the food industry as plastic constituents [7]. Phthalates used mainly as plasticizers, are ubiquitous and recognized as endocrine disruptors. According to the WHO (World Health Organization) definition, “endocrine disruptors” [8] are molecules that alter the functionality of the endocrine system causing adverse effects on the health of an organism”.
The tolerable daily intake (TDI) values established by the European Food Safety Authority (EFSA) for the phthalates DBP, BBP, DEHP and the phthalate DINP the values are 50 µg/kg of weight per day, while for the DIDP are 150 µg/kg of weight per day.
The objective of this study was to determine, through the use of SPME-GC/MS, endocrine disruptors, with particular attention to phthalates released from PET (polyethylene terephthalate) bottles in natural waters and from purifiers today increasingly used in domestic use, in drinking waters.

Experimental

Water samples from both plastic bottles and purifiers were extracted using SPME solid-phase microextraction and the analyses were carried out using a gas chromatography GC (Thermo Scientific, TRACE 1310) interfaced with a mass spectrometer (Thermo, TSQ 8000 triple quadruple MS).
Although phthalates are quite volatile and thermally stable, their extraction has been carried out using the direct-immersion mode (DI-SPME) using a three-phase fibre DVB/CAR/PDMS (Divinylbenzene/Carboxen/ Polydimethylsiloxane. Aliquots of 10 ml of water samples were placed in a 40 ml vial and an internal standard (Bisphenol A) of 300 ppb was added to each sample. The SPME needle was introduced through the septum and the the fiber was immersed directly in the aqueous phase. The analytes were extracted for 30 min at 25 ◦C with continuous magnetic stirring. After extraction, the fiber was retracted into the holder syringe needle, removed from the vial, and the SPME needle was manually introduced into the GC injection port [6]. Samples were analysed in triplicate.

Results

We analyzed the phthalates released from plastic bottles of different brands. Using SPME solid-phase microextraction and GC/MS analysis, we identified and quantified the following phthalates: DEP, DIBP, butyl octyl phthalate, DBP, and DEHP. The total concentration of phthalates released from PET bottles is different in the various samples (from 62.6 ppb in sample B1 to 148.2 ppb in sample B2).

Furthermore, we decided to expose two plastic water bottles to sunlight for several months to study the behaviour of phthalates and any other substances harmful to human health released into the water. We observed that the total phthalate concentration increased exponentially in both samples over time. This is a preliminary study to understand the release of PAEs until the expiration date reported in the bottles.
Finally, we analyzed the water from various purifiers, today increasingly used in domestic use, which are characterized by filters and other plastic components and therefore could release any PAEs and other substances harmful to human health. Also, in these samples, we identified and quantified the following PAEs: DEP, DIBP, butyl octyl phthalate, DBP and DEHP. We compared drinking water directly from the tap with purified water and we observed a decrease in the total concentration of phthalates in purified water. The TDI value established by EFSA for these phthalates (50 µg/kg of weight per day), considering the total volume of drinking water (≥ 2 L), no water samples analyzed exceed this limit.

Conclusion

A very simple extraction technique such as SPME solid-phase microextraction coupled with GC/MS analysis was used to identify and quantify, by the internal standard method, five phthalates released from PET bottles into natural water and from purified water into drinking water. Phthalates are synthetic chemicals widely used, mainly as plasticizers, recognized as endocrine disruptors. Finally calculating the tolerable daily intake (TDI) values, established by EFSA for the phthalates, we can state concentrations of phthalates released from PET bottles and purifiers in water samples don't exceed the limit expected.

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P16

Clams from Sicilian transitional water zones: mineral elements and safety

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Summary: *This study aims to evaluate the concentration of mineral elements by inductively coupled plasma mass spectrometry (ICP-MS) and direct mercury analyzer (DMA-80) of three clams species collected in 2023 and 2024 in two transitional water zones in the north-east of Sicily, the lagoons of Capo Peloro and Oliveri-Tindari.*

Keywords: *clams, minerals, potentially toxic elements, food safety, transitional water zones*

Introduction

"Clams" is a popular and commercial term indicating species of great interest because they have been exploited as food since ever and are good sentinel organisms for environmental pollution. They contain high quality nutrients and bioactive compounds that have great influence on human health, including essential minerals, such as calcium and iron [1]. However, clams absorb and bioaccumulate potentially toxic elements, a category of metals, metalloids, and nonmetals, known for their persistence in the environment, toxicity, and ability to accumulate and magnify within biological systems. As a result, the intake of these elements through the diet in certain quantities is associated with a risk to human health [2]. Three edible and indigenous clam species of commercial interest, *Rudipates decussatus* (Linnaeus, 1758), *Cerastoderma glaucum* (Bruguière, 1789), and *Polititapes aureus* (Gmelin, 1791), are found in the lagoons of Capo Peloro and Oliveri-Tindari, two important transitional water zones of natural and economic interest, located in the north-east of Sicily in the province of Messina. Transitional water zones are ecotones between terrestrial, freshwater and marine ecosystems where land, fresh and salty waters mix, forming a natural transition between land and sea. The high productivity and biodiversity of these areas has led to their exploitation by humans for various purposes [3].
In fact, the economy and the tradition of the urbanized Capo Peloro lagoon revolve around bivalve aquaculture while the Oliveri-Tindari lagoon are a famous and pristine natural attraction [3].

Experimental

The three clam species, *R. decussatus*, *C. glaucum* and *P. aureu*s, were collected during the 2023 and 2024 winter seasons in both transitional water zones, Capo Peloro Lagoon and Oliveri-Tindari Lagoon, in order to investigate the content of essential (Ca, Co, Cr, Cu, Fe, Mg, Mn, Na, Se, Zn), toxic (As, Cd, Hg, Pb) and potentially toxic elements (Al, Li, Ni, Sn, Sr, Tl). The analyses were carried out by inductively coupled plasma mass spectrometry (ICP-MS) and direct mercury analyzer (DMA-80). The aims were: i) to highlight the differences between clams collected in two different years and from two different transitional areas, and ii) to evaluate the safety of the commercialized clams from Capo Peloro lagoon.

Results

Among the macro elements, Na (752.31-1848.35 mg/kg) was present in the highest concentration followed by Ca (514.82-1167.28 mg/kg) and Mg (135.05-327.06 mg/kg). Fe (52.05-114.98 mg/kg) and Zn (7.99-16.40 mg/kg) were the essential trace elements present at the highest concentration. Among toxic and potentially toxic elements, those with higher concentrations followed this trend: Al (31.06-10.44 mg/kg) > As (3.04-0.77 mg/kg) > Sr (3.55-1.14 mg/kg).
The clams from Capo Peloro collected in 2023 had a slightly higher mineral content than those collected in 2024, while the clams from Oliveri- Tindari had a slightly lower mineral content than those collected in 2024. The clams from Capo Peloro collected in 2023 showed slight differences from the Oliveri-Tindari clams collected in the same year. In fact, clams from Capo Peloro had a higher content of As and Ca and a lower content of Mn and, only for *C. glaucum*, Na. Clams from Capo Peloro collected in 2024 had a lower content of minerals (especially Al, Fe, Mg, Mn, Na, Zn) than those from Oliveri-Tindari collected in the same year. However, despite the trend of the other minerals, the As content remains highest in Capo Peloro even in 2024. In all clam species analyzed, the concentration of Hg, Pd and Cd were always below the limits fixed by law, but no law indication is given for other toxic and potentially toxic elements [3].

Moreover, in order to evaluate the safety of clams, the Estimated Daily Intakes (EDIs) of minerals related to the consumption of clams (5.08 kg/capita/year, FAO [4]) were compared with the available values of Recommended Dietary Allowance (RDA) or Adequate Intake (AI) for essential elements and Tolerable Daily Intake (TDI), Tolerable Weekly Intake (TWI), Benchmark Dose Lower Confidence Limit (BMDL01), Reference Dose (RfD) for toxic and potentially toxic elements [5]. The results, expressed as a percentage of these values, showed that the consumption of clams covers about the 10% of Ca RDA and the 40% of Na AI. Moreover, the consumption of the commercialized clam species from the lagoon of Capo Peloro is safe for adults, since the consumption of any sample does not exceed the reference values.

Conclusions

The slight differences in mineral content between clam species collected in two different years and zones probably depend on diet and climatic and environmental fluctuations and are of no concern. The concentrations of toxic and potentially toxic elements are safe for the consumer. However, given the constant change and the anthropization of the transitional water zones, it is necessary to frequently monitor the mineral elements in these foods in order to always ensure the health of the consumers.

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P17

Fingerprinting of green and roasted coffee (*Parainema* and *Obata*) volatile organic compounds (VOCs): HS-GC-IMS and GC-MS

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Summary: *VOCs fingerprinting of wet- or dry-processed coffee samples from 10 different Honduran producers was obtained comparing SPME-GC-MS with HS-GC-IMS. Both analytical approaches permitted the clustering of the samples, leading information about aroma precursors. Moreover, HS-GC-IMS permitted the direct rapid analysis of green beans in vials, reducing sample pretreatment.*

Keywords: *Green coffee, Roasted coffee, VOCs, HS-GC-IMS, GC-MS*

Introduction

Once coffee cherries have been picked, they must be processed before being transported and sold. Processing should take place as soon as possible to prevent spoilage, and is typically done in one of two ways: washed or natural. Processing has a significant impact on the final cup profile, affecting everything from acidity and sweetness to body and clarity. The processing method used by producers usually depends on a number of factors, including resources, climate, and cost [1]. A coffee cherry is made up of various layers, including skin, fruit, mucilage, and parchment. Once the cherries have been harvested, they need to be processed to remove these layers, so as to be left with only the coffee bean.
One of the most widely used processing methods is “wet processing”. This method involves removing the skin of the cherries before submerging them in a trough of water to break down and remove the mucilage.
This can sometimes take up to 24 hours, allowing time for tiny microorganisms in the beans to create enzymes that break down the sticky outer layer. After fermentation, the beans are washed and dried, either under the sun or using dedicated drying machines (or sometimes a combination of the two). The result tends to be a coffee with high clarity, light body, and prominent acidity.
In contrast, natural processing is a method that has been used for centuries. Also known as “dry” processing, it involves spreading the harvested cherries out on a large surface to dry for several weeks with the fruit and skin intact. To avoid the build-up of mould and over-fermentation, they are regularly raked and turned. When the cherries reach a moisture level below 11%, the brittle outer layer is removed, and the bean within is kept for milling and sale.
Few data are available for green coffee VOCs analysis by HS-GC-IMS, particularly regarding the evaluation of VOCs related to the processing [2]. Main aim of this work was to evaluate the volatile fraction of different wet- or dry-processed coffee beans, directly analysed (head space) with HS-GC-IMS as well as sampled by headspace-solid phase microextraction (GC-MS).

Experimental

Samples: Fifteen different green wet- or dry-processed coffee samples (*Parainema* and *Obata*) from ten different Honduran producers were supplied by Andrej Godina. The same set of samples was subsequently roasted under controlled conditions in order to standardize the process.
HS-GC-IMS: 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 2.0 g of the sample. Then samples were treated for 5 minutes at 50 °C at 500 rpm. Then, in splitless mode, a 300 µL headspace sample was automatically delivered through a 70 °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 3 minutes, followed by a 17-minute rise to 25 mL/min and a 5-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.
SPME-GC-MS: the extraction of volatiles was performed with manual 50/30um DVB/CAR/PDMS SPME device (Supelco, Bellefonte, PA, USA) at 40°C for 10 minutes, after a 10 minutes equilibration time. The analysis was performed on a GCMS-QP2020 NX system (Shimadzu Co., Ltd., Tokyo, Japan), using 99.9995 pure helium as carrier gas. The fibre was desorbed in the injector of the GC in splitless mode at 250 °C. The separation was performed on a SH-I-5MS capillary column (30m x 0.25mm x 0.25um, Shimadzu Co., Ltd., Tokyo, Japan). The GC oven temperature was programmed as follows: 50°C held for 2 minutes, increased to

220 °C at a rate of 10 °C/min, held for 5 minutes. A constant flow of 1,5 ml/min was used. The volatile compounds were tentatively identified by comparing the spectral data obtained with the NIST database.

Results

The analysis of volatile profile of green coffee is of great interest, particularly regarding the evaluation of the fermentation process. The aim of this work was to establish (and compare) the usefulness of two hyphenated analytical techniques applied to fingerprint green coffee volatile compounds, evaluating the capacity to cluster specific samples subjected to different processing (wet and dry).

Moreover, the direct rapid analysis of green beans by HS-GC-IMS (here proposed as screening rapid method, compared to GC-MS) was functional to clearly recognize clusters of samples. HS-GC-IMS provided 2D chromatogram useful to quickly obtain very clear 2D patterns, avoiding any kind of sample pre-analytical handlings and processing. The main differences are found in the dry processed samples 183 and 554. They present a profile with a higher number of volatile compounds. The remaining 13 samples generally showed a similar profile, as the number 606 (Figure 1; left image).

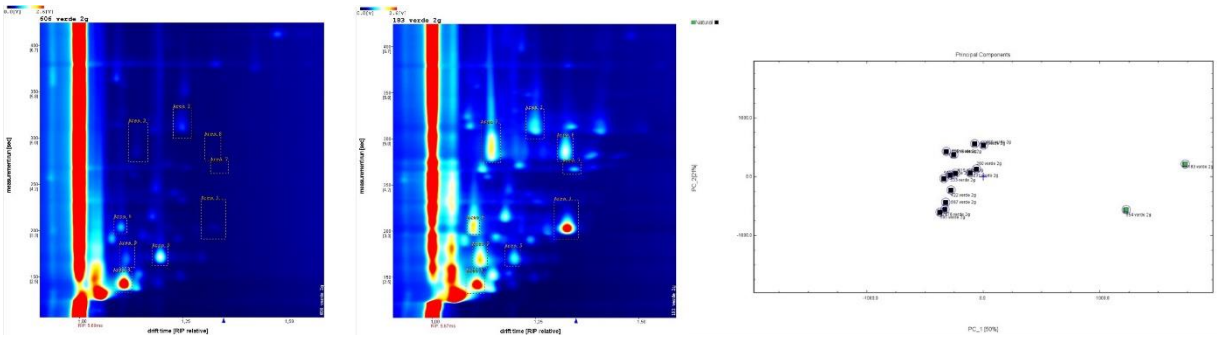

***Figure 1**. Fingerprint of two green coffee samples. Left (**wet** processing); center (**dry** processing). PCA (right) confirms how the two samples (green) subjected to the "dry" process cluster together.*

Substantially, even on the same set of roasted samples, the behavior detected is similar (data not shown for space reasons). The dry processed samples cluster compared to those obtained with wet processing. The latter, however, appeared less similar after the roasting process and their clustering is less evident.

Conclusions

These approaches emphasize the usefulness of the hyphenated multi-platform approach as analytical tool, preliminary to data mining. Both HS-GC-IMS and GC-MS analysis allowed to identify specific clusters of samples of known geographical origin, also permitting some considerations regarding the precursor of aroma. More particularly, rapid analysis using HS-GC-IMS reduced the time of the analysis, permitting the direct analysis of beans in vials without preliminary SPME extraction, opening new perspectives for green and/or roasted coffee quality control.

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P18

Effect of fermentation type on VOCs profile of table olives: a case study on 'Bella di Cerignola' cultivar using PTR-MS

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Summary: *Table olives are among the most popular fermented vegetables globally. This study examines the effect of fermentation on VOCs profile of "Bella di Cerignola" (Bella della Daunia PDO) olives using PTR-ToF-MS. The results reveal the key role of the fermentation method, while other productive parameters have a lesser impact on flavour.*

Keywords: *PTR-ToF-MS, VOCs,table olives, fermentation process*

Introduction

Table olives are one of the most popular fermented vegetables worldwide, with Spain leading in production, followed by Italy and, to a lesser extent, other Mediterranean countries such as Greece, Algeria, Turkey, Egypt, and Portugal. According to the International Olive Oil Council (IOOC)[1] there are four main traditional methods for producing table olives: *(i)* the Spanish style, *(ii)* the natural or Greek style, *(iii)* the Californian style, and *(iv)* other methods based on local practices.[2] Various factors significantly influence the organoleptic characteristics of the final product,[3] including NaCl content, processing temperature, and the origin of the olives, which affects the dominant microbiota during the fermentation.[4] The present study focuses on the characterization of volatile organic compounds (VOCs) in table olives of different sizes from the cultivar "Bella di Cerignola." This cultivar is widely available in the Italian market and received protected designation of origin (PDO) status in 2000 under the full name "Bella della Daunia olives variety Bella di Cerignola" (Regulation EC No 1904/2000). A proton transfer-reaction time-of-flight mass spectrometer (PTR-ToF-MS)[5] was used to screen VOCs in both olive fruit and brine samples. The aim was to demonstrate the potential of this technique to discriminate between different fermentation modes and to identify which compounds are involved in the production process.

Experimental

Plant material

Olives used in our study belong to Apulia variety of "Bella di Cerignola", of two different sizes (G = 71-80 pieces/kg, M = 91-120/121-180 pieces/kg) were subjected to two fermentation modes: SIV (*Sivigliano*, Spanish style) and NAT (*Naturale*, Natural style) (Table 1). The samples, named 1, 2, 3 and 4 (Tab. 1), were triturated and mixed with an antioxidant solution consisting of 0.25 g citric acid, 0.25 g ascorbic acid, 50 g water, and 20 g NaCl. Then, 3 g for table olives and 3 ml for brines were, separately, poured into 20 mL vials and closed with a screw cap with a silicon/PTFE septum. After being stored at 4 °C overnight, they were analyzed in triplicate to determine the volatile profile of the matrices and their respective brines using PTR-ToF-MS and GC-MS.

Table 1. *- Description of table olives (1, 2, 3 and 4) and respective brines (H1, H2, H3 and H4) under evaluation.*

Sample name	Matrix	*Size (pieces/kg)*	Fermentation mode
1	*olive fruit*	*M*	*SIV*
H1	*brine*	-	*SIV*
2	*olive fruit*	G	*SIV*
H2	*brine*	-	*SIV*
3	*olive fruit*	*M*	NAT
H3	*brine*	-	NAT
4	*olive fruit*	G	NAT
H4	*brine*	-	NAT

PTR-ToF-MS and data analysis

A multifunctional autosampler (Gerstel, Mülheim an der Ruhr, Germany) was loaded with three replicates for samples. Measurements of VOCs were performed in six technical replicates with a commercial PTR-ToF–MS 8000 apparatus (Ionicon Analytik GmbH, Innsbruck, Austria). PTR-ToF-MS was used in its standard configuration (V mode). The ionization conditions in the drift tube involved 110 °C drift tube temperature, 2.30 mbar drift pressure, 550 V drift voltage and and H_3O^+ as reagent ion. This led to an E/N ratio of about 140 Townsend (1 Td = 10−17 cm2 V−1 s−1) where E corresponds to the electric field strength and N to the gas number density. The inlet line consisted of a PEEK capillary tube (internal diameter 0.04 inches) heated at 110 °C. The inlet flow was set at 40 sccm. The device acquired one spectrum per second.

A multifunctional autosampler (Gerstel, Mülheim an der Ruhr, Germany) was loaded with three replicates of samples prepared by putting 3 g into 20 mL vials and closed with a screw cap with a silicon/PTFE septum. Measurements of VOCs were performed in six technical replicates with a commercial PTR-ToF–MS 8000 apparatus (Ionicon Analytik GmbH, Innsbruck, Austria). PTR-ToF-MS was used in its standard configuration (V mode). The ionization conditions in the drift tube involved 110 °C drift tube temperature, 2.30 mbar drift pressure, 550 V drift voltage and H3O+ as reagent ion. This led to an E/N ratio of about 140 Townsend (1 Td = 10−17 cm2 V−1 s−1) where E corresponds to the electric field strength and N to the gas number density. The inlet line consisted of a PEEK capillary tube (internal diameter 0.04 inches) heated at 110 °C. The inlet flow was set at 40 sccm. The device acquired one spectrum per second.

Results

The analysis performed on the PTR-ToF-MS mass spectra of the headspace of samples under study allowed the extraction of 296 mass peaks, from which were selected 167 mass peaks as significantly different from the blank. The PCA plot shows that the first two principal components, PC1 and PC2, together account for approximately 74% of the total variance in the dataset (Figure 1). The plot clearly separates the samples based on their fermentation methods, indicated by the shape of the points. Circular markers denote samples fermented using the SIV method, while triangular markers represent samples from NAT fermentation method. The separation is evident along the PC1 axis. A colour gradient approach was applied to connect every olive fruit and the respective brine; for example, 1 and H1 share the same hue, but H1 appears in a lighter/darker shade. Larger-sized olives (G) and medium-sized olives (M) are represented by symbols with different diameters. However, the size does not seem to contribute significantly to the variance explained by PC1 or PC2, suggesting that the fermentation method has a stronger influence on the volatile profile than olive size. Consequently, to better investigate the relationship between samples and VOCs, it was assigned each mass peaks to specific molecules, considering particularly on that already published tentative identifications[8] or based on GC-MS data experimentally produced on the sample.

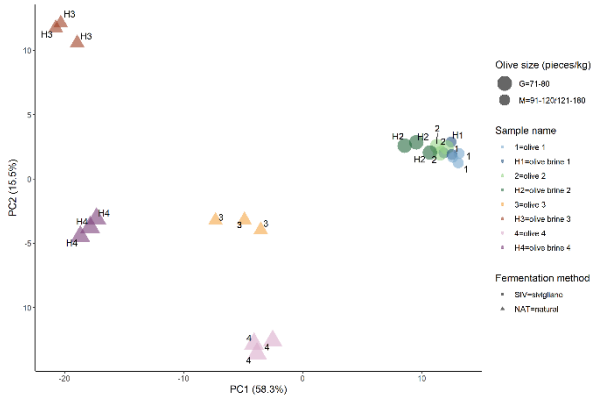

Figure 1. *Principal Component Analysis (PCA) of olive and corresponding olive brine samples (Tab. 1) based on volatile organic compounds (VOCs). The plot displays the first two principal components.*

Conclusions

The data demonstrate that the fermentation method is a dominant factor in the formation of olive flavour, overshadowing other productive parameters as olive size. The choice of fermentation technique significantly alters the volatile compound composition. The rapid, high sensitivity and non-invasive PTR-MS is a valuable tool to support the optimisation of the industrial production protocol in vegetable fermented products.

This work is supported by the projects ‘iNEST’, ‘ONFOODS’, and ‘SUS-MIRRI.IT’ [Italian National Recovery and Resilience Plan (NRRP) projects financed by the European Commission’s Next Generation EU programme].

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P19

Evaluating the effect of different cooking methods on polycyclic aromatic hydrocarbons formation in hamburger samples by means of GC-MS/MS

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Summary: *In this study the formation of 4 polycyclic aromatic hydrocarbons (Benzo[a]pyrene, Benzo[b]fluoranthene, Benz[a]anthracene and Crysene) was evaluated by analyzing samples of bovine and chicken hamburger after 6 different types of cooking treatment. The analytical determinations were obtained by means of validated GC-MS/MS analytical method. The study demonstrated that high temperatures and use of oil during cooking can cause slight formation of 3 polycyclic aromatic hydrocarbons.*

Keywords: *GC-MS, Meat safety, Polycyclic aromatic hydrocarbons*

Introduction

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations discourage high consumption of cooked meat, especially red meat. This suggestion is due to some toxic effects on humans, such as cardiovascular diseases and cancer risks that some chemical compounds that can be present especially after heating processes may exercise. On the other hand, meat consumption is increasing over the years, especially in developing countries, due to its high nutritional value (essential amino acids, bioactive compounds and micronutrients such as iron, zinc and vitamins B3 and B12). The heating process is a key phase for meat, since it makes foods microbiologically safe to consume and palatable. However, heating processes can lead to the formation of some harmful compounds, also known as “Processing contaminants”, such as heterocyclic amines, nitrosamines, acrylamide and polycyclic aromatic hydrocarbon (PAHs). The levels of such compounds in cooked meat rely to the specific heating treatment and to meat type [1]. Regarding PAHs, the maximum residue levels for the sum of 4 PAHs (Benzo[a]pyrene (BaP), Benzo[b]fluoranthene (BbF), Benz[a]anthracene (BaA) and Crysene (Cry)) and for Benzo[a]pyrene (BaP) alone, in several food products, have been established in Europe in the Regulation No. 915/2023/EC. Consequently, the occurrence of these classes of compounds in meat has been investigated during the last few years. However, the specific relationship between meat type, cooking method and PAHs formation is still a topic to be investigated, since few studies are available so far [1]. In this study, the formation of 4 PAHs (BaP, BbF, BaA and Cry) was evaluated by analysing samples of heifer and chicken hamburger after 6 different types of cooking treatment. The analytical determinations were obtained by means of validated GC/MS analytical method.

Experimental

Two kinds of burger samples were collected on the market: heifer and chicken. The samples were composed of meat, salt and pepper. Six different cooking techniques were compared: frying (sunflower oil) (T=180 °C; t=5’), frying (EVO oil) (T=180 °C; t=5’), baking (T=180 °C; t= 20’), grilling (T=200 °C; t=15’), electric grilling (T=200 °C; t=25’) and air frying (T=200 °C; t= 10’). Each sample was cooked twice and the results were expressed as the mean of two measurements. The samples, both raw and cooked, were analysed by using an optimised analytical method based QuEChERs with dispersive solid phase extraction, coupled to a double extraction in acetonitrile and the addition of a freezing step. The quantifications of analytes were obtained by using a GC-MS/MS system (Agilent 7000D). The method was previously validated, evaluating the following parameters: specificity, linearity, precision, recovery and robustness. The matrix extract was used for calibration in order to compensate for matrix effect [2].

Results

No PAHs residue was detected in raw samples (<LOQ). A slight formation of 3 PAHs (BaA, BaP and Cry) was verified in both types of burger samples, while BbF was never detected (< LOQ: 0.1 μg/kg) (Table 1). Taking into account the legal limits set in Europe (2 and 12 μg/kg for BaP and sum of 4 PAHs, respectively) the concentrations resulted quite low, below 0.2 μg/kg for each PAH. No significant differences were registered for two types of burger analysed, with higher levels quantified when using frying with EVO oil (sum of 4 PAHs: 0.400 and 0.404 μg/kg for heifer and chicken burger, respectively), followed by frying with sunflower oil and baking which resulted in comparable levels (sum of 4 PAHs in the range 0.217 – 0.271 μg/kg). Slightly lower concentrations were detected in samples cooked by grilling and electric grilling with lowest concentrations detected in samples cooked by air frying (sum of 4 PAHs ≤ 0.086 μg/kg). In Figure 1, a chromatogram example

where Cry was detected after cooking is shown.

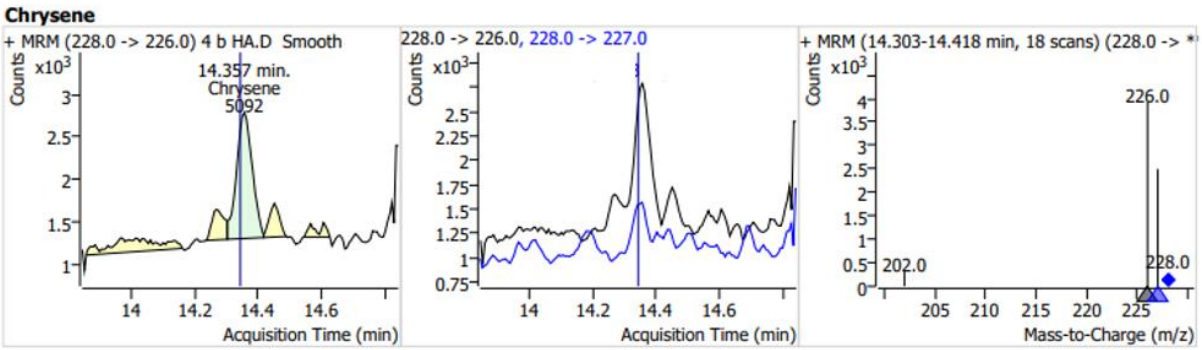

Figure 1 *- Example of Chrysene formation after frying a heifer burger using EVO oil (concentration: 0.027 μg/kg)*

Table 1 *- Results obtained by analysing burger samples after different cooking techniques (Results as μg/kg, mean of two measurements)*

SAMPLE TYPE	***COOKING TECHNIQUE***	**BaA***	**BaP***	**BBF***	**CHRY****	**Sum**
Heifer burger	*Frying (sunflower oil)*	0.171	0.100	<LOQ	<LOQ	0.271
	Frying (EVO oil)	0.175	0.199	<LOQ	0.027	0.400
	Baking	0.168	0.100	<LOQ	<LOQ	0.268
	Grilling	0.171	0.101	<LOQ	<LOQ	0.272
	Electric grilling	<LOQ	0.101	<LOQ	<LOQ	0.101
	Air frying	0.086	<LOQ	<LOQ	<LOQ	0.086
Chicken burger	*Frying (sunflower oil)*	0.182	<LOQ	<LOQ	0.039	0.220
	Frying (EVO oil)	0.177	0.200	<LOQ	0.028	0.404
	Baking	0.089	0.100	<LOQ	0.029	0.217
	Grilling	<LOQ	0.101	<LOQ	<LOQ	0.101
	Electric grilling	<LOQ	0.101	<LOQ	0.022	0.123
	Air frying	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

*** LOQ = 0.1 μg/kg**
**** LOQ = 0.02 μg/kg**

Conclusions

The results of this study confirmed that meat cooking can lead to the formation of low levels of BaP, BaA and Cry. No differences were observed between two types of burger tests (heifer and chicken). The use of oil during frying seems to slightly enhance PAHs formation. This result can be due to the close relationship between PAHs formation and fat presence in the samples. In conclusion, cooking methods which do not use oil and obtained at as lower temperature as possible seem preferable under a food safety point of view.

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P20

Phenols and VOCs fingerprint as a reliable tool for craft beer classification based on wheat origin

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Summary: *36 craft beers obtained by using different wheat varieties were analyzed by LC-MS/MS and HS-SPME combined with GC-MS. Both phenols and VOCs fingerprint proved to be a reliable tool for beers classification according to durum wheat yield.*

Keywords: *Beer, LC-MS/MS, HS-SPME-GC/MS*

Introduction

The origin of food, including species, variety, cultivation area, and potentially the production system, is the primary focus of food authentication and classification processes. In the case of wheat, the origin, defined by a combination of species, variety, and cultivation site, significantly influences the volatile profile of wheat kernels. The cultivation site's impact may also be linked to the altitude at which the wheat is grown. Profiling secondary metabolites, such as volatile organic compounds (VOCs), using high-throughput analytical techniques like gas chromatography coupled with mass spectrometry (GC–MS), is one of the most common methods for food identification and authentication.[1] This approach is popular in food authentication because volatile compounds play a key role in the flavour and aroma of foods, including craft beers. Beer is the second most consumed alcoholic beverage in Europe, making up 37% of total alcohol consumption in the EU, according to the European Spirits Organization. A deeper understanding of how wheat origin and yield influences the volatile organic compounds (VOCs) profile in craft wheat beer is essential for enhancing its quality and increasing the value of local products. In addition to VOCs, wheat could influence also the polyphenolic composition of craft beer.[2] Approximately 70–80% of the total polyphenol content in beer is derived from malt, with the remaining 20–30% coming from hops.[3] The aim of this study was to evaluate and classify craft wheat beer made from different durum wheat varieties at high and low yield, using a combined approach based on liquid chromatography with electrospray ionization hybrid linear ion trap quadrupole Orbitrap mass spectrometry and Solid Phase Micro Extraction followed with GC–MS (SPME/GC–MS).

Experimental

Beer polyphenols were analyzed through an LC-MS/MS method. Experiments were carried out using an HPLC system coupled to a LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany). Separation was achieved using a 150×4.6 mm i.d., 3.0 µm, 100 Å revered-phase Luna C18 (2) column (Phenomenex, Torrance, CA, USA), by using water (solvent A) and acetonitrile + 0.1% formic acid (solvent B), as mobile phases. The analysis of volatile organic compounds was carried out using solid-phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC–MS). A PDMS fiber (100 µm, Supelco-Italy) was exposed to the headspace, in order to adsorb the volatilized analytes. The SPME conditions were optimized by Response Surface Methodology.[4] The extracted analytes were desorbed into the injection port of the GC/MS system for 2 min at 260°C. GC/MS analyses were performed on an Agilent 8890 gas chromatograph coupled to an Agilent 5977B mass spectrometer, equipped with a single quadrupole analyzer. The chromatographic separation was carried out on (5%-Phenyl)-methylpolysiloxane Agilent HP-5MS UI capillary column-–30 m x 0.250 mm i.d. x 0.25 µm film thickness (Agilent Technologies, Santa Clara, CA, USA). A deactivated glass liner 105 × 8.0 × 0.75 mm (Supelco, Milan, Italy) was installed in the injector which was used in splitless mode. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}.

Results

36 craft beers obtained by using different durum wheat at high and low yield were analyzed by LC-MS/MS and HS-SPME combined with GC-MS. A comprehensive identification of beer phenolic profile using high-resolution mass spectrometry was obtained. In detail, linear ion trap quadrupole-Orbitrap mass spectrometry (LTQ-Orbitrap-MS) allowed single-stage mass analysis for molecular weight determination, as well as two-stage (MS/MS) and multi-stage (MS^n) mass analysis for obtaining structural information. The LC-MS/MS data proved

that the phenol composition was the result of the wheat type used for beer production, and more specifically of the wheat yield. VOCs extraction and analysis were optimized by experimental design, by applying a central composite design based on 20 runs. Most of the VOCs identified by GC-MS were predominantly produced during yeast fermentation of the different worts. Multivariate statistical techniques were used in order to study the most important sources of data variability and to classify beers of different origin, thus suggesting the phenolic and VOCs fingerprint as an effective means of beer authentication.

Conclusions

High-resolution and accurate mass measurement mass spectrometry techniques proved to be reliable a tool for elucidating the structure of unknown phenolic compounds in complex beer samples. Optimized solid-phase-micro extraction GC-MS by response surface methodology enhanced VOCs detection. Both phenols and VOCs fingerprint proved to be a reliable tool for beers classification according to wheat yield.

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P21

First steps of a journey to detect antibiotic treatment biomarkers in pig chains

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Summary: *The targeted methods employed to detect antibiotic residues in meat do not capture antibiotic exposure of pigs at all life stages. The current study proposes a novel untargeted HRMS-based metabolomic analytical strategy to investigate biomarkers as a proof of antibiotic treatment in pig chains.*

Keywords: *Food Authenticity, Untargeted Metabolomic, High Resolution Mass Spectrometry*

Introduction

Over the last decade, pig farms have introduced practices for the responsible use of antimicrobials, driven by the urgent need to reduce antibiotic consumption causing the selection of antimicrobial resistant bacteria. Pork from supply chains carrying voluntary label claims such as "Raised without Antibiotics" is increasingly common on the market. However, the authentication and traceability of these chains is an issue. Targeted analytical strategies implemented to assess compliance with mandatory EU regulations [1] in the field of antibiotic residues do not capture the antibiotic treatments that can occur in the whole life cycle of pigs. Therefore, the present study introduces an alternative analytical strategy based on the metabolomics analysis of tissues by ultra-high performance liquid chromatography−ion mobility−high-resolution mass spectrometry (UHPLC-IMS-HRMS) to detect antibiotic exposure during rearing, ranging from weaning to pre-slaughtering, with the final aim to investigate possible biomarkers proving that antibiotic treatment occurred in pigs. In a wider perspective, this study aims to provide a new tool to demonstrate the truthfulness of label claims and enhance the integrity of the pig supply chain.

Experimental

Ninety-one heavy pigs from commercial farms were considered to compare pigs exposed to antibiotics (ABT) from those never exposed to antibiotics (ABF). Liver, kidney, and diaphragm were sampled and submitted to analysis. For each matrix, 100 mg of sample were extracted according to the slightly modified Bligh & Dyer protocol [2]. Metabolomic and lipidomic extracts were obtained and transferred to glass vials. Metabolomic extracts were analysed with a binary Acquity UHPLC I-Class system (Waters, Milan, Italy) coupled with a Synapt G2-Si HDMS QTOF mass spectrometer (Waters). The HRMS was set to operate both in positive ESI+ and negative ionization ESI- modes operating in data independent High-Definition MSE acquisition mode [3]. Data were recorded in raw files by using MassLynx (v4.2) software (Waters) and the preliminary processing (auto-alignment of signals, peak peaking, deconvolution, and normalization) of raw data was performed by using the Progenesis QI software (Waters) considering proper adducts for ESI+ and ESI-, respectively.
The data were filtered by setting a maximum intragroup variability of 30%, and a minimum fold change of 3 compared to a method blank. The unsupervised Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were performed with SIMCA 17 software package (Sartorius Stedim Data Analytics AB, Umea, Sweden) to explore potential trends and outliers, and for classification purposes, respectively. Based on PLS-DA, variable selection was performed according to the Variable Importance in Projection (VIP) scores to extract those features responsible for the discrimination between ABF *vs* ABT, setting tailored VIP score cut-off. The resulted list of relevant features was imported in OriginPro 2019 software (OriginLab Corporation, Northhampton, MA, USA) to evaluate with the Receiver Operator Curves (ROC) analysis the ability of features to be considered as biomarker (confidence interval of 95%). All those features having values of Area Under the Curve (AUC) higher than 0.8 [4] were considered for the annotation.

Results

The three different metabolomic datasets were firstly individually scaled to unit of variance and then subjected to PCA by performing a 7-fold cross validation. The PCA algorithm for liver extracted 4 principal components (PCs), explaining 58.1% (R2X) of the overall variance with a coefficient of predictive ability (Q2) of 35.8%. Similarly, the PCA model for kidney was described by 5 PCs explaining 52.2% of the total variance (R2X) with

a coefficient of predictive ability of 20.6% (Q2). Lastly, the PCA model for diaphragm was described by 5 PCs with a score of goodness-of-fit of 63% (R2X) and predictive ability of 42.1% (Q2). For all models, the first two components were graphically plotted. A perfect trend in separation between ABF and ABT was achieved for liver, while for kidney and diaphragm the performances were considered discrete. The supervised PLS-DA models for liver (R2X=49.9; R2Y=99.4; Q2= 92.0%), kidney (R2X=28.7; R2Y=99.1; Q2= 90.0%), and diaphragm (R2X=51.3%; R2Y=94.2%; Q2=94.2%) demonstrated good performances. Following VIP analysis and setting VIP score >1.7, 151 and 162 features were filtered for liver and kidney, respectively; 271 features were obtained by VIP score>2.2 in the case of kidney. The assessment of the ROC curves considering an AUC value >0.8 on the extracted features provided three novel reduced lists (one for each of the three tissues) consisting of 126, 119, and 150 relevant features for liver, kidney, and diaphragm, respectively. The annotation of metabolites, based on the levels set by Metabolomics Standards Initiatives (MSI), is in progress by comparing the spectra with those stored in online libraries, namely, Human Metabolome Database, Food Metabolome Database, LIPID MAPS, KEGG database, Drugbank Food and Drug Administration database, ChEBI and Biocyc.

Conclusions

The current study proved that the investigation of metabolome of the liver, kidney, and diaphragm of heavy pigs may be useful in elucidating if pigs were exposed to antibiotic treatment during their life cycle. Furthermore, liver was more sensitive to antibiotic treatment, resulting in a distinct clustering of ABF from ABT that was easily detected by PCA. Ongoing annotation and subsequent biological interpretation of the annotated metabolites will allow the study to be finalized.

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P22

Veterinary drug residues in animal-origin food: an UPLC-MS/MS screening method for the determination of 21 beta-agonists in animal liver and lung

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Summary: *The misuse of β-agonist drugs as growth promoters in animal production has resulted in strict regulations in the European Union, requiring increasingly sensitive analytical methods. In this work, a screening method of 21 beta-agonists at half of MMPR was developed and validated on animal liver and lung by UPLC-MS/MS technique.*

Keywords: *β-agonists, UPLC-MS/MS, animal liver and lung.*

Introduction

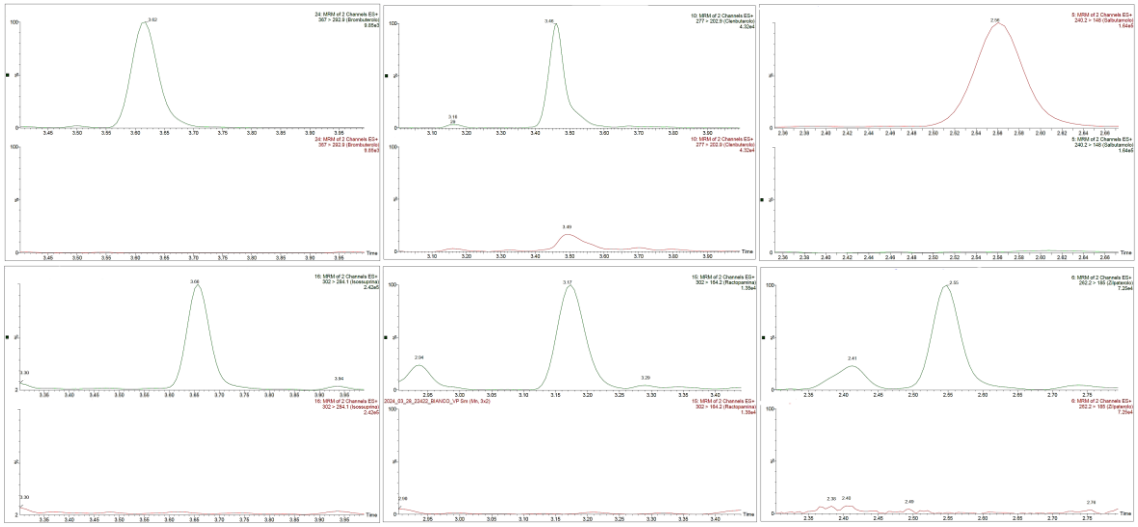
Beta-agonists are a group of phenylethanolamine compounds known in the therapeutic field for their bronchodilatory properties. Although they are banned substances, for decades these drugs have been largely used as growth promoters in farm animals, as evidenced by the cases of intoxication that occurred in Europe in the 1990s [1]. In order to tackle their illicit use, several directives and regulations have been signed by the European Union (EU) and the European Union control laboratories have started a process of developing high-performance analytical methods. To standardize their work, on the basis of Reg. (EU) 2021/808 [2], which specifies the requirements for the validation of analytical methods and the interpretation of results, the European Union Reference Laboratory (EURL) has stipulated the Guidance Document on Minimum Method Performance Requirements (MMPRs), describing the lowest recommended concentrations that control laboratories should achieve [3].
The aim of this work was the development and validation of an analytical method, to be used in official control, for the qualitative determination of 21 beta-agonists in animal liver and in animal lung by means of ultra performance liquid chromatography (UPLC) hyphenated to tandem mass spectrometry.

Experimental

Three grams of homogenized liver or lung was added with the enzyme β-glucuronidase-arylsulfatase (37°C, one night).
The samples were after corrected to pH between 6 and 7, and then cleaned up using Strata^{TM}-X-C strong cation exchange SPE (Phenomenex).
The purified extracts were evaporated, reconstituted and injected into UPLC-MS/MS.
A Waters Acquity BEH C18 column (100 mm × 2.1 mm, 1.7 µm) was used with mobile phases consisting in water and acetonitrile both with 0.1% of formic acid in gradient mode.
The LC system was coupled with a Waters Xevo TQ MS mass spectrometer equipped with an ESI source operating in positive ionization mode (ESI+) [4].
The validation was conducted in accordance with Reg. (EU) 2021/808 [2], analyzing twenty-four liver and twenty-four lung samples of different animal species. In each analytical batch the blank samples and spiked samples at the level of interest, corresponding to 0.05 µg/kg for eleven clenbuterol-like beta-agonists (clenbuterol, bromchlorbuterol, brombuterol, cimaterol, cimbuterol, clenpenterol, clenproperol, hydroxymethyl-clenbuterol, mabuterol, mapenterol, tulobuterol) and 0.25 µg/kg for ten isoxsuprine-like drugs (carbuterol, isoxsuprine, clencyclohexerol, ractopamine, ritodrine, salbutamol, terbutaline, zilpaterol, fenoterol, salmeterol) were processed. As internal quality control, two internal standards (clenbuterol-d_9 and isoxsuprine-$^{13}C_6$) were added to all samples at 0.50 µg/kg.

Results

The validation of a qualitative screening method requires the verification of four main performance characteristics such as Detection Capability CCβ, specificity, robustness, and stability, as stipulated by Reg. (EU) 2021/808 [2].
The basic principles for the evaluation/calculation of these parameters are detailed in the Guidelines for the Validation of Screening Methods for residues of veterinary medicinal products, developed by EURL.
For the substances analysed in this work, the CCβs were assessed at the Screening Target Concentration (STC) established on the basis of the fixed MMPRs. Having set STCs at half of the relevant MMPRs for each molecule, the lack of any false negative result demonstrated method compliance (percentage of false compliant results or beta-error ≤ 5%).



A B C

Figure 1. *UPLC–MS/MS chromatograms of a liver sample spiked at STC with the six mandatory beta-agonists [3]: (A) brombuterol, (B) clenbuterol, (C) salbutamol, (D) isoxsuprine, (E) ractopamine, (F) zilpaterol. Each chromatogram is associated to its corresponding blank sample.*

D E F

The specificity of the method was proved by the absence of any interfering peak at the retention times of analytes in the chromatograms of the twenty-four liver and twenty-four lung samples from different animal species (Figure 1). To test the robustness of the method, five potentially critical variables of the analytical method were identified: concentration of NH_3 (elution mixture), SPE conditioning, evaporation temperature, pH of the extract and sample weigh. These parameters were slightly changed to evaluate their effect. Data showed that small variations of the five selected factors did not significantly affect results, also proving method robustness to minor changes.

Conclusions

A multiresidual and fast screening method for the determination of 21 beta-agonists in animal liver and lung was developed and validated in accordance with Reg. (EU) 2021/808 [2]. The developed method fits for purpose, fulfilling the validation criteria established for screening analysis. With regard to Detection Capability CCβ, the results obtained permitted the qualitative determination of eleven clenbuterol-like beta-agonists at 0.05 µg/kg and for ten isoxsuprine- like beta-agonists at 0.25 µg/kg.

Accordingly, the screening method will be used for routine analysis in the framework of EU official controls.

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P23

Evaluation of toxicity profile of ClPFPECA-0,1 (N2) by lipidomics approach

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Summary: *ClPFPECA-0,1 (N2) belongs to ChloroPerFluoroPolyether Carboxylates (PFPECA) compound class and it was recently found in both environmental and biota samples. Herein, we report the evaluation of toxicity profile of ClPFPECA-0,1 (N2) by lipidomics approach in HepG2 cells. Results showed a dysregulation in TGs and CLs.*

Keywords: *ClPFPECA-0,1 (N2), lipidomics, toxicity*

Introduction

Per- and Poly-FluoroAlkyl Substances (PFAS) constitute a broad class of man-made chemicals that contain at least one fully perfluorinated methyl or methylene carbon atom. The chemical stability and amphiphilic nature of these compounds have given rise to their use in various industrial and consumer product applications. Unfortunately, it is well demonstrated that PFAS are related to several human diseases [1] such as cancer, hepatic diseases (e.g. NAFLD, NASH…), insulin resistance and more. The environmental persistence of these compounds in combination with the ever-increasing body of evidence of their potential adverse health effects has led to regulatory efforts and voluntary initiatives to implement their elimination/reduction. In the recent years, alternative compounds deemed safer than the called "legacy" PFASs (PFOA, PFOS...) were synthetized. An example is the "emerging" class called ChloroPerFluoroPolyether Carboxylates (ClPFPECA), which were recently found in environmental [2], biota [3,4] and food [4] samples. The presence of ClPFPECA in food matrices involves an involuntary intake of these compounds, whose toxicity has not yet been studied at the best of our knowledge. Herein, we report the evaluation of the toxicity profile of ClPFPECA-0,1 (N2) by lipidomics approach. Moreover, in perspective of TEF-based legislation, lipidomics analysis combined with other tools (e.g. QSAR) can provide a quickly way to assign a toxic equivalency factor to newly discovered PFAS in food matrices.

Experimental

HepG2 cells were treated with different concentrations (160 nM, 800 nM, 4 µM, 20 µM and, 100 µM) of ClPFPECA-0,1 (N2) for 24h in triplicate. Pellets were extracted with MMC (methanol/MTBE/chloroform 40/30/30 v/v/v). After evaporation, the residue was resuspended in IPA and then injected in UHPLC-HRMS (Q Exactive, Thermo Fisher Scientific, Waltham, Massachusetts, United States). For lipids separation, an Accucore C18 column (150 x 2.1 mm 2.6 µm) LC column (Thermo Fisher Scientific, Waltham, Massachusetts, United States) was employed using a mixture of H2O/ACN 50/50 v/v and a mixture of H2O/ACN/IPA 5/10/85 v/v/v as mobile phase A and B, respectively, both containing 5mM of ammonium formate and 0.1% of formic acid. For the analyte detection, full MS/dd-MS^2 (TopN) approach was used. Obtained data were elaborated using Lipostar2® [5] software.

Results

After elaboration, a total of 306 lipids were definitely identified. The obtained results shown lipids dysregulation for ClPFPECA-0,1 compared to the control samples. Particularly, MUFA and PUFA cardiolipins and triacylglycerols were the lipidic classes mainly responsible for the separation in the multivariate statistical analysis (PCA and PLS-DA) between treated samples and control (Figure 1) as confirmed by univariate statistical analysis generated comparing CTRL versus 100 µM treated samples.

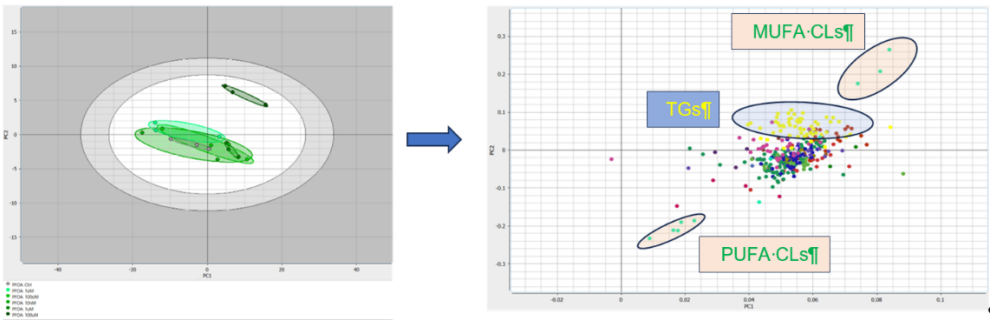

Figure 1. *Multivariate statistical analysis (PCA) of HepG2 cells treated with ClPFPECA-N2.*

Conclusion

Lipidomics analysis of treated HepG2 cells with ClPFPECA-0,1 (N2) demonstrated a dysregulation of both triacylglycerols (TGs) and cardiolipins (CLs). TGs alteration involves some multivarious pathological conditions such as obesity, type 2 diabetes, coronary heart disease, hypertriglyceridemia, NAFLD and NASH [6]. Dysregulation between MUFA CLs (immature cardiolipins) and PUFA CLs (mature cardiolipins) is related to several mitochondrial dysfunction such as mitochondrial-dependent steps in apoptosis and mitochondrial membrane stability and dynamics [7]. Our results highlight that ClPFPECA-0,1 (probably others ClPFPECA too) shows a similar toxicity profile of perfluorocarboxylic acids (e.g. PFOA) so it is important to monitor their concentrations to evaluate the human exposure to these substances. Furthermore, the time required to obtain results (<3 weeks) demonstrates the power of lipidomics as rapid and predictive toxicological assay.

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P24

Targeted and untargeted metabolomics profiling and evaluation of fungal communities in Italian wheat grains to assess the food security

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Summary: *Climate change and mycotoxigenic fungi threaten wheat crops. Screening 24 Italian wheat varieties with HRAM-Orbitrap and HPLC-MS/MS revealed minimal mycotoxins* traces in a few samples*. Investigating endophytic fungi, 452 strains were isolated in vitro. Phenotypic observation of the mycelia grown on PDA revealed common and specific distributions of the fungal communities. Characterization studies of these fungal isolates are currently ongoing.*

Keywords: *mycotoxins, endophytic fungi, wheat*

Introduction

With a production of about 6.8 million tons in 2023, Italy is one of the most important European countries for wheat production. Wheat crops are greatly challenged by climate change and phytopathogenic fungi outbreaks that are evolving and spreading to new geographical areas, posing a significant threat for global food security and environmental sustainability. Furthermore, fungi such as Alternaria, Aspergillus, Fusarium and Penicillium produce mycotoxins that contaminate the major cereal crops [1]. Prolonged consumption of mycotoxin contaminated food and feed can lead to acute or chronic health problems to both human and animal health, due to chronic exposure, underestimated dietary intake of masked mycotoxins, and the synergistic threat of co-contaminations by multiple mycotoxins [2].

Experimental

In this project, the grain of 13 durum wheat varieties and 11 soft wheat varieties was studied. These samples were harvested in 2023 in different regions of the Italian peninsula in the context of the wheat chain project that lies within the Spoke 9 of the PNRR National Centre CN2 - Agritech. An “untargeted” approach was initially performed using HRAM-Orbitrap and Compound Discoverer™-Thermo Scientific™ to screen for the presence of potential contaminants (chemical, microbiological) in cereals. Afterwards a “targeted” approach was performed using HPLC-MS/MS to screen for the presence of the main mycotoxins in cereals: Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), fumonisins (e.g., fumonisin B1, FB1, fumonisin B2, FB2), trichothecenes (e.g., DON, nivalenol, NIV, 15-acetyldeoxy nivalenol, 15-AcDON, 3-acetyldeoxy nivalenol, 3-AcDON, deoxynivalenol 3-glucoside, D3G, HT-2 toxin, HT-2, T-2 toxin), ochratoxin A (OTA) and zearalenone (ZEA), alternariol (ALT) [3]. To investigate the possibility of contamination we assessed the distribution of endophytic fungal communities through an in vitro selection.

Results

The “untargeted” approach was initially performed using HRAM-Orbitrap and Compound Discoverer™-Thermo Scientific™ to screen for the presence of potential contaminants (chemical, microbiological) in cereals, which led to the detection of traces of some mycotoxins such as deoxynivalenol (DON). Afterwards a “targeted” approach was performed using HPLC-MS/MS to screen for the presence of the main mycotoxins in cereals: AFB1, AFB2, FB1, FB2, DON, NIV, 15-AcDON, 3-AcDON, D3G, HT-2, T-2 toxin, OTA, ZEA, ALT [3]. The targeted HPLC-MS/MS analysis confirmed the absence of the previous mycotoxins, with only minimal traces detected in a few samples, confirming the quality and safety of these samples of Italian wheat, but it will be necessary to consider other crop seasons to confirm the results. In fact, climate conditions can be conductive or not according to the different crop season. Therefore, to investigate the possibility of contamination we assessed the distribution of endophytic fungal communities through an in vitro selection. This led to the isolation of 452 strains from the caryopses of the 24 wheat varieties considered. The initial phenotypic observation of the mycelia grown on PDA revealed common and specific distributions of the fungal communities: some isolates were abundant in all wheat samples, while others were unique to specific samples. Characterization studies of these fungal isolates are currently ongoing.

Conclusions

The isolation of pathogenic and/or mycotoxigenic fungi and the metabolomic profile of wheat grains, in relation to the geographical origin and the agronomic practices used for wheat cultivation represents an important step for preserving food safety and security in a warming climate contest.

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P25

Indirect recycling of fast food in insect feed: the use of UHPLC-MS/MS proteomics to ensure food safety

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Summary: *As part of the EU Green Deal, the European Commission is promoting the reuse of food waste in animal feed. The EU's goal is to reduce dependence on critical feed materials like soya while simultaneously decreasing food waste. However, former foodstuffs containing meat or fish remain prohibited. In this study, insect meals were produced on substrates containing fast food products and evaluated by MS-proteomics to detect the presence of meat residues. Collagen peptides appear to be effective markers to monitor the use of prohibited products and to distinguish them from authorised by-products such as dairy products*

Keywords: *insect, proteomics, food waste*

Introduction

Since 2021, insect meal has been authorised in feed intended for pigs and poultry, in addition to fish feed [1]. Despite the regulatory changes that seem to increase the insect market, the insect meal market is still limited and more expensive compared to traditional protein sources. The high cost is partly due to the restricted substrates allowed for insect breeding under current legislation. From a legislation point of view, edible insects are considered farmed animal and, therefore, must comply EU animal by-products regulation.
Following the same sustainability strategy, the EU has also adapted legislation to promote the use of another feed source, former foodstuffs (FFS). FFS retain a significant nutritional value and their use aligns with the current trend of circular economy.
However, FFS containing meat or fish remain prohibited.
Currently, there is no method capable of controlling what type of substrates insects have been raised on and therefore detecting this type of fraud, particularly in processed products imported from third countries in the form of insect meal.
The objectives of this study were (a) to evaluate and select peptide markers suitable for this research and to adapt the MS-proteomics protocol according to the materials; (b) to rear insects on substrates adulterated or not with fast food products and to prepare insect meals; and finally (c) to evaluate the substrates and insect meals with the UHPLC- MS/MS proteomics method for the detection of prohibited products (meat residues).

Experimental

For this study, three different materials were used:
(a) The preliminary evaluation of the peptide markers was carried out on raw, cooked and industrial meat, targeting three species: beef, pork and chicken;
(b) The substrates used for insect rearing consisted on a chicken feed free of animal ingredients adulterated with ground cheeseburger, pizza or chicken nuggets at a 10 % adulteration level;
(c) *Hermetia illucens* (10-day-old larvae) was used for its ability to convert food waste and because its authorisation for use in animal feed. Insects were reared 10 days on the different substrates and were then processed to prepare insect meals.
The sample preparation and MS analysis followed a previously published protocol [2]. Sample preparation included protein extraction in a buffer containing 200 mM TRIS-HCl pH 9.2 and 2 M urea, followed by trypsin digestion and purification with tC18 SPE (Waters). Analyses were performed by liquid chromatography (Acquity UHPLC system, Waters) coupled with a triple quadrupole mass spectrometer (Xevo TQ-XS, Waters). Peptide markers identified in previous studies [2-4], targeting haemoglobin, collagen, casein and β-lactoglobulin of ruminant origin, as well as collagen of pig and poultry origin, were simultaneously monitored.

Results

To *select the peptide markers*, different types of meat products were analyzed (raw, cooked and industrial). Industrial products included beef burgers, ham and chicken burgers for beef, pork and chicken meat detection, respectively. Ruminant haemoglobin peptides were detected in all beef meats. Collagen peptides were detected in their respective meat, except in raw meat where the peptides were not detected. Milk proteins were also identified in certain industrial products, as expected given the labeling "may contain traces of milk".
For the *rearing trial*, four rearing boxes were prepared: one for each adulterated feed and one for the control feed. Each box received 100 g of larvae and was placed in a climatic chamber at 24 °C for 10 days. After this

period, the larvae were rinsed on a sieve to remove as much substrate as possible, kept without feed for 24 hours and then frozen to kill them. Following a 3-day drying period at 40°C, both the substrate and larvae were ground before analysis. Portion of the initial substrate and larvae were also processed.
At the end of the trial, the substrates and the insect meals were analyzed by MS-proteomics. For the *substrates*, ruminant haemoglobin peptides were identified in the cheeseburger substrate but were no longer detected after the 10-day rearing period. Collagen peptides were detected in the respective adulterated meats, showing a decrease in peak area by the end of the trial, with some ruminant and poultry collagen peptides no longer detectable. Milk proteins (casein and β-lactoglobulin) were initially detected in substrates containing cheese burger and pizza, and β-lactoglobulin was still detected in both substrates after 10 days.
In the *insect meals*, only collagen peptides were still detected at the end of the trial. Ruminant and pig collagen peptides were found in insect meals produced on substrates containing cheeseburgers and pizza, but poultry collagen peptides were no longer detected in the case of nuggets. Ruminant haemoglobin and milk proteins were also no longer detected.

Conclusion
In the context of research of new protein sources, insect meal is a promising alternative. However, due to the high production costs, the fraudulent use of prohibited food waste is tempting, making strict control essential. Current official analytical methods cannot distinguish between the use of prohibited food waste and authorised animal by- products. In this study, MS-proteomics succesfully detected the prohibited substrates in insect meat residues. After the insect rearing process, insects were separated from the feed substrate, but residual feed materials remained stuck on the larvae.
Proteins degradation varies among the different proteins (haemoglobin *vs* collagen *vs* milk proteins). Collagen appears to be a reliable marker for monitoring the use of prohibited products. MS- proteomics enables the distinction between the presence of authorized ingredients (milk) and prohibited ingredient (meat).

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P26

Iterative data dependent analysis by LC-QTOF for untargeted approach in food matrices

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Summary: *DDA with iterative by LC-QTOF followed by data elaboration is a new and efficient approach to comprehensive chemical characterize food matrices. This approach was applied to olive oil mill wastewater (OMWW) and truffle. In OMWW samples were annotated 364 compounds while 413 in truffles, in which three compounds were found for the first time.*

Keywords: *DDA, untargeted analysis, UHPLC-QTOF.*

Introduction

The liquid chromatography hyphenated with high-resolution mass spectrometry together with bioinformatics platforms are the most suitable option to deal with the variety of small molecules with distinct physicochemical properties in complex matrixes [1]. Among the various acquisition strategies to acquire data for untargeted analysis, data dependent analysis (DDA) and data-independent analysis (DIA) are two powerful tools for identification of unknown compounds [2]. In this work, DDA with iterative were used to characterize the highest possible number of molecules from two food matrices: olive oil mill wastewater (OMWW) and truffle. This untargeted UHPLC-QTOF approach aimed to discover new recoverable compounds for the nutraceutical, pharmaceutical and cosmetic industry.

Experimental

Three different samples of OMWW were collected in a local olive mill in the Marche region during the production of extra virgin olive oil: Cultivar Sargano di Fermo, Cultivar Peranzana and Multivarietal. The remaining oil fractions in the OMWW samples, were removed by sample defatting and each sample together with pooled sample (quality control, QC) and blank were injected into UHPLC-QTOF system. A total of 30 white truffle samples (*Tuber magnatum* Pico) were collected in Acqualagna, Marche region, and compounds were cold extracted with 80% methanol solution. Then, extracts were injected into UHPLC-QTOF system. For the untargeted approach, the high resolution instrument worked in SCAN and in DDA mode, i.e., Auto MS/MS mode. The DDA acquisition algorithm was applied in the iterative mode for five consecutive injections in order to acquire MS/MS data from more precursor ions avoiding important data losing. MS-DIAL was employed for peak-picking (features), alignment, integration and annotation (performed by matching with NIST 2020 high-resolution MS/MS library). MetaboAnalyst 6.0 [3] and XLSTAT were used for statistical analysis.

Results

More than twenty thousands features resulted from the positive and negative acquisitions of the OMWW samples. After data filtering (5000 features for each polarity were obtained), ANOVA was applied to reveal statistically significant features (4713 in positive and 4703 in negative) among the three OMWW samples. From Ward hierarchical clustering method a great similarity among two OMWW coming from the two cultivars of Marche region (Multivarietal and Sargano di Fermo) emerged. A total of 364 compounds were annotated reaching a Level 2 of confidence [4] setting a total identification score cut off at 60%. On the other hand, in truffle samples a total of 413 compounds were annotated. Among them, 67 compounds had a total identification score higher than 90%. Some bioactive molecules were found for the first time in truffle and were confirmed with analytical standards such as riboflavin, azelaic acid and 2-isopropylmalic acid.

Conclusions

The progress of analytical technologies together with more and more efficient and fast software and algorithms will lead to a more comprehensive chemical characterization of food matrices and will determine a series of discoveries. Data dependent analysis with iterative followed by data elaboration is an efficient approach to characterize as higher number of molecules as possible in food matrices. Moreover, the use of high resolution instrument combined with the most modern bioinformatics platforms will open an enormous way for foodomics.

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P27

Aroma Profiling of Hops and Beer Using High-Capacity Sorptive Extraction with GC×GC–FID/TOF MS/SCD

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Summary: *This study demonstrates an advanced analytical system for comprehensive aroma profiling of hops and beer, providing sensitive detection of trace odor taints on a fully automated platform.*

Keywords: *Aroma profiling, GC×GC–FID/TOF MS/SCD, Hops and beer*

Introduction

Beer contains hundreds of organic ingredients, and the combination of these results in its unique aroma and flavor. Many of the aroma-active compounds come from hops, with monoterpenes (C10) and sesquiterpenes (C15) providing much of the characteristic 'bitterness' of the finished beer.

These compounds can span many orders of magnitude in concentration and may have very low odor thresholds, necessitating robust and highly sensitive analysis to assess the quality of the hops before brewing commences. Traditional solid-phase micro-extraction (SPME) has limitations in sensitivity and reproducibility, which high-capacity sorptive extraction overcomes by providing a larger volume of PDMS stationary phase, resulting in higher sample loadings.

Experimental

Samples: American hops ('Citra', 'Mosaic', and 'Amarillo') and American pale ale.

Extraction and Enrichment: The Centri® platform with HiSorb™ PDMS probes was used for automated high-capacity sorptive extraction. Parameters included 60 minutes of equilibration at 35°C with 400 rpm agitation.

GC×GC Analysis: Flow modulator INSIGHT® was used, with FID for robust quantitation, TOF MS for identification, and SCD for specific sulfur detection. ChromSpace® software handled full instrument control and data processing.

Results

Screening by FID: The monoterpenes and sesquiterpenes in beer often present at high concentrations can mask important trace-level species. GC×GC with parallel detection allows capturing these high-loading species. The major terpenes in the three hop varieties showed β-myrcene as the most abundant, with other terpenes present in much lower concentrations.

Table 1. *Percentage composition of key mono- and sesquiterpenes in the three hop varieties*

Compound	*Amarillo (%)*	*Mosaic (%)*	*Citra (%)*	*Aroma*
β-Myrcene	88.01	95.95	96.67	Spicy
β-Pinene	3.39	0.30	0.38	Herbal
Limonene	3.00	1.85	1.52	Citrus
α-Humulene	2.62	0.85	0.46	Woody
β-Caryophyllene	1.20	0.38	0.39	Spicy
α-Pinene	1.02	0.56	0.44	Herbal
Linalool	0.47	0.11	0.13	Floral
β-Farnesene	0.28	0.00	0.01	Woody

Confident Identification by TOF MS: The enhanced separation achieved by GC×GC–TOF MS avoids co-elution that would occur in 1D GC, providing excellent matches to reference spectra and enabling the identification of aroma- active species.

Sensitive Sulfur Detection by SCD: SCD provides highly selective analysis of sulfur species, which cause undesirable odors in food and beverages. This study found dimethyl disulfide and dimethyl sulfide in the highest abundances, with 'Amarillo' having the lowest overall sulfur content.

Conclusions

This study demonstrates that high-capacity sorptive extraction combined with GC×GC–FID/TOF MS/SCD offers robust and sensitive sample enrichment, cryogen-free preconcentration, comprehensive aroma profiling, and fully automated workflows. These capabilities make it a powerful tool for quality control and flavor profiling in the brewing industry.

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P28

Characterization of the natural vanilla samples of different botanical and geographical origin based on the aromatic profile

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Keywords: *Vanilla pods, VOCs, characterization*

Vanilla is one of the most popular flavouring agents in various foods, beverages, pharmacies, and cosmetics. Natural sources of the vanilla flavour are the pods of tropical orchid plants: *Vanilla planifolia*, *Vanilla tahitensis*, and *Vanilla pompona*. *Vanilla planifolia* is the most widely traded species followed by *Vanilla tahitensis*. Although the characteristic vanilla aroma is mainly due to vanillin (4-hydroxy-3-methoxybenzaldehyde), the vanilla pods consist of many other volatile compounds.
Like vanillin, ethyl vanillin (3-ethoxy-4-hydroxybenzaldehyde), which is 3 to 4 times more intense than vanillin, is also widely used as a less expensive flavouring agent than vanilla extracts to imitate or enhance the vanilla aroma. Currently, all ethyl vanillin supplied to the global market is produced by chemical catalysis, and up to now the presence of ethyl vanillin in natural vanilla extracts has not been reported yet.
The aim of this study was to characterize the aroma profile of vanilla pod extracts to discriminate between different geographical origins and different botanical species. Ten vanilla samples from Madagascar, Mexico, French Polynesia, Papua New Guinea and La Réunion were collected. These samples were of two different botanical species: *Vanilla planifolia* and *Vanilla tahitensis*.
The hydroalcoholic extracts were analysed by GC-MS/MS and about 50 volatile organic compounds (VOCs) belonging to different chemical classes were quantified. The different content of VOCs was able to distinguish the two botanical species, but not the geographical origins.
Moreover, the GC-MS/MS analysis did not detect ethyl vanillin above the detection limit in any of the vanilla pod samples. The absence of ethyl vanillin in vanilla pods was also confirmed by researching its glycosylated precursor by means of UHPLC-HRMS.

P29

Comparison of dried thyme from different geographical locations with GC-TOFMS and software tools designed to rapidly determine similarities and differences

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Keywords: *Non-Target Aroma Profiling, Geographical localisation, GC-TOFMS.*

Determining the aroma-contributing compounds of natural products is important for many tasks in the flavor, fragrance, and food industry. This type of information can be helpful for quality control of raw materials or finished products, authentication, product development or optimization, and determining if or how to adjust processes and solve problems.

Gas Chromatography (GC) and Time-of-Flight Mass Spectrometry (TOFMS) are well-suited for this non-target aroma profiling, as analytes of interest can be separated, identified, and discovered. Software to compare analytes and their trends across sample sets facilitates differentiating and characterizing the samples.

In this work, we use the Pegasus® BTX and ChromaTOF® Sync software to compare the aroma profiles of dried thyme from different geographical locations (France, Morocco, Poland, and Spain) to understand analyte similarities and differences in the chemical profiles that may impact their aroma.

P30

Impact of stabilizers on ice cream's aroma release

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Summary: *This study explores how various stabilizers influence ice cream's aroma release of vegan and dairy bases recipes. The effect of twelve stabilizer blends was investigated by PTR-MS headspace, revealing significant differences in aroma release. The findings aim to enhance ice cream formulations for an optimal sensory experience*

Keywords: *Food hydrocolloids, PTR-MS, Headspace*

Introduction

Ice cream's physical and sensory attributes are influenced by its microstructural components, including the ice phase, air phase, and fat phase. To enhance these attributes and maintain stability through shelf life and temperature fluctuations, stabilizers such as hydrocolloids are used.[1] This ingredient adds viscosity to the unfrozen portion of water, preventing water migration within the product, enhancing gel structure, and delaying ice crystal formation. Collectively, these effects contribute to a smoother texture and improved stability.[2]
Polysaccharides, while often showing smaller interactions with volatiles, can still affect the dynamic headspace concentration due to their gel properties, which slow the mass transfer of volatiles from the food phase into the headspace.[3] Dynamic studies have shown that increasing the gel strength of biopolymers, as indicated by the strength of interactions (such as hydrogen bonds, covalent bonds, and hydrophobic interactions) correlates with decreased sensory intensity and volatile release. For instance, research by Carr *et al*.[4] demonstrated that different biopolymers affected volatile release differently as stronger gels result in decreased sensory perception. Additionally, it has been observed that thickened solutions of similar viscosity, but with different thickening agents, do not induce the same flavor release, highlighting the specific interactions between stabilizers and aroma compounds.[5]
This intricate balance between enhancing texture and preserving flavor release is a key focus of ongoing research, aiming to optimize ice cream formulations for sensory appeal.[6-9] This study employs an array of different stabilizer combinations to explore the synergy and rheological behavior of the stabilizers. Answering how stabilizer blends impact ice cream aroma release and influence rheological properties would be a key improvement for product developers.

Material and Methods

Ice cream samples
The dairy and vegan ice cream ingredients were added sequentially based on their solubility temperature, starting from dry ingredients to oils, with the temperature controlled up to 70°C using a liquid-kitchen thermometer. The tested recipes were similar in terms of macronutrients (fat, sugar and protein contents) with varying blends of stabilizer reported in table 1. The recipes included vegetable fat, vegetable oil, sugar, glucose syrup, skimmed milk powder, mono- and diglycerides, water, and stabilizers, all provided by Sorermartec internal suppliers.
The ice cream recipes were prepared in phases: Control, phase 0, phase 1, phase 2, and phase 3 (Table 1). After preparation, the mixture underwent homogenization followed by a rapid decrease in temperature to 10°C by ice bath. The mixture was then aged for at least 24 hours. Following the aging period, two flavors (milk and peach-type) of known composition, including flavoring substances from categories such as lactones, esters, terpenes, carboxylic acids, aldehydes, alcohols, and ketones, were incorporated at concentrations of 1% and 0.5% (*w/w*) to the ice cream liquid before blast chilling. The final product was prepared using a benchtop ice cream machine set to a 30-minute program. Subsequently, the ice cream underwent a hardening step, remaining at -19°C for at least 5 days before the headspace measurements were taken.
PTR-MS analysis and Data Analysis
Samples were weighed (1.5- 1.7 grams) and placed into 20 mL vials for Proton Transfer Reaction Mass Spectrometry (PTR-MS) headspace measurements. The vials were frozen overnight to ensure headspace equilibration and the frozen state of the ice cream.

Table 1. *Summary of stabilizer blend type*

Sample group		Information	
Blends			
a)	Blank	a)	Empty Frozen headspace vials
b)	Control	b)	Recipe without stabilizer
c)	Phase 0	c)	Best on literature: Guar, LBG, carrageenan kappa
d)	Phase 1	d)	Singular stabilizer: Alginate, LBG, xanthan, guar
e)	Phase 2	e)	Alginate+xanthan, alginate+Guar, xanthan+guar
f)	Phase 3	f)	(Alginate + carrageenan kappa) with xanthan or guar or LBG

The ice cream samples containing milk and peach volatile compounds were analyzed using PTR-MS at two-time points: time one (frozen dessert at 4°C) and time twenty (melted ice cream at 30°C to simulate consumption). With ionization conditions set to drift tube temperature of 110°C, pressure of 2.80 mbar, and voltage of 628 V, E/N ratio of 128 Townsend. The PTR-MS data were processed with in-house software from the Sensory Quality Unit at Fondazione Edmund Mach. Peak identification was performed using an in-house library and literature research. The ANOVA analysis was done to determine which sample signals (*m/z*) were significantly ($p < 0.05$) higher than those detected from the blanks, the outcome was the peak selection of 66/215 *m/z* for the vegan base and 114/240 *m/z* for the dairy base. Principal Component Analysis (PCA) was performed on all samples using the selected peaks (*m/z*) concentration, to highlight differences between samples formulated with different blends of stabilizers.

Results

Different stabilizer blends exhibited distinct impacts on aroma release, particularly when flavor was added. Unflavored samples clustered together, whereas flavored samples differentiated based on the stabilizer used showing a clear impact of the stabilizer's type/combination. Notably, dairy ice cream without stabilizers (NSF) showed the highest release of peach flavor volatiles, tentatively identified as linalool (*m/z* 155.14), hexenyl acetate (*m/z* 143.12), and isoamyl acetate (*m/z* 131.12). This indicates that the absence of stabilizers enhances the release of certain aroma compounds confirming the idea that a stronger matrix may have a negative impact.

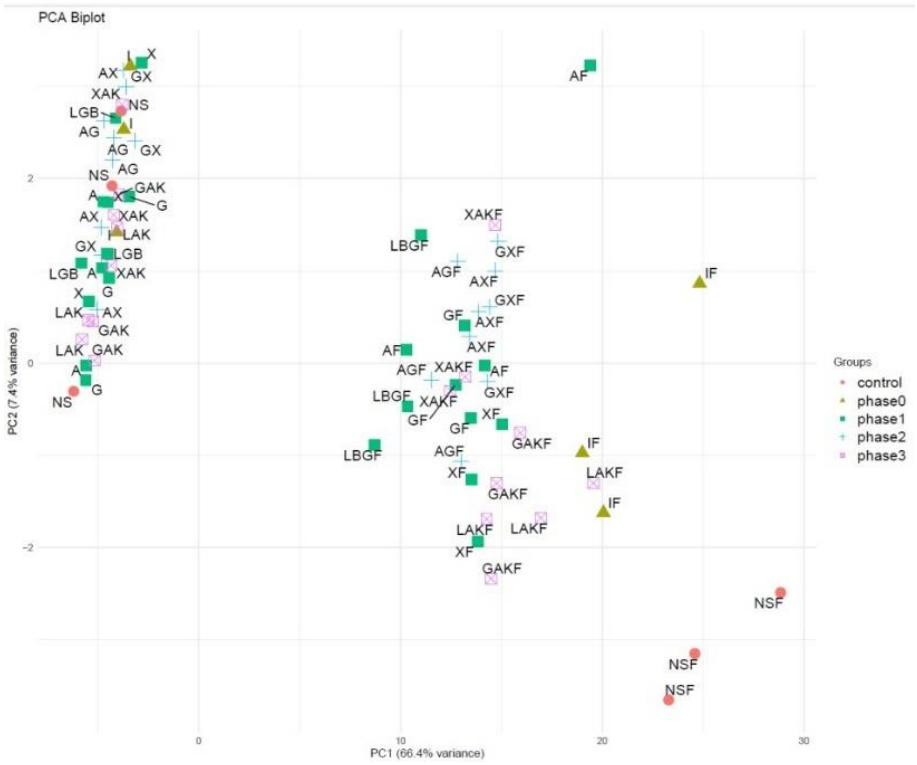

Figure 1. *Dairy Ice cream peach flavored and unflavored recipes measured at minute 20. Colors and shapes mean different groups of stabilizer blends. I: Guar +Locust Bean + Carrageenan kappa, A: Alginate, LBG: Locust bean gum, X: Xanthan, G: Guar, K: Carrageenan kappa, NS: No stabilizer, F: for flavor added*

However, this effect is clear only on dairy ice cream, whereas vegan ice cream samples containing a blend of alginate and carrageenan with locust bean gum and xanthan showed the highest release of milk flavor volatiles, tentatively identified as decanoic acid (*m/z* 173.15) and ethyl lactate (*m/z* 119.08). This research contributes to the broader understanding of the interactions between stabilizers and volatile compounds, in which significant difference were observed within the phases attributed, with the complex blend of three stabilizers having overall a higher release of aroma compounds.

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P31

Qualitative and quantitative characterization of free amino acids in green coffee by UPLC/ESI-MS

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Summary: *This work, born from the collaboration between illycaffè S.p.A. and the University of Parma, is focused on the qualitative and quantitative characterization of free amino acids in green coffee by UPLC/ESI-MS to identify possible patterns able to act as markers of quality, origin, botanical species and/or cultivation practices*

Keywords: *green coffee, free amino acids, UPLC/ESI-MS*

Introduction

Coffee is one of the world's most exported commodities and one of the most consumed beverages globally. The coffee fruit is a drupe and, when ripe, is generally red. Each drupe is composed of several layers [1]: the outer skin (exocarp), the mucilage or pulp (mesocarp), the "pergamino" (endocarp), the "silverskin" a thin tegument that covers the green coffee beans, being the latter the most investigated among the different coffee tissues. Differently from others coffee bean metabolites, including alkaloids, polyphenols, lipids and carbohydrates, free amino acids have not been the subject of detailed investigations. However, free amino acids may be a promising class of compounds for the sake of authenticity and traceability purposes. In this regard early studies put in evidence different free amino acids profile depending on botanical species. The best-known and most commercially exploited *Coffea* species are *Coffea arabica* (Arabica) and *C. canephora* (Robusta) followed by a negligible production of *C. liberica* and *C. excelsa*. The aim of this work, born from the collaboration between illycaffè S.p.A. and the University of Parma, is the qualitative and quantitative characterization of free amino acids in green coffee resorting to an *ad hoc* optimized analytical method. The study was undertaken to identify possible markers able to trace defected beans, different geographical origin, or different agronomical practices in addition to confirm different profile depending on botanical species.

Experimental

This study was structured according to the following operational phases: 1. development and optimization of the extraction and derivatization method of free and total amino acids; 2. analysis of selected green coffee samples by UPLC/ESI-MS; 3. Statistical analysis of the data to evaluate any significant differences between samples.

The characterized green coffee samples can be divided into three macro groups:

- Paired Arabica Brazilian samples that differ in agronomical practices.
- Arabica samples of different geographical origin, including samples of sensorially perceived defected beans and positive controls;
- Samples of *C. canephora* of different geographical origin and samples of *C. liberica* var. dewevrei (Excelsa) post-harvest processed with two different methods.

The optimized method for the extraction of free amino acids involves the addition of 10 mL of 4% 5-sulfosalycilic acid (SSA) solution to 1 g of previously ground sample [2]. Nor-leucine is added as an internal standard. To perform amino acids analysis with UPLC/ESI-MS, 10 µL is taken for derivatization. To derivatize free amino acids, an AccQ•Fluor Tag derivatization kit (Waters, Milford, MA, USA) is used. After UPLC/ESI-MS analyses, i.e. ultra-high performance liquid chromatography combined with mass spectrometry, significant differences between coffee samples are evaluated using SPSS data analysis software.

Quantification of total amino acids consists of three procedures:

- acid hydrolysis, performed to determine all amino acids, except Asn, Gln, Cys, Met, Trp;
- oxidation followed by acid hydrolysis, performed to determine sulfur amino acids (Cys, Met);
- alkaline hydrolysis, performed to determine Trp.

The sum of these results gives the total amount of amino acids in the samples.

Results

In the case of Arabica Brazilian samples that differs in the adopted agronomical practices, the most abundant free amino acid fractions are Glutamic Acid, Asparagine, and Aspartic Acid, while Glycine, Isoleucine, Leucine, Lysine, Methionine, and Threonine are the least significant (Figure 1).

The total amino acids fraction is 9-12% p/p, with Glutamic Acid, Asparagine, and leucine being the most relevant [Figure 2]. In all cases the agronomical practices do not affect the amino acid profile. The evaluation

of the total amino acid fraction allows to establish the conversion factor F on green coffee beans for Kjeldahl analyses as 5,84.
Principal Component Analysis (PCA) was performed to identify the relationship between amino acid content, coffee botanical species, and geographical origin. This analysis revealed a strong correlation between free amino acid content and coffee botanical species (Figure 3), as well as geographical origin (same coffee botanical species) [Figure 4].

Conclusions

The *ad hoc* optimized method with SSA allows to extract all the free amino acids present in the examined green coffee samples. The quali-quantitative determination of the free amino acid fraction of green coffee and the subsequent statistical analysis highlights the potential to use this class of compounds to discriminate different botanical species as well as different geographic origins (same botanical species). Agronomical practices seem to not affect amino acid profile, but in this regard further studies are necessary.

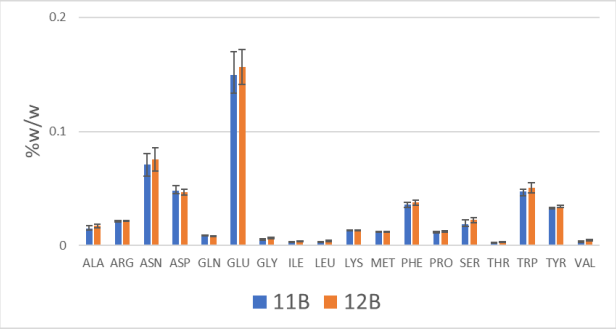

Figure 1. *Free amino acid fraction identified in Arabica Brazilian samples 11B and 12B (same genotype but different agronomical practices)*

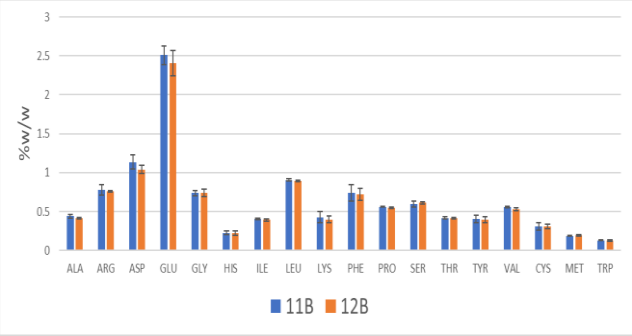

Figure 2. *Total amino acids fraction identified in Arabica Brazilian samples 11B and 12B (same genotype but different agronomical practices)*

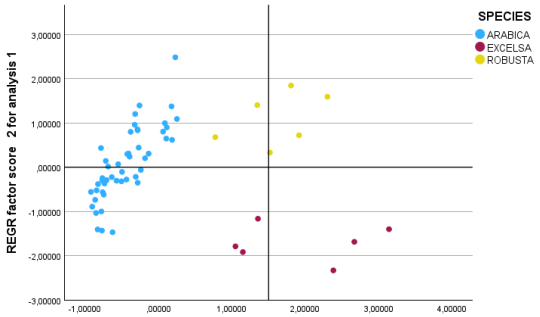

Figure 3. *PCA on species (cluster of samples including Arabica, Excelsa, and Robusta coffee)*

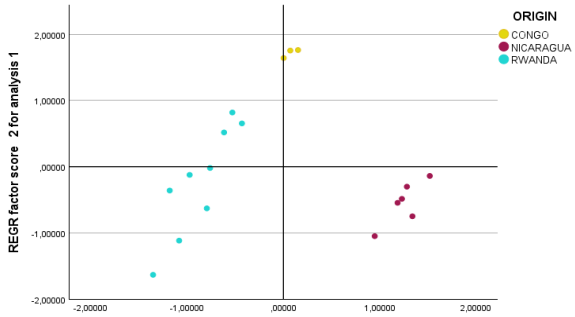

Figure 4. *PCA on geographical origin (cluster of Arabica including coffees from Congo,Nicaragua and Ruanda)*

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P32

LC-MS as a useful tool for coffee chemotaxonomy: the case of *Coffea racemosa* and *C. zanguebariae* Mozambican accessions

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Summary: *Coffea racemosa and C. zanguebariae are two indigenous coffee species of Mozambique. Very recently, the botanical classification of these plants was confirmed by DNA sequencing on a screening of Mozambican accessions. However, the seeds of these coffee species are still extremely difficult to be discriminated. In support of genetic analyses and in order to explore possible chemotaxonomical markers able to discriminate the seeds, a detailed chemical characterization by means of both UHPLC-DAD and LC-MS was performed. LC-MS proved to be very useful in assessing the presence of important secondary metabolites in trace amount which may assist the seeds classification.*

Keywords: *Coffea racemosa, Coffea zanguebariae, chemotaxonomy*

Introduction

Mozambique lacks a tradition of farming *Coffea arabica* or *C. canephora*, the two species dominating the global coffee market. However, native coffee plants have been growing spontaneously and, in some cases, have been cultivated on the Ibo and Quirimba islands in the north of the country and in the Inhambane province in the south. According to recent studies the coffee indigenous to central and southern Mozambique including the Inhambane province is *C. racemosa*, whereas *C. zanguebariae* is indigenous to northern Mozambique including Ibo and Quirimba islands [1]. For many years there has been confusion about botanical classification of these indigenous coffee plants due to the lack of literature data and a very restricted number of examined coffee samples. Very recently, Navarini et al. [2], reported on a screening of Mozambican accessions by DNA sequencing, aimed to determine the most likely taxonomical classification of coffee samples from Ibo and Quirimba islands. The obtained results identified *C. zanguebariae* as the taxonomical reference for both Ibo and Quirimba samples, while *C. racemosa* as the counterpart from the south of Mozambique. From the chemotaxonomical point of view, *C. racemosa* and *C. zanguebariae* has not been extensively investigated and very scarce data are available in the literature often restricted to few samples per taxa. In order to explore possible markers able to distinguish the seeds of the two coffee species which are still subjected to confusion, we identified and quantified the main seed secondary metabolites such as caffeine, trigonelline, chlorogenic acids and diterpenes (cafestol, kahweol and 16-O-methylcafestol (16OMC)), of a wide range of Mozambican accessions of *C. racemosa* and *C. zanguebariae*. In support of the quantification carried out by UHPLC-DAD, an LC-MS approach to quantified selected secondary metabolites usually present in traces (eg. kahweol,16OMC or minor chlorogenic acids) in coffee samples has been performed. This study also provides, for the first time, a detailed chemical characterization of *C. zanguebariae*.

Experimental

The green coffee beans were ground using a Retsch MM400 ball mill which was carefully cleaned after grinding each sample.

For the determination of diterpenes, coffee samples were saponified and analyzed in triplicate. For the determination and the quantification of 16OMC, an UHPLC-MS/MS analyses were performed with an Agilent 1290 UHPLC interfaced to a SCIEX Triple QuadTM 4500 [3]. The determination and the quantification of cafestol and kahweol were performed following the instrumental parameters as described in the "DIN method 10779 – Determination of 16-O-methylcafestol content in roasted coffee by high-performance liquid chromatography". For chlorogenic acids characterization grinded samples were extracted with water/methanol (30/70 v/v) for 30 min at 60°C in an ultrasound cleaning bath the ratio of sampling weight to extraction solution volume was 20. After extraction the sample was centrifuged (5 min, 8602 × g RCF at 20°C) and filtered with regenerated cellulose (RC) membrane 0,20 µm and diluted with water if needed. Analyses were performed with UHPLC-DAD and UHPLC-MS/MS as previously reported in literature [4].

Results

The identification and quantification of seed secondary metabolites are usually useful for the discrimination of coffee species. In particular the diterpenes content and the cafestol/kahweol ratio allow us to understand, from

an initial analysis of the sample, which species of coffee it might belong to. Moreover, the 16OMC is an ideal molecular marker for the discrimination of the main commercial coffee species: large amounts were determined in Robusta coffee, whilst it is present in very low traces in Arabica ones. From the perspective of the discrimination of *C. racemosa* and *C. zanguebariae* seeds, we performed a very detailed characterization of secondary metabolites including caffeine, trigonelline, chlorogenic acids and diterpenes. Both coffee species may be considered as low caffeine coffee, however the caffeine content of *C. racemosa* was systematically found lower than that of *C. zanguebariae*. Trigonelline and main chlorogenic acids content is not significantly different between the two coffee species. On the other hand, minor chlorogenic acids and diterpenes profile seems to be very promising chemotaxonomical markers. Particularly the latter shows a very different cafestol/kahweol ratio between the two coffee species.

Conclusions

In addition to the quantification carried out by UHPLC-DAD, an LC-MS approach to quantified selected secondary metabolites usually present in traces proved to be a useful tool for coffee chemotaxonomical purposes. In addition to slight difference in caffeine content, the different diterpenes profile may assist the classification of the seeds of these Mozambican coffee species.

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P33

Monitoring of roasted coffee bean freshness during storage by HS-GC/MS analysis

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Summary: *An HS-GC/MS method was developed to measure three volatile compounds, i.e. 2-Butanone, 2-Methylfuran and 2,3-Butanedione, in roasted Arabica coffee beans. Two different indexes were used by monitoring the 2-Butanone/2-Methylfuran and 2,3-Butanedione/2-Methylfuran ratios over 30 days, with the aim to evaluate their reliability as indicators of coffee freshness during storage.*

Keywords: *Coffee freshness, freshness index, HS-GC/MS analysis*

Introduction

Coffee is among the most extensively consumed drinks globally and its aromatic characteristics intricately linked to the roasting stage, during which significant alterations in the chemical composition of the coffee occur. Most aromatic compounds are unstable and highly volatile, tending to decrease post-roasting due to evaporation or chemical interactions. Consequently, freshness is a key criterion for evaluating coffee quality, thus defining its aromatic profile [1,2].

In this study, a headspace-gas chromatography-mass spectrometry (HS-GC/MS) method was developed for determining 2-Methylfuran (2M), 2-Butanone (2B), and 2,3-Butanedione (2-3B) in roasted coffee beans. These volatile organic compounds (VOCs) commonly found in the aroma profiles of coffee can be used to evaluate its freshness during storage. Notably, 2M and 2B are known to be relatively stable concerning chemical reactions, while 2-3B serves as an indicator of intrinsic reactivity. For this reason, these markers were monitored in Arabica roasted coffee stored for 30 days in four different conditions as whole coffee beans in sealed vials, open vials and in the tank of a household automatic coffee machine and as ground coffee collected from the bottom of the machine.

This study focused on evaluating the reliability of using two indexes to predict the freshness of Arabica coffee: the ratio 2B/2M (I1) and the ratio 2-3B/2M (I2).

Experimental

Samples and experimental conditions

Samples of 100% Arabica coffee beans were collected. The analyses were performed over a period of 30 days (sampling times: t_0, t_1, t_2, t_6, t_{10}, t_{21}, t_{29}) on four different conditions, defined as Closed (C), Open (O), Manual (M), and Ground (G). Specifically, for the first two conditions, 2 g of whole coffee beans were stored in sealed headspace vials (C) and open vials (O) until the day of GC/MS analysis (under dark conditions). In the condition M, the GC/MS analysis was carried out by manual injection of the headspace sampled in the tank of a household automatic coffee machine (ECAM 320.60 Magnifica Plus, De' Longhi Group, Italy) containing about 270 g of coffee beans. A cup of coffee per day was dispensed during the period of sampling (30 days) simulating a constant low domestic consumption. In the condition G, freshly ground coffee (2 g) was collected from the bottom of a second equivalent machine prototype, placed in a headspace vial and analyzed by HS-GC/MS. Headspace vials used were 20-mL.

HS-GC/MS

The analysis of 2M, 2B and 2-3B was carried out using the GC/MS system (Agilent GC8890-MSD5977C, Agilent Technologies, USA) with PAL SYSTEM autosampler fitted with heating block and 2-mL gas-tight heating syringe (CTC Analytics AG, Switzerland). A manual gas-tight syringe (2.5 mL, Agilent) was used for the manual injection. The separation was performed with a Agilent VF-WAXms column (60m;0.25mm ;0.25µm) and using He as carrier gas (flow 1 mL/min). The injection volume was 1 mL. The temperature program started at 30°C (held for 5 min) and raised with 8°C/min to reach 70°C. It continued at 10°C/min, until 170°C, and then to 230°C at 10°C/min with the total run time of 30 min. The injection port, quadrupole, source and transfer line temperatures were 250, 150, 230 and 280°C, respectively. Selecting ion monitoring (SIM) mode was used for the detection (72m/z for 2B; 82 m/z for 2M; 86 m/z for 2-3B) Standard solutions were supplied by Sigma-

Aldrich (Italy). The GC/MS peak area of the monitored VOCs were used to calculate the two freshness indexes (I1 and I2).

Results

Variation of the three VOCs

The variation of VOCs during the 30 days of storage is reported in the Figure 1, expressed as peak areas ratio between peak area at sampling point (t) and peak area at the starting period (t_0).

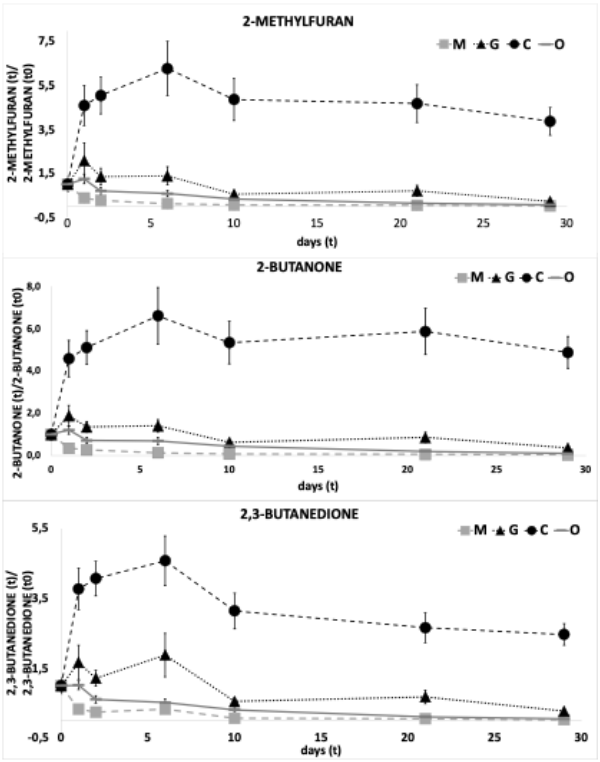

Figure 1. *Variation of the three VOCs in function of storage time for coffee stored in the 4 different conditions. Bars indicate standard deviation.*

The curves are positioned in the following order: C > G > O > M. The storage of coffee beans in C simulates an almost ideal system that limits volatilization, allowing only intrinsic reactivity and reactivity with the O_2 present in the headspace. In C, after an enrichment of three VOCs until t_6, small changes were observed. On the contrary, the storage of coffee beans in the condition O simulates inadequate storage conditions, allowing either the volatilization of VOCs and compounds reactivity. All the O curves show a peak at time t_1, followed by a constant trend. In the condition M, we are dealing with monitoring of VOCs in the tank of the coffee machine that is much larger than the vials, with a headspace that increases over storage time. The 2-3B curves, after t_6, exhibit an exponential decrease during monitored storage time. In condition G, curve is interposed between curves of C and O. This could be explained by the reduced exposure of VOCs to O_2 during storage, due to their being dispensed from the bottom of the machine, as well as by their higher volatility in ground coffee. All the G curves seem show higher values from t_1 to t_6.

Evaluation of freshness indexes

Indexes I1 and I2 calculated during storage time for the four different conditions are reported in Figure 2. Although I1 showed an initial rapid decrease at t_1, the condition G an increase of I1 was observed in all tested conditions. In the condition O a plateau after t_{10} were observed. The curve of I1 of condition M is positioned between C and O, with two rapid increases between t_1 and t_{10} and between t_{21} and t_{29}. This behavior is reasonable when considering that the volatility of the VOCs is high in this type of system, as indicated for individual VOCs. These results are in agreement to those reported in literature [2], for storage systems with double-layer packaging and for single-layer storage systems, which exhibit trends similar of I1 in C and O conditions, respectively.

In the case of I2 the trend was more complex. In the condition C a slight decrease of I2 up to t_{21}, as observed by [2] for double-layer systems, indicating the intrinsic reactivity of 2-3B which decreases over time even in the presence of a small amount of O_2. The trend of I2 in C is also in agreement with [1] and [4]. The O, after the first few days of sampling, showed a nearly constant trend. The I2 in M had a maximum at t_6, probably

related to the increase of the 2-3B with constant values from t_{10} to t_{29}. The curve of I2 in G was between those of O and M and very close to that of M.

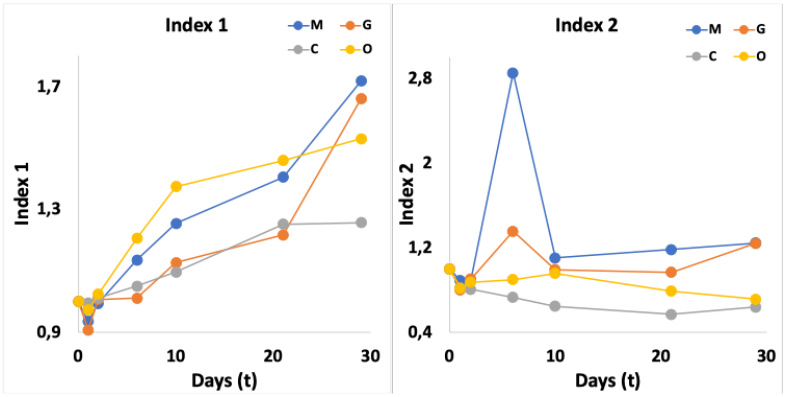

Figure 2. *Dependence of Index 1 and Index 2 on storage time for coffee stored in the 4 different conditions.*

Conclusions

In this study a method for measuring 2B, 2M and 2-3B in roasted coffee beans was developed. Moreover, the profile of the two indexes to predict coffee freshness was assessed in different conditions including a coffee machine. Future activities will focus on the study of other types of coffee and conditions to evaluate the robustness of the obtained results.

Acknowledgment

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P34

UHPLC-QTOF characterization of Coffea racemosa husks for an adequate valorization of a coffee by-product

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Summary: *Coffea racemosa is a wild coffee species not yet commercially exploited, indigenous to central and southern Mozambique including the Inhambane province. In the present study, C. racemosa husks (cascara) samples from 4 different areas of Inhambane province were characterized by UHPLC-QTOF-MS and HPLC-DAD analysis with a particular focus on bioactive compounds. A low caffeine content and the presence of several flavonoids make C. racemosa cascara a promising by-product to be exploited for possible food applications.*

Keywords: *coffea racemosa, coffee husks, cascara, bioactive compounds.*

Introduction

In the coffee post-harvest processing known as dry processing, coffee cherries are dried in the sun before the coffee beans are mechanically separated from the dried husks. These husks, also known as cascara (from the Spanish "cáscara," meaning husk), consist of skin (exocarp), pulp (mesocarp), mucilage (pectin), parchment (endocarp) and a portion of the silver skin of the coffee fruit and are an impacting by-product in the coffee sector. To date, there has been research activity mostly on *Coffea arabica* L. (Arabica) husks, focusing on both potential food and non-food applications particularly in the framework of circular economy. From food application point of view, it has to be highlighted that for centuries, the cascara has been used to prepare medicinal or refreshing infusions in producing countries, where they received different names. Examples are "hashara" in Ethiopia, "qishr" in Yemen, "sultana" in Bolivia, and "cascara" in El Salvador and Colombia [1]. In 2022, dried husk of the fruit of *Coffea arabica* L. has been considered to be safe as a novel food (to be used to prepare beverage by infusion in water) [2]. Nutritionally, Arabica coffee husks have been found to contain 8%–11% protein, 0.5%–3% lipids, 3%–7% minerals, and 58%–85% total carbohydrates. In terms of bioactive compounds, Arabica coffee husks have been found to contain caffeine, chlorogenic acid isomers and flavonoids including catechin and quercetin derivatives. A great variability of bioactive compounds content in coffee husks has been reported depending on the botanical species as well as on the geographical origin [3]. As regards the botanical species, in addition to Arabica, *Coffea canephora* (Robusta) was taken into consideration for its commercial exploitation parallel to that of Arabica. *Coffea racemosa* is a wild coffee species indigenous to central and southern Mozambique including the Inhambane province, not yet commercially exploited in spite of its claimed flavour quality [4]. In the present preliminary study, *C. racemosa* cascara was characterized by UHPLC-QTOF-MS and HPLC-DAD analysis. Four different samples of cascara from southern Inhambane province of Mozambique were studied with a focus on bioactive compounds.

Experimental

Four samples of coffee husks derived from *Coffea racemosa* were obtained from Mozambique by dry processing of coffee cherries. The coffee husk was manually separated from coffee seeds and the four samples identified as R1CH, R2CH, R3CH and R4CH. For the preparation of coffee husk extract each sample was finely grounded and extracted with methanol/water (80:20 v/v) by vortexing for 2 min followed by 10 min in an ultrasonic bath and overnight on a horizontal shaker. The solution was centrifuged and then filtered through a 0.2 µm RC filter and stored at 18°C until analysis.
Quantification of trigonelline, caffeine, and main chlorogenic acids was performed by UHPLC-DAD analysis. UHPLC-QTOF-MS/MS identification of compounds was performed by using the methods previously reported [5].

Results

The CGAs identified and quantified with UHPLC-DAD showed a profile similar to that of Arabica coffee husk published in literature [3,6]. In all samples the 5-O-caffeoylquinic acid was the major CGA present with a

maximum content of 1.76 mg/g. 3,5-dicaffeoylquinic acid was the major of the di-CQAs. Caffeine content lies between 0.32 and 0.87 mg/g, that results confirm that *Coffea racemosa* is a natural low caffeine species not only for beans content but also for coffee pulp.
In all the analysed samples other identified compounds resulting more interesting as far as biological activity or nutraceutical properties are concerned are two anthocyanins (cyanidin-monoglucoside and cyanidin-rutinoside), several flavonols such as quercetin and kaempferol and their derivatives, flavanol monomers (catechin and epicatechin) and dimers (procyanidins). As far as we know this is the first time that *C. racemosa* husk is characterized from bioactive compounds point of view. Unfortunately, the lack of reported data cannot allow any intraspecific comparison. However, an interspecific comparison with Arabica and Robusta husk put in evidence some differences in the bioactive profile. This study is an important step to investigate possible differences in cascara chemistry among different coffee species.

Conclusions

Coffee husk represents a great source of bioactive compounds, and this preliminary works shows the characterization of *Coffea racemosa "*cascara", enlighten a discrete amount of interesting bioactive compounds. Using this coffee by-product for food ingredients or herbal infusion, thanks to the low content of caffeine, could be an interesting possibility for developing countries like Mozambique where this coffee naturally grows.

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P35

Influence of geographical origin, post-harvest processing and brewing methods on quercetin derivatives in roasted *arabica* coffee

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Summary: *This study quantified six quercetin derivatives in coffee, highlighting the influence of geographical origin and post-harvest processing methods. Ethiopian coffee showed the highest flavonol content, particularly quercetin-3-glucuronide. Natural and honey processing methods preserved more quercetin derivatives compared to washed methods. Espresso preparation resulted in higher flavonols content than moka.*

Keywords: *Coffee, Quercetin derivatives, Geographical origin, Post-harvest processing.*

Introduction

Geographical origin and post-harvest processing methods significantly influence the chemical composition of coffee, particularly the levels of bioactive compounds like phenolic acids and flavonoids. Literatures have shown that environmental factors and processing techniques affect the flavonoid profiles of coffee beans (*Kumar & Sharma, 2024; Várady et al., 2022*). This study aims to quantify, for the first time, six quercetin derivatives in coffee from various geographical origins and post-harvest processing methods using newly developed extraction techniques and advanced analytical instruments.

Experimental

New extraction procedures were developed and validated for roasted coffee and coffee beverages (espresso and moka). The analysis was performed by HPLC-ESI- MS/MS to quantify quercetin, rutin, isorhamnetin, quercetin-3-glucuronide, hyperoside, and quercitrin. The coffee samples for the geographical origin section were selected from Brazil, Colombia, Ethiopia, Guatemala, and India, while for post-harvest processing section, natural, honey, and washed methods were chosen.

Results

Ethiopian coffee exhibited the highest total flavonols content, with quercetin-3-glucuronide levels reaching ± 5.95 ng/g, followed by Colombian and Guatemalan samples (Figure 1). Natural and honey processing methods preserved more quercetin derivatives compared to washed methods. Additionally, espresso extraction yielded higher flavonols content than moka, particularly for Ethiopian and Guatemalan samples (p-value < 0.05).

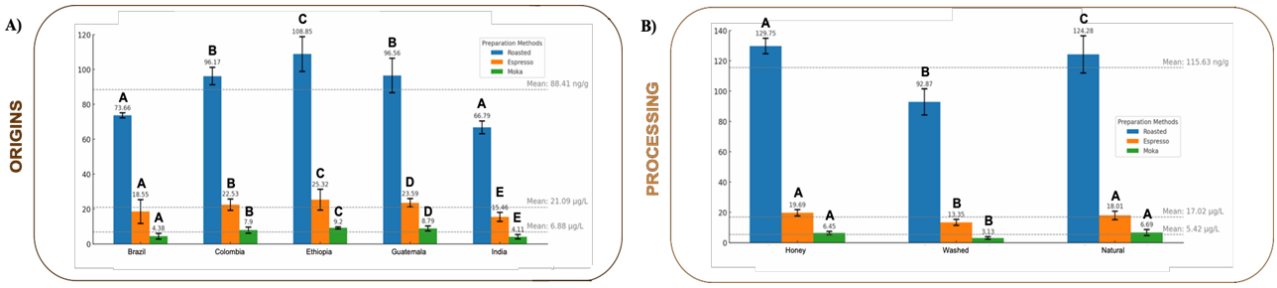

Figure 1. *Concentrations of total flavonols (rutin, quercetin-3-glucuronide, hyperoside, quercitrin, quercetin, and isorhamnetin) in coffee samples. (**A**) Flavonols content in roasted, espresso and moka coffee from different geographical origins (Brazil, Colombia, Ethiopia, Guatemala and India). (**B**) Flavonols content in roasted, espresso and moka coffee prepared from different post-harvest processing methods (natural, washed and honey). The columns represent the sum of the analytes, with error bars indicating standard deviations calculated from the triplicate measurements. The horizontal line represents the mean value. Different letters above the columns denote statistically significant differences (p-value < 0.05) according to one-way ANOVA and Tukey's test.*

Conclusions

This research underscores how geographical and post- harvest processing variables influence the quercetin and its derivatives compositions in coffee beans and cup. Moreover, the present work suggests valuable insights for potentially predicting the coffee chemical composition and quality by accurately selecting the origins and processing.

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P36

Supercritical CO_2 extraction of lipids from Coffee Silverskin: from laboratory to Industrial scale

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Summary: *This work focused on the scaling up of the optimized lab-scaled supercritical CO2 extraction of lipids from coffee silverskin (CS). This is the following step of the rECOBIOpack project, which aims to develop a packaging material from CS.*

Keywords: *coffee silverskin, lipids, supercritical CO2*

Introduction

Coffee is the most important food commodity worldwide and ranks second, after crude oil, among all commodities1. Since more than 50% of the coffee bean fruit is not used for production of the commercialized green coffee and, therefore, is discarded during processing, it should be interesting to find applications for these by-products. Coffee silverskin (CS) is the thin tegument that covers each hemisphere of the green coffee beans and is the only waste that comes from coffee roasting. Despite promising applications, CS is still underutilized and nowadays still discarded by the roasters or used for fuel or for composting. In terms of composition, CS displays an interesting content of dietary fibres, proteins, carbohydrates, lipids and antioxidants. Considering its composition, the rECOBIOpack project (funded by MUR, PRIN 2022), promoting a strategy close to “zero waste”, aims to develop a functional food packaging material completely exploiting the CS added value, in the sense that it uses its 5 main components: cellulose as polymeric backbone and lipids, proteins and polyphenols as additives.
The aim of this work was the scaling-up of the lipid extraction from CS (Illycaffè S.p.A) using supercritical CO_2 (sc-CO2). Laboratory extraction was scaled up at ILSA S.p.A. (Arzignano, Italy) using an industrial sc-CO2 apparatus.

Experimental

The biomass used as starting material was CS derived from a mixture of Arabica coffee beans. CS has been kindly supplied by the Italian coffee company Illycaffè S.p.A (Trieste, Italy).
The optimal extraction conditions in term of yield of extraction were defined at lab-scale as 50 ºC and 400 bar, using Design of Experiments. The industrial extraction was performed at ILSA S.p.A. at 400 bar and 50-60 °C for 3 h and in dynamic conditions. The biomass was extracted using two vessels (10 L) in series each one containing 0.7-0.9 kg of CS. Five replicates of the extraction were performed and a total of 8 kg of biomass were processed.
The extract was characterized in term of free fatty acid (FFA%) content using an acid-base titration with NaOH 0.1 M.
The fatty acid composition of the lipidic extract was evaluated using GC-MS (Bruker Scion SQ Instrument, Milan, Italy) equipped with a quadrupole mass spectrometer, after derivatization (both transesterification and sylilation were employed).
The quantification of the caffeine present in the extracts was performed, using a Waters ACQUITY UPLC system (Waters corp., United States) equipped with a UV–Vis photo diode array (PDA).

Results

The industrial extraction was thus performed using the optimal conditions determined in a lab-scale optimization trial.
The extract obtained at ILSA comprised two phases, as shown in Figure 1: a solid fraction and a liquid aqueous fraction.

***Figure 1**. CS lipid extract*

8 kg of CS were extracted in different batches, obtaining an average yield of 4 ± 1%, considering only the solid fraction. The FFA% was determined at 14±1 %. The fatty acid composition results are shown in Figure 2.

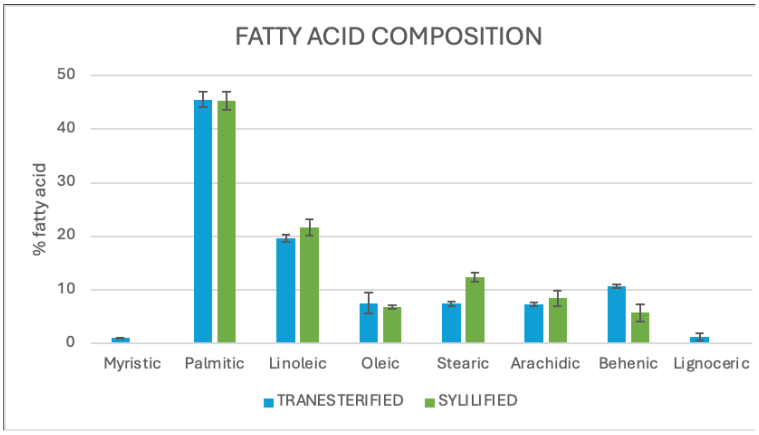

***Figure 2.** Composition of the main fatty acids in the CS lipid extract.*

In agreement with literature data2 and with the lab-scale extractions, the fatty acids found in the extracts were palmitic acid, linoleic acid, oleic acid, stearic acid, arachidic acid, behenic acid and lignoceric acid. It is worth noticing that the heavier fatty acids show, at first glance, no significant variability between the two types of derivatizations, an aspect that did come up in the lab-scale extractions. A relevant aspect was the presence of needle-shaped crystals in the liquid (aqueous) extract after storing it overnight at +4ºC.
The crystals were separated and analyzed with UPLC-PDA to identify and quantify the caffeine content, observing a purity equal to 99.4% and thus confirming their nature.

Conclusions

The scale-up of the extraction of lipids from CS using supercritical CO2 extraction was successful.
The industrial-scale extraction showed higher extraction yield (4 ± 1%) than the optimized lab-scale extraction (2.5%).
The fatty acid profile of the extracts consists of palmitic acid, linoleic acid, oleic acid, stearic acid, arachidic acid, behenic acid and lignoceric acid.
Caffeine was found in the liquid part of industrial extracts, easily separated in pure crystals.

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P37

Exploring the impact of roasting conditions on hazelnut quality by GC-IMS and sensory analysis

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Summary: *This study used SHS-GC-IMS to analyze hazelnut paste from different origins, examining the effects of roasting conditions and storage time on VOCs. It demonstrated the technique's efficacy in monitoring roasting by correlating VOCs with sensory data, color, roasting profiles, and moisture content.*

Keywords: *hazelnut, roasting, VOCs, sensory, SHS-GC-IMS*

Introduction

Hazelnuts (Corylus avellana L.) are of significant industrial importance, particularly in the form of processed products derived from roasted kernels. The unique flavor developed during roasting is a key driver of hazelnut consumption and their use in confectionery products. The sensory quality of roasted hazelnuts is largely determined by their volatile organic compounds (VOCs), which can be influenced by factors such as the geographical and botanical origin of the kernels, as well as technological processes like storage and roasting conditions. Gas Chromatography coupled with Ion Mobility Spectrometry (GC-IMS) is an increasingly popular analytical technique for food flavor analysis. Utilizing static headspace (SHS) sampling, SHS-GC-IMS requires minimal sample preparation, eliminates preconcentration steps, and offers relatively short analysis times. This makes SHS-GC-IMS a promising and rapid analytical tool, especially for applications where simplified protocols are advantageous.

Results

In this study, SHS-GC-IMS was used to characterize hazelnut paste samples from two different geographical and botanical origins. The research examined the impact of various roasting conditions and the storage time of raw kernels. A targeted approach was employed to monitor key odorants and roasting conditions, while an untargeted strategy, based on an automated peak detection workflow, provided insights into the complex nature of the volatilome of roasted hazelnuts. The VOC datasets were analyzed and correlated with sensory data from a panel trained in hazelnut evaluation, as well as with other measured parameters such as color, roasting temperature profile, and moisture content.

Conclusions

This research demonstrates the feasibility of using SHS-GC-IMS for efficient monitoring of industrial hazelnut roasting by integrating color assessment, roasting profiles, sensory evaluation, and VOC analysis.

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P38

Characterization of process dependent Maillard adducts and chemical modifications in wholemeal pasta by untargeted HR-MS/MS

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Summary: *The proteomic profile of spaghetti prepared with different mixtures of wholemeal semolina and using different drying cycles will be presented. Focus was placed on a comparative investigation by high-resolution mass spectrometry and software-based identification of process-dependent formation of Maillard adducts and chemical modifications (deamidation and oxidation) correlated to semolina composition and drying cycles.*

Keywords: *Maillard adducts, pasta drying cycles, proteomic profile, protein heat-damage*

Introduction

Traditionally made from durum wheat, pasta is a source of complex carbohydrates, and has been thoroughly investigated in terms of starch digestibility. On the contrary, only a few studies focused on protein characterization and digestion, although proteins represent the major determinants of the nutritional value and functional properties of pasta.
In this contribution, the proteomic profile of pasta prepared with different mixtures of wholemeal semolina and using different drying cycles will be presented. Focus was placed on a comparative investigation by high-resolution mass spectrometry of process-dependent formation of Maillard adducts and chemical modifications (deamidation and oxidation) correlated to either semolina composition or drying cycles.

Experimental

Two durum wheat mixtures with different protein content (13-14%) were selected and used to make as wholemeal pasta labelled as medium quality (MQ) and high quality (HQ), respectively. Spaghetti was selected as model pasta shape and produced in a pilot-scale, using different drying cycles: (i) 21h drying cycle at low temperature 50°C (labelled as 'LT') and 6h drying at high temperature 85°C (labelled as HT).
Extraction of total protein was carried out according to previous protocols with few modifications [1]. Briefly, 1g of ground sample was extracted with 20 mL of 100 mM Tris-HCl at pH 8.5 under strong denaturant (8M urea) and reducing (100 mM dithiothreitol) conditions. The extraction was assisted by ultrasounds with probe immersion [2]. Protein quantification was accomplished by colorimetric assay (RC DC™ Protein Assay, BIORAD) according to the producer instructions. An aliquot of the extract was diluted 1:10 in enzyme working buffer (Tris HCl 100 mM, pH 8.0 added with 10mM of $CaCl_2$) and digested overnight with chymotrypsin (minimum enzyme/protein ratio of 1:100 for each sample). The resulting peptide pool was purified and preconcentrate by solid phase extraction on disposable cartridges with polymeric stationary phase (Strata X, Phenomenex srl). The purified samples were analyzed by untargeted high-resolution MS/MS analysis on a hybrid quadrupole-Orbitrap™ mass spectrometer Q-Exactive Plus (Thermo Fisher Scientific). The chromatographic separation was accomplished with an Acclaim PepMap100, C18 column (3 µm, 100 Å, 1 x 150 mm). The settings for Full-MS/dd-MS^2 analysis mode were described elsewhere [1]. The raw data were processed by Proteome Discoverer v.3.0 (Thermo Fisher Scientific) for peptide/protein identification. The Sequest HT searching algorithm was applied against the *Triticum turgidum ssp. Durum* reference proteome (UP000324705). The workflow for sequence identification was specifically tailored to highlight Maillard adducts on lysine residue [3] and chemical modifications (oxidation and deamidation) on labile amino acids.

Results

Pasta samples were produced in a pilot-scale from whole meal semolina having different protein content. In particular, the high-quality semolina (HQ) presented a protein content of 14,2% and, the medium quality semolina (MQ) of 13,2% on dry matter. Given that pasta quality may be modulated by the drying step and increased drying temperature may impair protein digestion and/or affect nutritional properties, two drying cycles were used in pasta-making for comparative purposes: high temperature drying cycle (HT) and low

temperature drying cycle (LT).
First, total protein extraction was accomplished under strong denaturant and reducing conditions for all sample types and the amount of extracted proteins was estimated by colorimetric assay (see Figure 1). Three independent samples were prepared for each sample and the differences in averaged contents among the four types resulted to be not statistically significant, even if a general trend confirming higher protein content for HQ pasta than MQ pasta could be envisaged. Such results confirmed comparable and satisfactory extraction yields for all samples.

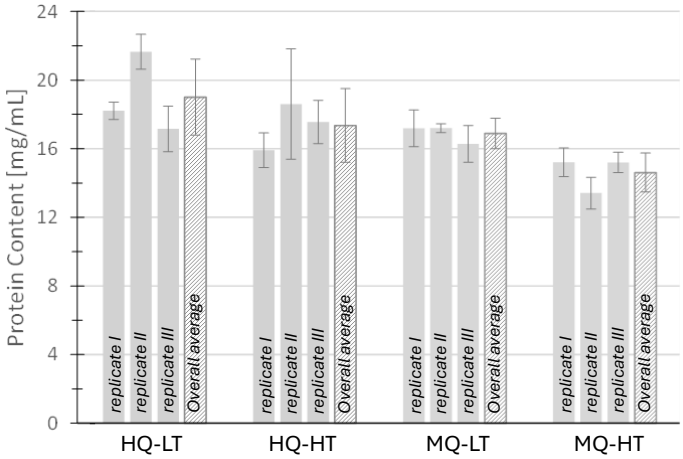

Figure 1. *Total protein content of pasta extracts by colorimetric Lowry modified assay. high temperature drying cycle (HT); low temperature drying cycle (LT)*

Discovery high resolution MS/MS analysis was carried out with a two–round identification protocol previously described [4]. In addition, the identification workflow set up on commercial software was tailored to disclose the presence of Maillard adducts (MA) on the lysine, as well as chemical modifications of methionine, glutamine, asparagine and arginine.
More than four thousand peptide sequences have been identified for each sample, with on average half of them presenting at least one modified amino acid. As an example, typical distribution of modifications among targeted amino acids was reported in Figure 2a for sample HQ-HT. Most of the modification were accounted by MAs on lysine residues (48,87%), and by deamidation of glutamine (28,74%) and asparagine (13,91%). In Figure 2b the specific modifications were reported together with the peptide counts for each modification. In particular, advanced glycation end products (AGE-MGH1, Δm = +96,0318), carboxymethyl-lysine (Δm = +58,0049) and carboxyethyl-lysine (Δm = +72,0206) containing peptides resulted to be the most common MAs. Detailed discussion about difference among the four types of pasta samples will be presented in the contribution in correlation with the semolina composition and the drying cycles.

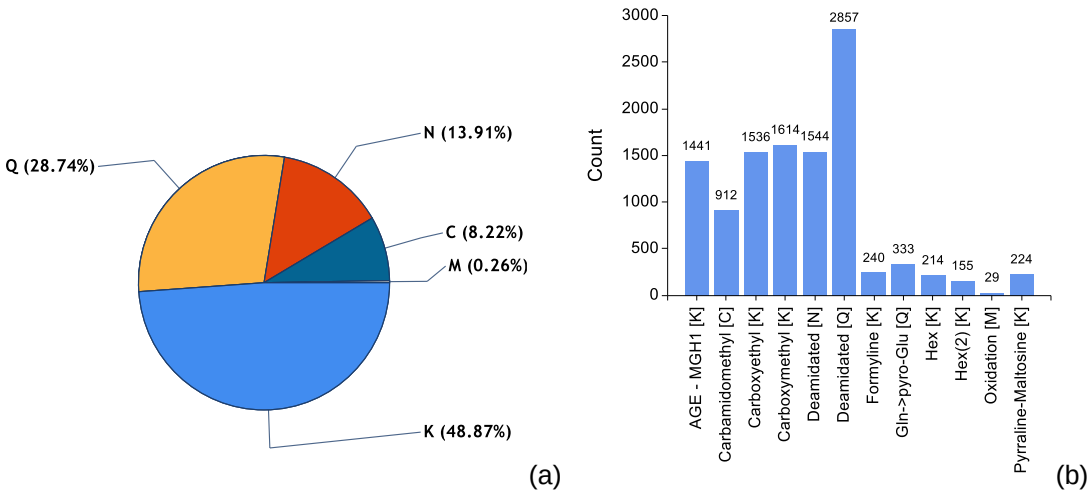

Figure 2. *(a) Relative distribution of the identified modifications over targeted amino acids, sample HQ-HT. (b) Qualitative evaluation (peptide count) of specific modifications (Maillard adducts and chemical modifications) identified for the sample HQ-HT.*

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P39

Glycerol as a precursor in the Maillard reaction: focus on alkylpyrazines

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Summary: *Glycerol, naturally present in food, along with α-amino acids (AA) and reducing sugars participates to Maillard reaction as modulator and precursor of several aroma compounds. In this study, glycerol and AA model systems have been thermally treated in the absence of reducing sugars. Volatile organic compounds profile has been investigated by HS-SPME-GC-MS with focus on alkylpyrazines. The role played by glycerol as a flavor precursor has been highlighted.*

Keywords: *glycerol, α-amino acids, Maillard reaction, alkylpyrazines*

Introduction

Glycerol, a ubiquitous polyol present in natural oils and fats, serves as a crucial intermediate in the metabolism of all living organisms. Widely distributed in various food sources, glycerol finds extensive use in the food industry as a versatile additive, functioning as a humectant, moisturizer, filler in low-fat products, thickening agent, and solvent for flavor extracts. Cerny and Guntz-Dubini (2005) explored the impact of glycerol on the Maillard reaction by analyzing the volatiles produced in the headspace above a solution containing [$^{13}C_6$] fructose and alanine in glycerol/water [1]. Their investigation, conducted under defined conditions (130 °C for 2 hours) within a sealed vial, revealed that glycerol influences the Maillard reaction not only through modulation of water activity and the physiochemical environment but also as a precursor molecule. In particular, several alkylpyrazines including 2,5-dimethyl-3-ethyl pyrazine, formed in the reaction of alanine and fructose in ^{13}C-labeled glycerol were triply labeled in significant percentage (ranging from 11% to 27%), proving unambiguously the inclusion of glycerol carbons in the molecules, presumably via 1-hydroxy-2-propanone or its oxidation product 2-oxopropanal as an intermediate. The latter can be transaminated by the Strecker reaction resulting in 2-aminopropanal which can form the dihydropyrazine derivative by condensation [2]. Addition of acetaldehyde (Strecker aldehyde of alanine) to the ring followed by subsequent dehydration reactions is supposed to give rise to 2,5-dimethyl-3-ethyl-pyrazine. According to this mechanism, glycerol is able to provide the α-dicarbonyl compound for Strecker degradation reaction in the absence of typical carbohydrate precursors. The role played by glycerol as a flavor precursor in the Maillard reaction has been put in evidence by investigating a proline/glycerol system and by comparing several volatiles generated both in presence and absence of various reducing sugars [3].
In this contribution different systems of single α-AA and glycerol were heated to give rise to the Maillard reaction products, and pyrazines profile investigated by HS-SPME-GC/MS.

Experimental

400 mg of glycerol and 7 mg of a single α-AA were thoroughly mixed in a 20 ml vial. After sealing with a crimp seal closure, the model systems were heated at 130°C for 2 hours. 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 to 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 um 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. Among several aroma compounds, 10 different alkylpyrazines were identified (resorting to both standards and library) and semi-quantified by peak area integration.

Results

In the thermally treated model systems (glycerol + α-AA) headspace, several volatile aroma compounds were found including alkylpyrazines as expected. 10 of the latter were identified, and the corresponding peak areas integrated in all the obtained samples (see figure, data are reported as a percentage of ASP, considered equal to 100%). 2,5-dimethylpyrazine is usually the most representative; in general, aspartic acid demonstrated to provide the most abundant quantity of alkylpyrazines followed by serine and tryptophane, while proline was the least efficient in their formation. Distribution of pyrazines was shown to be affected by the α-AA type. In particular, 2,5-dimethyl-3-ethyl pyrazine is very abundant in ASP, THR and ALA system whereas trimethylpyrazine is very largely produced in the GLY system. Unsubstituted pyrazine is particularly present in the SER system. 2,6-dimethylpyrazine and methylpyrazine were also systematically detected in all the investigated model systems apart from proline. During the thermal treatment and in the presence of air, glycerol

may be oxidized into dihydroxyacetone, which can then be dehydrated to form 2-oxopropanal, the α-dicarbonyl leading to 2-aminopropanal through the Strecker degradation of α-AA. 2,5-dimethylpyrazine may be then generated by 2-aminopropanal condensation and this mechanism is consistent with the observed prevalence of this alkylpyrazine in almost all investigated model systems. In the lack of glycerol, SER only is able to generate the full range of alkylpyrazines previously observed.

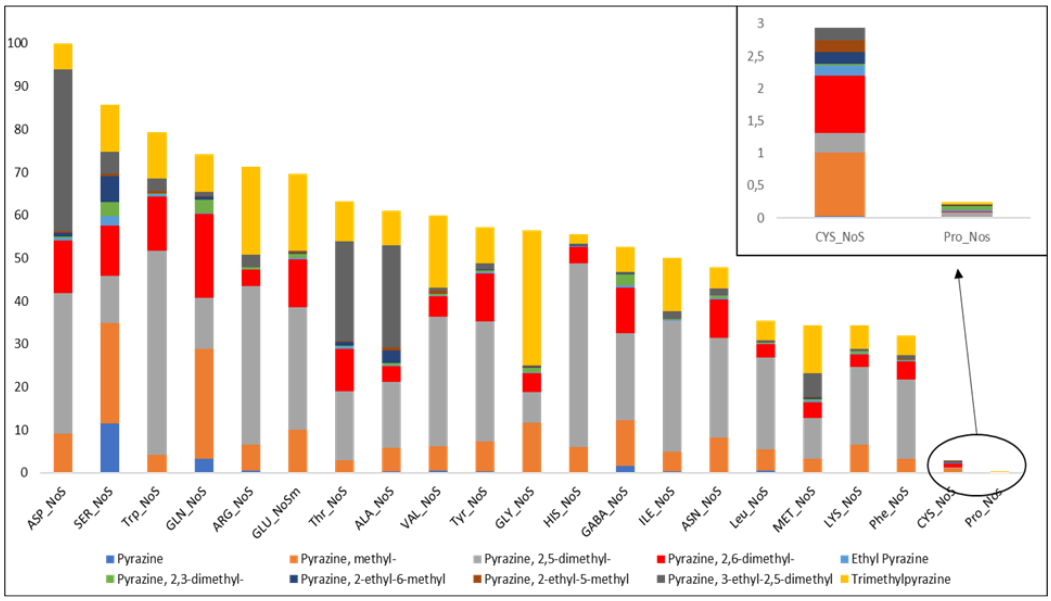

Figure 1. *ASP = aspartic acid, SER = serine, TRP = tryptophan, GLN = glutamine, ARG = arginine, GLU = glutamic acid, THR = threonine, ALA = alanine, VAL = valine, TYR = tyrosine, GLY = glycine, HIS = histidine, GABA = g-amino-n-butyric acid, ILE = isoleucine, ASN = asparagine, LEU = leucine, MET = methionine, LYS = lysine, PHE = phenylalanine, CYS = cysteine, PRO = proline.*

Conclusions

In the present study several α-amino acids have been thermally treated in the presence of glycerol and the resulting volatiles have been investigated by HS-SPME GC/MS. We focused our attention to alkylpyrazines which play a relevant role in the aroma of several roasted products including coffee. The importance of glycerol as a precursor in the Maillard reaction is emphasized.

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P40

Lipidomics of edible insects: exploring Maillard reaction products with advanced chromatography and mass spectrometry

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Summary: *This study explores the Maillard reaction products of phosphatidylethanolamines and their plasmalogen forms in the edible insect Acheta domestica. We used two chromatography columns with different selectivities: HILIC and C30 reversed-phase. By HR-MS we identified various lipid derivatives, offering insights into their potential impact on food quality and human health.*

Keywords: *Lipidomics, Insects, HILIC, C30, High-Resolution Mass Spectrometry*

Introduction

As the global population continues to grow, nutritional challenges are becoming increasingly significant, making edible insects a sustainable alternative to traditional meat sources [1]. Despite their potential, the consumption of these insects remains limited, highlighting the need for further research into their nutritional and health benefits. To ensure safety for human consumption, pre-treatments such as blanching, pasteurization, roasting, and drying are essential to eliminate pathogenic bacteria and viruses and inactivate enzymes [2]. However, these processes can lead to nutrient modifications. One notable reaction, the Maillard reaction (MR), involves complex interactions between reducing sugars and amino groups in proteins or lipids such as phosphatidylethanolamines (PE) and their plasmalogen forms (PE-O) when exposed to heat. Initially, a sugar molecule reacts with an amino group to form a glycosylamine, which then undergoes various chemical transformations including dehydration and fragmentation. This process results in a diverse array of by-products that may impact food quality and human health [3]. This study investigates MR by-products in PE and PE-O from the edible insect *Acheta domestica.*

Experimental

This study employed two different liquid chromatographic columns: (i) a hydrophilic interaction liquid chromatography (HILIC) and a C30 reversed-phase, both utilizing core-shell particles of 2.7 µm (2.1 mm and 15 cm). These separation techniques were coupled with high-resolution mass spectrometry (HR-MS) operating in both positive and negative electrospray ionization (ESI) modes. This comprehensive approach, together with MS/MS experiments, enabled the detailed identification and characterization of MR by-products, specifically focusing on derivatives of PE and PE-O.

Results

The HILIC column effectively separated lipid classes based on their modified polar heads, while the C30 column provided additional separation and refinement (Figure 1).

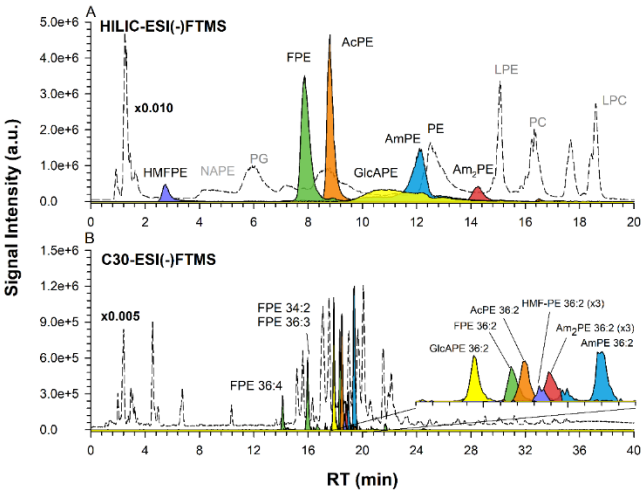

Figure 1*. Multi-extracted ion current chromatograms in negative ion mode by (A) HILIC-ESI-FTMS and (B) RPLC-ESI-FTMS.*

This dual approach allowed for an in-depth analysis of Maillard reaction by-products. We identified at least six distinct lipid classes derived from PE and PE-O, including formyl-PE (FPE), acetyl-PE (AcPE), Amadori products (AmPE and Am_2PE) [4], hydroxy methylfurfural-PE (HMF-PE), glucuronosyl-PE (GlcA-PE) and lyso forms. The most prevalent species contained 34 and 36 carbon atoms. To better understand the formation of these by-products, we developed a reaction scheme and included fragmentation rules for regiochemical attributions.

Conclusions

As edible insects are poised to become a significant food source, thorough research into their health implications is crucial. This study represents a pioneering effort to explore Maillard reaction products in edible insects, offering critical insights into their potential effects on food quality and human health. Understanding these by-products will help ensure that the benefits of edible insects are realized without compromising safety or quality. The MR-modified lipids hold potential significance also for biofuel production [5], as future agricultural by-products from edible insect processing could undergo similar modifications. Therefore, thorough characterization of these compounds is crucial for optimizing their use in biofuels and other sustainable applications.

Acknowledgements

This work was supported by the project 2023-UNBACLE-0241870 - Lipid7, financed by Uniba ERC SEEDS project and by the project PON Ricerca E Innovazione– Azione IV.6 “Contratti di ricerca su tematiche Green” - 2014-2020 DM 10/08/2021 N.1062 MISURA GREEN H95F21001190006, 02-G-14853-2, financed by the MIUR.

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P41

Analysis of the phenolic compounds, volatile profile and evaluation of the antioxidant activity of different varieties of honey in Italy

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Summary: In this study commercial honey (different botanical and geographical origin, different brands and different years of production) were studied in terms of antioxidant activity, polyphenol content, colour and volatile profile in order to have more information and find possible correlations between composition and origin.

Keywords: honey, polyphenols, volatiles.

Introduction

The interest in studying honey is continuously increasing alongside its increasing global production [1]. Honey is "the natural sweet substance produced by Apis mellifera bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature" [2]. It is composed mainly of sugars (80-85%), water (15-17%) and proteins (0.1-0.4%), while in small part by enzymes, organic acids, vitamins, and phenolic compounds [3]. All these compounds are responsible for the physical and biochemical properties of honey, especially anti-inflammatory, antioxidant, antimicrobial, anticarcinogenic, anti-diabetic properties, which give to this natural product a great potential as a health-promoter [4,5]. Physical, biochemical and sensory properties are influenced by the botanical origin, the geographical area, flora and entomological sources and processing factors.
In addition, several studies demonstrated that volatile compounds of honey can be used as fingerprints to determine the geographical origin and to expose any adulterations [6-9]. However, it's difficult to find specific chemical markers for honey [10,11] as the composition of honey is influenced not only by the botanical source, but also by geographical origin, harvesting season, storage conditions, possible interactions between chemical compounds in the honey that occur naturally and also during thermal processing [12,13].
Given the high number of variables that influence the composition of honey, it's difficult to find criteria to determine the origin of the honey or detect any fraud. In this study different types of commercial honey (different botanical and geographical origin, different brands and different years of production) were studied in terms of antioxidant activity, polyphenol content, colour and volatile profile in order to have more information and find possible correlations between composition and origin.

Experimental

Eighteen honey samples were used for all analysis: nine samples of unifloral honeys (2 chestnut, linden, strawberry, sunflower, coriander, and 3 acacia samples) and 9 samples of a multifloral honey were analyzed. Antioxidant activity (by TPC, TFC, AOA), colour, polyphenol content by HPLC-MS/MS and volatiles by GC/MS were determined in honey samples.

Results

TPC, TFC and AOA were related each other and with the colour (expressed in PFUND) of honey and results showed that honey samples with high TPC values had a very dark colour (such as chestnut, strawberry honey samples), while those with a low TPC value resulted to be very light (acacia honey samples). The composition of honey, in terms of polyphenols and volatiles was quite different among the samples, confirming the great influence not only of the botanical origin but also of other factors, such as the geographical origin, harvesting season and storage conditions.

Conclusions

This study confirmed the great potential of honey as natural antioxidant source and its potential use for nutraceutical purposes. However, it is complex to determine specific quality marker, due to the high variability of composition. Further studies are needed to find specific molecules that are affected by fewer variables.

Acknowledgment

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P42

Amino acids, phenolic and flavonoid contents in two diverse extracts of *Spinacia Oleracea L.*: evaluation of in vitro antioxidant and enzyme inhibitory activity

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Summary: *The aim of this work is to evaluate the profile amminoacids, phitochemical compounds and analyze antioxidant and enzyme inhibitory activity in samples of Spinach obtained by two different methods of extraction.*

Keywords: *Amminoacids, Antioxidant, Enzyme inhibitory activity.*

Introduction

Spinach (*Spinacia oleracea L.*) is a green herbaceous annual leafy vegetable cultivated in many parts of the world, characterized by low cost and widely used in many traditional dishes.
It is considered a functional food for its nutritional composition, phytochemicals and bioactive compounds that contribute to reduce oxidative stress, inducing secretion of satiety hormones, helping to promote protection mechanisms against hypoglycaemia, cancer and obesity [1].

Experimental

Plant materials were collected for the protein extraction process. Two extraction processes were conducted in which one involved the use of $CaCl_2$ (sample called S2), while the other one was extracted as such (sample called S1).
Then the amino acid content was determined in both samples using HPLC-DAD technique with the aim to investigate the phytochemical profile, together with the phenolics and flavonoids compounds. To allow the identification and quantification of amino acids using HPLC-DAD, derivatization with the fluorenylmethyloxycarbonyl (Fmoc) group was carried out following a previously described procedure [2].

Results

Data reveal that sample S2 presents Isoleucine (12.16 µg/mL) while, the sample S1 contains Lysine (4412,6 µg/mL) and Tyrosine (9,02 µg/mL). Both of them, present a comparable total phenolic content instead of the total flavonoid content in which sample S1 shows a higher quantity (3.7 mg RE/g). Biological assays show a higher antioxidant activity in sample S1 by ABTS (21,3 mg TE/g) and metal chelating (32,3 mg EDTAE/g) assays, than sample S2 in antioxidant tests by CUPRAC (21,6 mg TE/g) and FRAP (13,6 mg TE/g). Finally, sample S2 exhibits greater inhibition of tyrosinase, than sample S1. Sample S1 exhibits greater inhibition of the glucosidase enzyme than sample S2.

Conclusions

In conclusion, sample S1 reveals better amino acid content, antioxidant activity and enzyme inhibitory activity among them. Further studies are view to improve the protein extraction method promoting the development of enriched foods and beverages.

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P43

Characterization of flavonoids in ancient Lucanian plum varieties using RPLC-ESI-FTMS: a rapid screening approach

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Summary: *This study characterizes flavonoids in ancient Lucanian plum varieties using RPLC-ESI-FTMS. It highlights the method's ability to distinguish flavonoid isomers and epimers and introduces a rapid screening approach for compound detection, supporting genetic preservation efforts within the BioDruBa project.*

Keywords: *Flavonoids, RPLC-ESI-FTMS, Lucanian plum varieties*

Introduction

The antioxidant activity of phenolic compounds in fruits and vegetables, including flavonoids, significantly contributes to their recognized beneficial effects [1-3]. This study focuses on developing an analytical method for identifying these plant secondary metabolites, formally classified as lipids, using reversed-phase liquid chromatography coupled with high-resolution mass spectrometry (RPLC-ESI-FTMS). The primary goal was to characterize flavonoids present in ancient plum varieties of *Prunus domestica* [4] cultivated in the Basilicata region, South of Italy.

This research aims to highlight the unique features of these varieties and enhance their qualities as part of the BioDruBa project, in collaboration with the Institute of Biosciences and BioResources (IBBR) of Bari. While plum cultivation in Basilicata is not quantitatively significant, these ancient varieties hold immense natural heritage value. Therefore, genomic and metabolomic characterization based on unbiased criteria is essential [5,6].

Experimental

To identify the most suitable RPLC conditions, a standard mixture of flavonols was analyzed using a core-shell ODS column (150 x 2.1 mm ID, 2.7 µm). The gradient elution was performed with water and acetonitrile, both containing formic acid, and the column temperature was systematically investigated. All ion fragmentation (AIF) mode is a technique used to generate comprehensive fragmentation data for all ions entering the mass spectrometer. In AIF mode, instead of selecting a specific precursor ion for fragmentation (as in targeted MS/MS), all ions generated in the ion source are subjected to fragmentation in the higher-energy collisional dissociation (HCD). This Orbitrap-exclusive analysis mode was used to promptly identify the various phenolic classes.

Results

Flavonoids, a class of polyketides that includes anthocyanins, were investigated. Due to the common occurrence of isomers and epimers among these compounds, well-performed chromatographic separation is essential for accurate identification, as tandem MS spectra alone are insufficiently distinctive. Ethanolic extracts of Lucanian plums were then analyzed using RPLC-ESI-FTMS, and their chromatographic profiles were compared and the presence of both known and unknown flavonols was ascertained (Figure 1). The presence of flavonoids was confirmed, and their identification was discussed in terms of confidence levels [7]. Neochlorogenic acid, catechin, epicatechin, rutin, chlorogenic acid, and procyanidin b were identified and quantified using standard compounds, while the structure of other phenolic derivatives was investigated by MS/MS data. Furthermore, the acquired data in AIF mode provided a rapid screening method to detect and identify the occurrence of certain flavonoid compounds, including isomeric species.

Conclusions

The developed analytical method successfully characterized the flavonoid content in ancient plum varieties from Basilicata, revealing their potential as valuable genetic and natural resources. Using RPLC-ESI-FTMS combined with AIF MS/MS data proved to be a powerful approach for identifying and distinguishing different flavonoids, including isomers. This method not only helps preserve these unique plum varieties but also offers a rapid screening tool to assess the presence of specific compounds, supporting the broader objectives of the BioDruBa project.

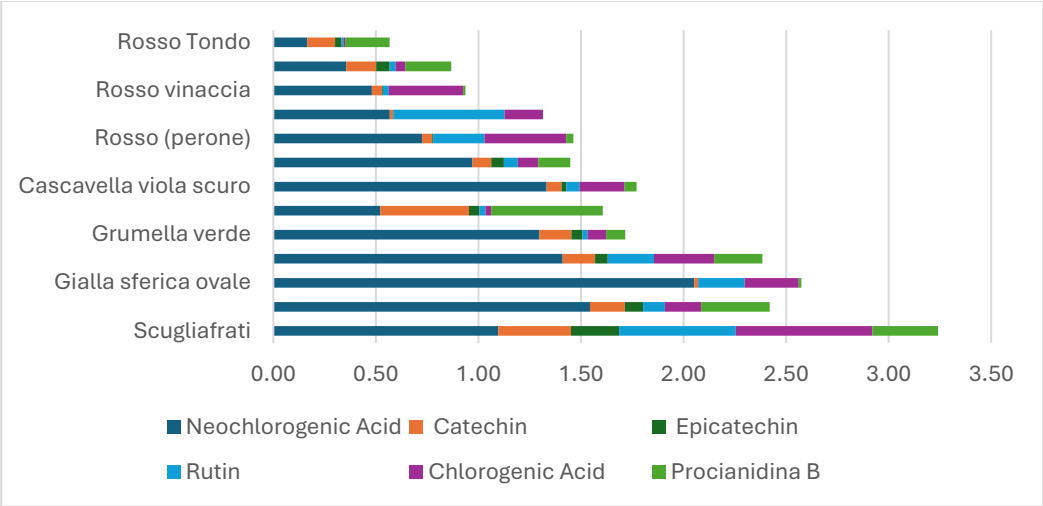

Figure 1. *Distribution of antioxidant compounds in representative plum samples.*

Acknowledgements
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P44

Antioxidant activity and MS-analysis of milk and cheese produced by ewes fed with silages of prickly pear by-products (*Opuntia ficus-indica*)

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Summary: *By-products of Opuntia ficus-indica are rich in bioactive substances (proteins, vitamins, minerals and polyphenols), which can be used to improve the chemical-nutritional characteristics of milk and cheese samples from sheep fed with these wastes.*

Keywords: *food wastes, fatty acids, polyphenols*

Introduction

Industrial and technological growth has led to an increase in waste and by-products, which requires green and circular economy approaches. The waste resulting from Opuntia ficus-indica can exceed 40% up to 55% of the total weight of the fruit. These by-products lend themselves well to ensiling1 and are rich in bioactive substances such as proteins, vitamins, minerals, carbohydrates and polyphenols, and have multiple physiological activities such as antioxidant, anti-inflammatory and antimicrobial2. The aim of this study was to determine the chemical-nutritional characteristics of prickly pears by-products silages (PPS) (peels+12% of wheat bran and prickly pears peels, pulp, seeds + 12% of wheat bran), bulk milk and cheese samples from ewes fed with these PPS, in order to evaluate their effects on their quality.

Experimental

Milk, cheese and PPS samples were subjected to qualitative-quantitative chemical characterizations on the lipidic and phenolic fraction. Cheeses samples were subjected to triangular test. PPS, milk and cheese samples were freeze-dried before analysis. All the samples were analysed for their antioxidant activity by DPPH and ABTS tests, total phenolic content (TPC) by Folin-Ciocalteau test, phenolic profile by UHPLC-ESI/MS-MS analysis and fatty acid profile by GC- MS method.

Results

PPS showed considerable antioxidant activity, especially the PPS peels, which was reflected in the milk and cheese samples of the ewes fed with this by-product. In particular, cheese samples showed higher anti-radical values (0.375-0.710 mmol TEAC/100 g) respect to the control (0.122 mmol TEAC/100 g). Analyses carried out on TPC showed a higher value in milk and cheese samples (2.04-3.41 mg GAE/g), respect to the control samples (0.24 mg GAE/g). Supplementation of PPS in the sheep's diet, however, did not significantly influence the fatty acid profile of milk and cheese samples and the sensorial test (P>0.10).

Conclusion

The feed supplementation with Prickly pear by-products silage represents an optimal strategy to increase the antioxidant activity of ewe milk and, consequently, the production of innovative cheeses enriched with bioactive molecules.

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P45

Acorn flour extracts: analysis of bioactive compounds by HPLC-ESI-MS/MS and evaluation of their antioxidant and enzyme inhibitory activities

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Summary: *In this study acorn flour extracts (obtained at 20, 60, 80 and 100 °C) were analyzed in terms of polyphenolic content, antioxidant activity and enzyme inhibitory activity to exploit possible applications in the nutraceutical, pharmaceutical and cosmeceutical fields.*

Keywords: *Acorn, enzyme inhibitory activity, antioxidant activity.*

Introduction

Acorns, nuts derived from *Quercus Genus*, are known for their nutritional benefits, mainly related to the presence of polyphenols, which exhibit strong antioxidant, antibacterial, and antitumor properties [1-3], and unsaturated fatty acids, which are critical components in the prevention of chronic diseases such as obesity and cardiovascular disorders [4]. Recently, acorn flour has also gained attention for its potential in gluten-free baking [5]. This research aimed to characterize, for the first time, acorn flour extracts (obtained at 20, 60, 80 and 100 °C) in terms of polyphenolic content, antioxidant activity and enzyme inhibitory activity in order to exploit possible applications in the nutraceutical, pharmaceutical and cosmeceutical fields.

Experimental

Acorn flour consisted, according to the product's technical specifications, in dried and ground acorns of the pedunculate oak *Quercus robur* (AF, Dary Natury, Koryciny, Poland; https://darynatury.pl/). Acorn flour contains these ingredients: roasted and ground acorns 100% product of organic farming. Four flour extracts (obtained at 20, 60, 80 and 100 °C) were studied to determine the polyphenolic content (by HPLC-ESI-MS/MS), the antioxidant activity (by TPC, TFC, DPPH, ABTS, CUPRAC, FRAP, MCA and PBD) and the enzyme inhibitory activity (by cholinesterase, butyrylcholinesterase, tyrosinase, amylase, and glucosidase inhibition tests). Results were subjected to statistical analysis (One-way analysis of variance ANOVA, Fisher's LSD post-hoc test and Pearson correlation analysis).

Results

The study identified a diverse range of phenolic acids, flavonoids, and other bioactive compounds, with significant variations in concentrations influenced by the temperature of extraction, as previously reported [6]. The highest concentration of all phenolic compounds was found in acorn flour extract at 60 °C, mainly due to the presence of gallic acid, rutin, ellagic acid, quercetin, kaempferol and isorhamnetin. This result was in agreement with results of the antioxidant and enzyme inhibitory activities, which revealed the great potential of these extracts (mainly that obtained at 60 °C) as natural antioxidant sources and as adjuvants for the treatment of some pathologies influenced by disorders of enzymatic activity. Pearson correlation's results were reported in Figure 1.

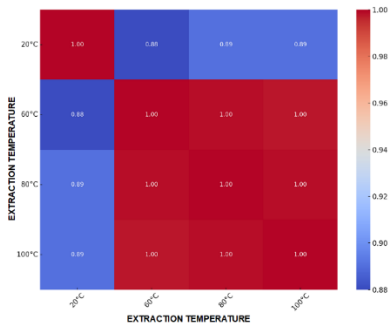

Figure 1. *Pearson correlation matrix of bioactive compound profiles and bioactivity assays at different extraction temperatures. The color intensity and numerical values indicate the strength of the correlation between different temperatures. High correlations were observed between extracts at 60 °C and 80 °C, 60 °C and 100 °C, and 80 °C and 100 °C, suggesting similar extraction efficiency and bioactivity profiles across these temperatures.*

Conclusions

In this study, acorn flour extracts of *Quercus Robur* species were analysed, for the first time, to determine the polyphenolic content, the antioxidant activity and the enzyme inhibitory activity. Results showed a strong relationship between extraction temperatures, analyte profiles and bioactivity assays, indicating 60 °C as the optimum temperature to obtain the highest polyphenol content, antioxidant and enzyme inhibitory activities. This result was also confirmed by the correlation analysis, which provided insights into optimizing extraction conditions for maximizing the yield of beneficial compounds from acorn flour. The research not only provides a deep identification of acorn flour's bioactive components but also establishes its potential for health-promoting uses, suggesting its potential use in food, nutraceutical and cosmeceutical products.

Acknowledgment

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P46

UHPLC/QTOF study of chemical markers in grapevine leaves as a response of plant water stress

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Summary: *Climate changes and pathogens control can be mitigated using vine varieties high-tolerant to fungal diseases and hot/dry climates. Secondary metabolites in leaves of two resistant vine varieties undergone to different water supply were determined by UHPLC/QTOF. Targeted/untargeted data analysis provided hundred formulae with score >99% which are under study to identify putative markers of water stress.*

Keywords: *UHPLC/QTOF; grapevine leaves; water stress marker*

Introduction

Currently, the main problems in viticulture are 1) the increase in temperature and heatwaves and decrease in rainfall resulting in increasing water stress, 2) control of the pathogens and consequent environmental and health impact due to the many phytosanitary treatments/year which are often needed. In the last century, new grape varieties characterized by high tolerance to powdery mildew and downy mildew were produced by crossing *V. vinifera* and other *Vitis* varieties. Recently, many wine farms mainly sited in Northern Italy have planted some of these varieties (e.g., in regions such as Trentino-Alto-Adige, Veneto, Emilia Romagna, Friuli Venezia Giulia) and other farms sited on middle-south are interested to do it. Anyway, to promote their diffusion also in the southern regions, it is essential to know their tolerance to warmer/dry environments. Assessment of water content in vegetative tissues associated to the quality of grapes can provide important information on the attitude of these varieties to hot/dry climates, and secondary metabolites in leaf can potentially be studied as markers of water stress and to generate a picture of the metabolic pathways involved in the physiological response of vine to water stress effects.

Experimental

Samples

Cabernet Volos (a red-berry grape *cv*) and Sauvignon Rythos (a white-berry grape *cv*) vines used in the study are planted in a 6-year vineyard/guyot trained sited in a hot/dry environment in Southern Italy (Due Palme Co, Brindisi, Puglia) and grafted on rootstock (P1103). 120 vines of each variety were grown in 2022 and 2023 by providing weekly two different water supplies starting from April till the harvest: full-watered (control) and 50% water irrigation deficit (thesis). Soil water content was monitored by FDR system (EM50 datalogger) with sensors “10 HS” buried at a 30 and 50 cm depth and a WatchDog Weather Station 2900 ET was used for monitoring rainfall, temperature, solar radiation, relative humidity, wind speed and direction, dew point, and evapotranspiration. Leaves were collected in the two years at different vegetative stages and the samples were immediately frozen.

Sample preparation

Leaves (2 g) were homogenized by crushing using liquid N_2 and 1 g of powder was extracted with 10 mL ethanol/H_2O 8:2 solution under stirring for 20 min. One milliliter of water was added to 1 mL extract, followed by 100 µL of 3,7 dihydroxy flavone 20.33 mg/L as IS, and 50 mg of NaCl. The solution was washed with 2 mL of hexane and the aqueous phase was recovered. The solution was filtered on a Clarify-PTFE 0.22 µm filter (Phenomenex, Torrance, CA, USA) and the sample was collected in a vial for LC analysis. Two extracts of each leaf sample, were prepared.

UHPLC/QTOF analysis

LC/QTOF MS negative-ionization analysis was performed using an ultra-high-performance liquid chromatography (UHPLC) 1290 Infinity pump coupled to 1290 Infinity Autosampler (G4226A) and 6550 accurate-mass quadrupole time-of-flight (QTOF) mass spectrometer (40,000 resolving power FWHM) equipped with Dual Agilent Jet Stream Ionization source (Agilent Technologies, Santa Clara, CA). Chromatographic and instrumental conditions were the same previously reported [1,2].

Metabolites identification

Agilent MassHunter Qualitative Analysis Software B.05.00 (5.0.519.0), was used. First *targeted* identification of putative metabolites was performed using the algorithm *Find by Formula* and the homemade of grape

compounds database *GrapeMetabolomics* using the chromatographic retention times. A second-step identification was performed to identify new putative molecular formulae using the algorithm *Find by Molecular Feature* and confirming the structures by HR-MS/MS. $[M-H]^-$ signals of metabolites were normalized to the IS $[M-H]^-$ signal [3,4].

Results

Targeted identification using *GrapeMetabolomics* provided tens metabolites mainly belonging to the chemical classes of flavanols and flavonols, such as catechin and glycosidic derivatives of quercetin, isorhamnetin, and kaempferol. The algorithm *Find by Molecular Feature* provided the identification of around 240 formulae (MF) with score >99% calculated on the isotopic pattern, which included 136 with MF $C_nH_mO_p$, 46 with MF $C_nH_mO_pN_k$, and 8 with MF $C_nH_mO_pS_q$. Main MFs identified are summarized in the Table 1.

Currently, the presence/absence of MFs and differences in signals intensity in samples collected from vines grown with different water supply are under study to identify possible putative markers and understand the metabolic pathways induced by water stress.

Table 1. *Molecular formulae identified in vine leaf extract with score >99% by the algorithm Find by Molecular Feature*

MF id. (score %)	MFs	MF range	heteroatoms		
			O	N	S
99.0 - 99.8	136	$C_{11}H_{20}O_n$ - $C_{39}H_{48}O_n$	3 - 18		
99.0 - 99.8	46	$C_{10}H_{13}N_mO_n$ - $C_{37}H_{59}N_mO_n$	3 - 17	1 - 10	
99.1 - 99.7	8	$C_{10}H_8O_nS$ - $C_{27}H_{46}O_nS$	3 - 11		1

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P47

SPME-GC-MS investigation on Sicilian hemp seed cake flour, with a preliminary evaluation of its sensory properties

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Summary: *Scope of the present study was the evaluation of the physical, chemical and sensorial properties of some samples of Hemp Seed Cake Flour produced in Sicily. Beyond the flour, bread samples were prepared by using mixtures of wheat flour and HSCF at different ratios: 0%, 10%, 20%, and 30% HSCF, respectively.*

Keywords: *Cannabis sativa,* hemp seed cake flour, GC-MS

Introduction

Hemp seed cake flour (HSCF), a by-product of the hemp oil production process from the seeds of *Cannabis sativa*, has been widely demonstrated to contain important bioactive molecules: up to 50% proteins, 9-20% lipids, 6-7% dietary fiber, considerable amounts of minerals. In fact, hemp seeds are mainly used as animal feed, but there is growing interest in their usage for human nutrition as a source of nutrients, for its nutritional and medicinal properties [1].
The European Industrial Hemp Association (EIHA), the only pan-European membership organization in the industrial hemp sector, whose main objective is to monitor EU hemp related policies, on November 2023 has published a revised version of the Novel Food Catalogue which includes cannabinoids and cannabidiol as new entries, confirming the importance acquired by hemp products in the Union [2]. The proven health-promoting effects of hemp and hemp-based products have become strongly appealing to the public interest. As an example, hemp seed oil (HSO) represents an active ingredient in the formulation of food supplements and cosmetic products, useful for the relief from a variety of ailments, such as psoriasis, dermatitis, hypertension, dyslipidemia. In line with sustainable food production and a circular economy, this research aims at evaluation of the physical, chemical and sensorial properties of some samples of HSCF produced in Sicily.

Experimental

The hemp flour was obtained from stone-ground hemp seeds and it was provided by a local producer, namely Molino Crisafulli, located in Caltagirone, Catania, Italy.
Moisture, ashes, fiber, carbohydrates, proteins, polyphenols, tocopherols, sterols, and fatty acids were assessed. Additionally, Headspace-Solid-Phase Microextraction (HS-SPME) coupled to GC-MS was applied to the analysis of the volatiles released by flour samples. Beyond the flour, bread samples were prepared by using mixtures of wheat flour and HSCF at different ratios - 0%, 10%, 20%, and 30% HSCF, respectively. Then, a panel test was carried out by trained personnel in order to evaluate the sensory quality of bakery products containing HSCF, hence, consumer's acceptance.

Results

Moisture (%)	6.44 ± 0.31
Protein (%)	27.37 ± 0.12
Lipid (%)	10.44 ± 0.43
Fiber (%)	46.27 ± 0.26
Ash (%)	4.53 ± 0.22
Carbohydrate (%)	4.73 ± 0.51
Total polyphenols (mg/Kg flour)	815 ± 33
α-tocopherol (mg/Kg fat)	18.85 ± 0.28
δ-tocopherol (mg/Kg fat)	76.76 ± 0.39
γ-tocopherol (mg/Kg fat)	9.70 ± 0.17

Figure 1. *Proximate composition of hemp seed cake flour*

Compound	RI DB	RI exp	Mean	RSD	Compound	RI DB	RI exp	Mean	RSD
b-ionone epoxide	-	-	4.69	0.71	2-ethylhexanol	1030	1037	0.47	0.06
ethyl alcohol	446	-	2.42	0.47	(E)-b-ocimene	1044	1040	0.48	0.07
acetone	481	-	4.56	0.58	g-hexalactone	1060	1052	0.23	0.03
acetic acid	640	-	19.95	1.59	g-terpinene	1054	1056	0.85	0.12
a-pinene	932	932	0.93	0.05	(E)-caryophyllene	1417	1420	3.52	0.49
(2E)-heptenal	956	960	0.24	0.10	a-trans-bergamotene	1432	1435	0.58	0.03
hexanoic acid	979	978	1.07	0.15	(Z)-b-farnesene	1440	1440	0.15	0.03
2-pentylfuran	991	989	6.37	0.34	a-humulene	1452	1456	1.08	0.14
decane	1000	1000	0.82	0.03	alloaromadendrene	1458	1462	0.28	0.02
octanal	1006	1005	0.30	0.04	b-selinene	1492	1495	0.40	0.02
d-3-carene	1008	1010	0.19	0.06	a-selinene	1501	1505	0.22	0.07
p-cymene	1025	1026	2.15	0.19	caryophyllene oxide	1582	1579	0.26	0.04
limonene	1030	1031	1.05	0.17	isoamyl laurate	1814	1809	0.21	0.03
1,8-cineole	1032	1035	0.60	0.07					

Figure 2. *VOCs distribution in hemp seed cake flour*

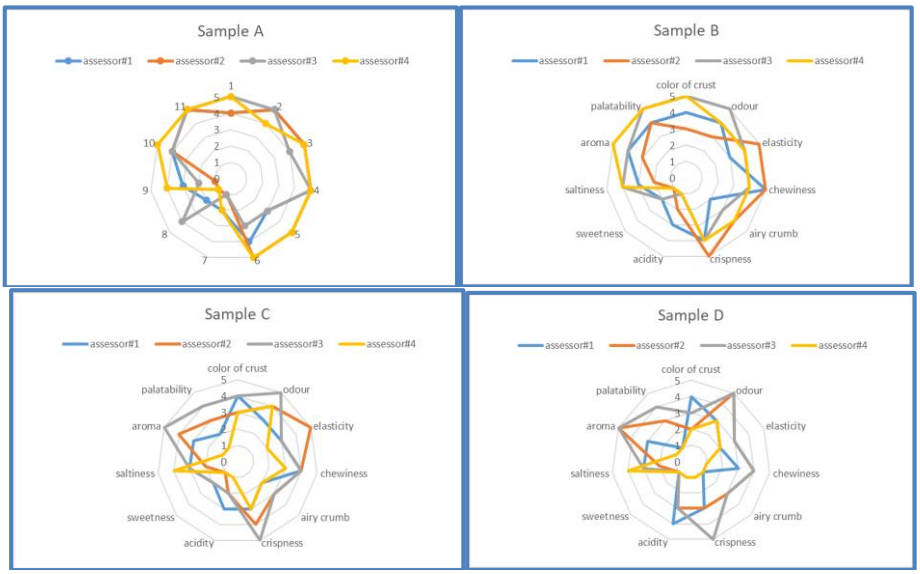

Figure 3. *Sensory panel of bread sample made with hemp seed cake flour.*
Sample description: ***A****, 0% hemp flour;* ***B****, 10% hemp flour;* ***C****, 20% hemp flour;* ***D****, 30% hemp flour.*

Conclusions

Hemp (*Cannabis sativa L.*) seeds contain a high concentration of proteins and biologically active compounds. The chemical composition of this material and its low cost make it an attractive ingredient for the development of value-added food products.

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P48

Cyclic ion mobility mass spectrometry: advances in metabolite separation and identification and high spatial resolution using a desorption electrospray ionization (DESI) source

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Summary: *Separation and accurate identification of metabolites are crucial points in mass spectrometry analysis. Cyclic ion mobility mass spectrometry helps on deep metabolomic characterization, separating isomers and isobars, adding another level of identification and allowing to perform high spatial resolution metabolomics when coupled to a desorption electrospray ionization (DESI) source.*

Keywords: *Ion mobility (IM), Mass spectrometry imaging (MSI), Desorption electrospray ionization (DESI)*

Introduction

The use of mass spectrometry with cyclic ion mobility technology (Cyclic IMS) [1,2] is a powerful tool to assign molecule identification more accurately than classical high-resolution spectrometry, based on Collisional Cross Section (CCS) values, that are values independent from the chromatographic method used derived from to the drift time of ions within the ion mobility cell, correlated with the shape, size and charge of the ion. Furthermore, this technology allows the separation of molecules having the same m/z ratio and the same chemical-physical properties, and the identification of a greater number of metabolites in the analyzed matrices. The ion mobility technology can be coupled to an electrospray ionization desorption interface (DESI) [3] allowing non-destructive and spatially highly resolved analyses to be carried out on whole samples/tissues or semi-processed materials "spotted" on special slides, which will allow rapid analysis of the metabolic content of the matrices of interest without the need of chromatography.

Experimental

To test the potential of the new acquired instrument, SELECT SERIES Cyclic IMS (Waters) (Figure 1), we performed bulk and spatial metabolomic analyses on different plant material followed by untargeted analysis. We analysed different plant materials by liquid chromatography and electrospray ionization (ESI) source or by direct analysis on the DESI source. Spatial analysis included tomato fruit imprinted in membrane, curcuma rhizome ultrathin cross-section and *Bixa orellana* seeds and non-polar extract spotted on multi well glass plates (Figure 2).

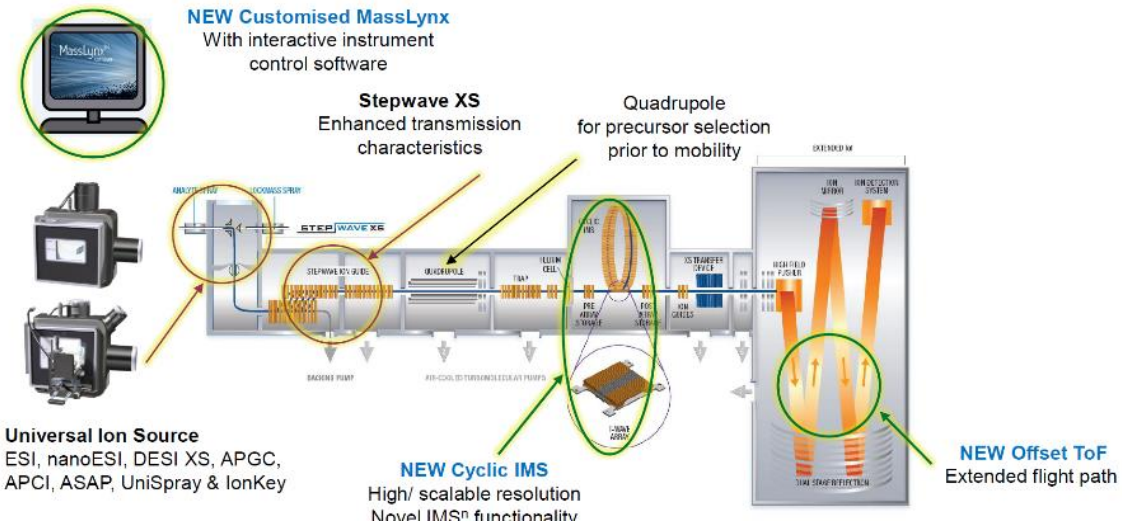


Figure 1. *Representation of the SELECT SERIES Cyclic IMS (Waters) mass spectrometer components*

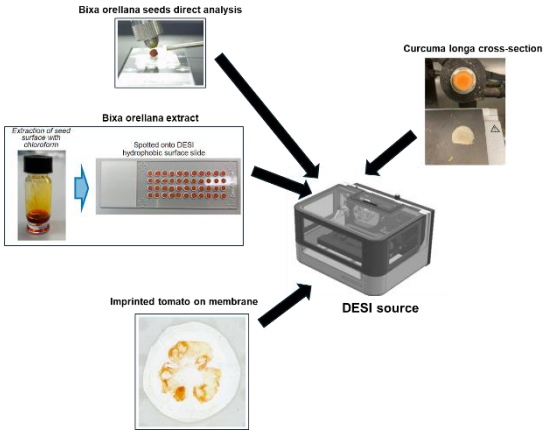
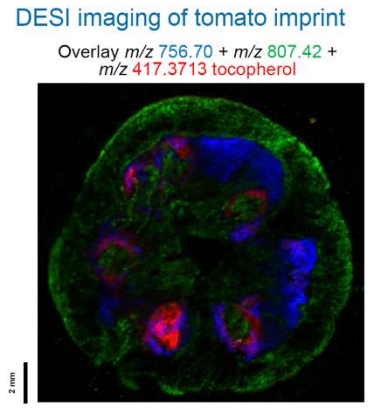


Figure 2. *Examples of samples analysed using the DESI source*

Results

Separation and identification of isomers and isobars and accurate assignment of different metabolites based on CCS values were performed for different plant extract including saffron and tomato semi-polar extract.
MS images obtained using the DESI source showed great detail of spatial localizations of a wide variety of molecules with high accuracy (see Figure 3 as an example).



Metabolite	Chemical formula	adduct	Calculated ion mass (m/z)	Observed ion mass (m/z)	Mass delta (ppm)
tocopherol	C28H48O2	H+	417.3727	417.3713	-3.4

Figure 3. *Tocopherol (in red) spatial distribution in tomato imprint using the DESI source*

Conclusions

The request for cyclic ion mobility is necessary due to the complexity of the mixtures analyzed which are rich in isomeric/isobaric compounds, identifiable only thanks to the separation by ion mobility. In particular, cyclic ion mobility currently represents the technology with the highest mobility resolution value. The ion mobility cell allows to separate structural isomers, isobars and conformers based on their cross section (CCS), greatly improving peak capacity, resolution, spectral quality, result reliability and detection limits, avoiding interferences in complex and varied matrices.

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P49

An untargeted metabolomic study on Melinda *Golden Delicius* apples: a comparison between commercial bio-packaging at different times

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Summary: *An untargeted study was performed on Melinda Golden Delicius apples using three polymeric films at different times. Direct infusion electrospray ionization Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry (MS) and targeted chromatographic assay by HPLC-PDA/MS were exploited to afford the phytochemical complexity and monitor the quality and preservation of the extracts.*

Keywords: *metabolomics; FT-ICR; apples.*

Introduction

Food packaging plays a crucial role in maintaining food quality during its distribution, nevertheless storage and packaging conditions may largely affect the composition of foods. The conventional use of plastic materials for quality preservation contributes significantly to environmental pollution and may pose a not negligible food contamination risk. in this context, it is becoming increasingly urgent the usage of proper biofilm materials to maintain food quality, ensure environmental and human safety, and improve consumers' health [1]. The present study is aimed at the metabolite profiling and the preservation monitoring of conventional apples (Melinda *Golden Delicious*). Three different commercially available packages were chosen for the study: i) ECO, from corn starch, cassava and eucalyptus, ii) PLA, made of polylactic acid from corn starch, and iii) RIF, a polyethylene film used as reference. Apple slices were stored in each package and the chemical analyses at three time points (0, 14 and 21 days) were carried out by means of ESI FT-ICR and HPLC-PDA/MS. Thanks to the high resolution and the extreme accuracy of this mass spectrometer it has been possible to obtain a univocal molecular formula which has been gathered to several metabolites in the range of less than 1ppm [2].

Experimental

Dried samples were pooled and ground and then extracted by Bligh-Dyer procedure to obtain hydroalcoholic (HA) and organic (Org) phases. The diluted solutions were directly infused in an ESI source and analysed by means of FT-ICR mass spectrometer (7T SolariX, Brüker Daltonics), using electrospray in positive ionization mode (ESI(+)). Spectra were acquired in the *m/z* 100-1000 mass range and each sample was analysed in three technical replicates. Then, the spectra were processed by using the software MetaboScape 5.0 (Brüker Daltonics) and the compounds were annotated with HMDB and LOTUS database uploaded to Metaboscape.

Results

The high-resolution mass analysis enabled to reveal more than 600 chemical formulas for each sample, showing a richness in both primary, such as organic acids, sugars, amino acids, and fatty acids, and secondary metabolites, including vitamins, terpenes, and polyphenols. Hazardous compounds like pesticides and mycotoxins were also detected, and quantified by HPLC-MS, below the maximum residue levels established by the EU regulation. To achieve a comprehensive qualitive evaluation of the samples chemical profile, several graphical visualisation tools, including van Krevelen diagrams and elemental composition histograms, were employed. These tools allowed to obtain interesting information about density of molecular classes, metabolic pathways among metabolites and correlation between samples. Indeed, RIF packaging showed a decrease in the region of terpenoids, polyketides and polyphenols at 21 days compared to the ones at 14 days. Instead, PLA and ECO retained the same amount of hits. Generally, the apples preserved in PLA and ECO packaging displayed a great abundance of terpenoids and polyketides compared to fresh apples (0 days). Moreover, the histograms revealed that the samples contained a higher percentage of CHO components, followed by CHNO, and in smaller amount, CHOP and CHNOP. In particular, at 14 and 21 days, the ECO biofilm presented more CHO formulas, mostly polyphenols and fatty acids, than both other biofilms and fresh apples; therefore, this evidence could suggest a better in the conservation and maturation of the fruit in ECO biofilm.

Conclusions

The high sensitivity and mass accuracy typically achieved with FT-ICR MS have allowed that a broad chemical and metabolomic profile have been gathered to monitor traceability and quality of apples. Overall, elemental formulas of many metabolites and harmful compounds present in trace amounts, like pesticides, agrochemical derivatives and metals, has been determined. In addition, this study indicates the ECO packaging to have the best capacity to maintain the good state of conservation of apples, especially in apple samples packaged for 21 days.

Acknowledgment

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P50

Metabolomic characterization of raw materials and novel food products for the promotion of Mediterranean lifestyle and healthy diet

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Summary: *This study is focused on the metabolomic profile of food ingredients and newly developed healthy snacks with high adherence to the Mediterranean diet. By Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) we characterized metabolites levels in different plant varieties and their changes in processed novel healthy food products. The findings emphasize the potential of Mediterranean ingredients in creating functional, health-conscious snack choices using advanced analytical methodologies.*

Keywords: *Metabolite profiling, Mediterranean Diet (MD), Healthy snacks*

Introduction

Despite the mediterranean diet's well-documented benefits, mediterranean countries are seeing significant nutritional changes that are compromising public health. It is necessary to modernize food production processes, preserve the nutritious value of local foods, and simplify supply chains from farm to fork [1]. The project PROMEDLIFE [2], aims to increase adherence to the mediterranean diet (MD) by encouraging the adoption of a healthy eating lifestyle while decreasing the environmental and economic impact of food production and processing. We characterized selected raw material and newly developed food products using a metabolomic approach to define nutritional, quality and sensory values while emphasizing nutritional and bioactive compounds. We also exploited new agronomical technologies of soil-less culture for saffron production from corms of various origins.

Experimental

LC-PDA-HRMS using a Q-Exactive mass spectrometer followed by targeted analysis was used to investigate the metabolite profiles of selected raw materials and of novel food products. We analyzed different varieties/ecotypes of argan oil, almonds, tomatoes, saffron and dates, and final products such as i) micro texturized and bioactive veggie snacks based on tomato fruits, ii) date bars with natural sugars iii) amlou, a Moroccan spreadable cream made of argan oil, almonds and honey, iv) biofortified yogurt with saffron syrup (Figure 1). For saffron we developed smart farming technologies for sustainable hydroponic cultivation.

Figure 1. *Raw material and final products characterized by metabolomic analyses.*

Results

We set up a sustainable production of saffron through hydroponic cultivation in controlled conditions using Italian and Moroccan corms. The saffron obtained from the hydroponic cultivation and from the classic cultivation in soil showed similar metabolomic profiles and high levels of saffron specific bioactive compounds of the apocarotenoid class (crocins and picrocrocin) in all cases (Figure 2).

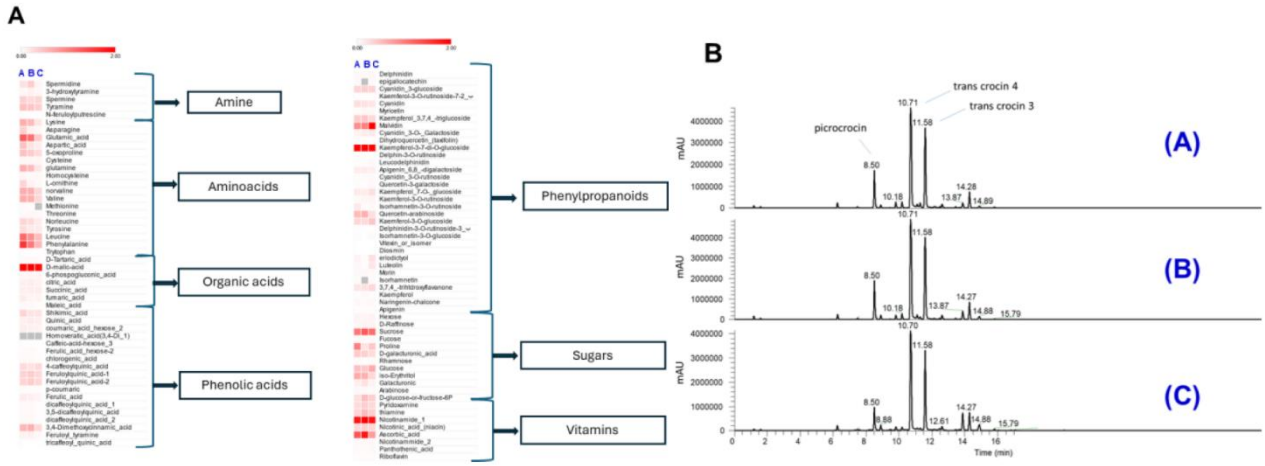

Figure 2. *Metabolomic analysis of saffron cultivated in hydroponics and in soil. A: heat map of primary and secondary metabolites levels. B: PDA chromatograms of crocins and picrocrocin.(A) Moroccan saffron grown in hydroponics.(B) Italian saffron grown in hydroponics.(C) Moroccan saffron grown in soil*

The analysis of the other raw materials and final products was performed using the same approach shown for saffron, and gave important information on the metabolic content in different varieties, that are useful for the formulation of the snacks.

Conclusion

PROMEDLIFE project aims to increase adherence to the MD through a multi-actor approach by encouraging the adoption of a healthy eating lifestyle while decreasing the environmental and economic impact of food production and processing. Within the project the ENEA team worked on the soil-less cultivation of different saffron ecotypes demonstrated that they are a valid alternative to the classic soil cultivation allowing to obtain high quality saffron in terms of bioactive compounds. The metabolic characterization of raw materials and final snacks products allowed to compare nutritional values of different varieties from different location of the Mediterranean basin, determining antioxidant/bioactive molecule levels and their changes in processed foods.

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P51

A targeted approach by HPLC-ESI-MS/MS (MRM) for tryptophan metabolites in food & beverages

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Summary: *A targeted HPLC-ESI-MS/MS (MRM) method for profiling TRP and ten related metabolites was developed, validated, and applied to various matrices differing for origin - vegetal or animal - and processing (fermentation). Different profiles were evidenced, suggesting a good versatility for application in food, agro-by-products, nutraceuticals, and botanical studies.*

Keywords: *sleep herbal teas; fermented drinks; serotonin*

Introduction

Besides acting as building block in protein synthesis, tryptophan (TRP) has a complex metabolism occurring through different pathways, the kynurenine, the serotonin and the indole ones, leading to a cascade of metabolites with different biological functions, in plants and animals [1]. Among the others, the neuro-transmitter serotonin (SER) and the neuro-hormone melatonin (MEL) are well-known crucial TRP metabolites in humans. TRP is bio-synthesized from plants and microbes; conversely, it is an essential amino acid for humans that need to intake it with diet. The higher levels of free TRP are found in soy and pumpkin seeds and in spirulina (https://ndb.nal.usda.gov/ndb/); TRP and metabolites have been found in vegetables, honeys, milk, wine [2], in malt and spent malt [3], in herbal products [4]. Moreover, the biotransformation of TRP into MEL by yeast strains has been reported, with application for the production of pharmacologically active metabolites [1].

Although some methods based on LC-MS/MS have been reported for the analysis of TRP metabolites [1,2,5], the phytochemical characterization, in terms of TRP metabolites distribution, of plants, plant foods, agro-food products, by-products and wastes, is limited and focused on few compounds.

A method for the simultaneous analysis of TRP and its key metabolites of the serotonin and indole pathways has been developed by a HPLC targeted approach using the multiple reaction monitoring (MRM) tandem mass technique for the selective and sensitive detection and quantitation of TRP and ten metabolites, namely 5-hydroxytryptophan (5-HTRP), SER, tryptamine (TRY), 5-methoxytryptamine (5OMETRY), N-acetyltryptamine (ACTRY), N-acetyl-serotonin (ACSER), MEL, L-tryptophan ethyl ester (TEE), 3-indoleacetic acid (IAA) and 5-hydroxyindole-3-acetic acid (5-HIAA). The validated method has been applied to a sleep herbal tea with added melatonin and to commercial samples of beer and kefir.

Experimental

Analyses were performed by a Water 1525µ HPLC connected to a Quattro Micro Tandem MS/MS with an ESI source. A Waters C18 XBridge column was used for the chromatographic separation, with the mobile phase A (water/0.02% formic acid) and B (acetonitrile/0.02% formic acid) flowing at 0.2 mL/min. The gradient elution was optimized by using a starting stock solution containing the 11 analytical standards (1 mg/mL STD in MeOH) for the 11 compounds. The MS parameters for the MRM method were optimized by direct infusion of the STDs solution, selecting the best ion transitions in ES+ or ES-. The calibration curves in the concentration range 10-80 ng/mL were calculated analysing the MRM peak area against the STD nominal concentration. All the quality parameters of the optimized method were evaluated. MRM chromatograms are shown in Figure 1. Commercial sleep herbal tea was prepared according to the instructions. A commercial beer was degassed by 10 min sonication. A commercial kefir was centrifuged and the supernatant recovered. All the samples were filtered 0.22 µm, diluted with the mobile phase and injected in triplicate for the analysis.

Results

A very good linearity was found, with $R^2 \geq 0.99$ for all of them. Analogously, good quality parameters were found for all compounds. LOD and LOQ values were found in the range 5.64-30.93 ng/mL and 17.08-93.74 ng/mL, respectively. 5-HTRP, SER, TRP, TRY and MEL were detected in all tested matrices, namely sleep herbal tea with added melatonin, commercial beer and kefir. Conversely, 5-HIAA, ACTRY and ACSER were not found in any samples. 5-OMETRY and TEE were found only in the fermented beverages beer and kefir. Last, IAA was found only in beer. Results are resumed in Table 1.

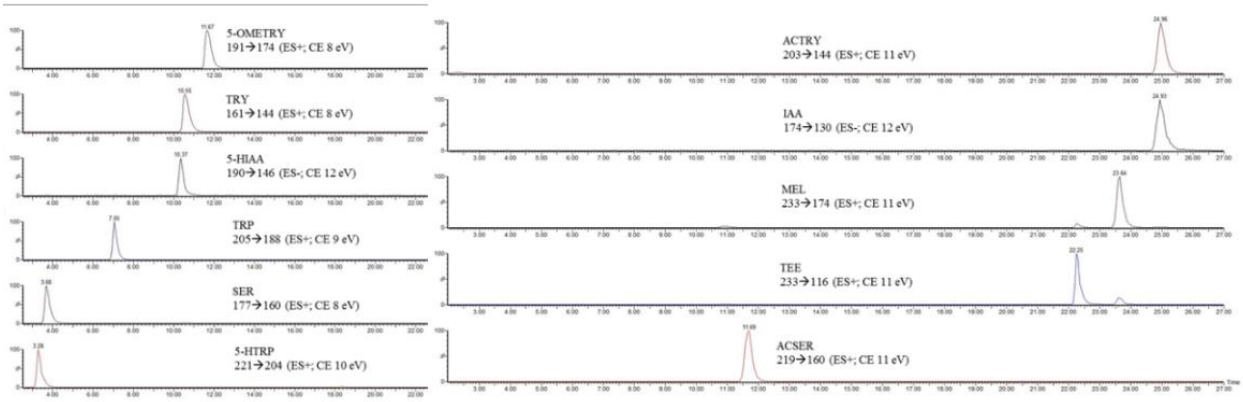

Figure 1. *MRM chromatograms of TRP and its metabolites, including ion transition, ionization polarity and collision energy*

Table 1. *TRP and its metabolites distribution in vegetal (sleep herbal tea, beer), animal (kefir) and fermented (kefir and beer) matrices.*

Compound	t_R (min)	Sleep herbal tea	Kefir	Beer
5-HTRP	3.28	x	x	x
SER	3.68	x	x	x
TRP	7.05	x	x	x
5-HIAA	10.37	-	-	-
TRY	10.55	x	x	x
5-OMETRY	11.67	-	x	x
ACSER	11.69	-	-	-
TEE	22.25	-	x	x
MEL	23.64	x	x	x
IAA	24.93	-	-	x
ACTRY	24.96	-	-	-

Conclusions

A targeted HPLC-ESI-MS/MS (MRM) method for the analysis also in low amount (ng/mL) of TRP and 10 metabolites belonging to the serotonin and indole pathways was developed and validated. The method was successfully applied for the analysis of various matrices differing for origin, vegetal or animal (herbal tea, beer and kefir), and processing (herbal tea and fermented beverages). Different profiles were evidenced: strong analogies were found between kefir and beer, in which the fermentation allows the way to melatonin; conversely, the serotonin pathway including TRY, 5-HTRP and SER resulted characteristic of the herbal tea, in which MEL is only an added ingredient. The indole pathway seems not to be characteristic of the chosen matrices, except for IAA found in beer.

Up to these preliminary results, the proposed method appears versatile for fast, sensitive and selective profiling of TRP and related bioactive compounds for application in food, by-products, nutraceuticals, as well also for botanical studies.

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P52

Unraveling the prebiotic effect of *Ulva lactuca* L. by *in-vitro* colonic fermentation and metabolome investigation

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Summary: *This study investigated the potential prebiotic effect of ulvan polysaccharide through in-vitro colonic fermentation. Results show increased short-chain fatty acids production and distinct changes in the gut metabolome, including elevated kynurenine and indole-3-propionic acid levels. This study provides insights into ulvan's impact on gut health and function.*

Keywords: *mass spectrometry, sea lettuce, ulvan*

Introduction

Ulva lactuca L., also known as sea lettuce, is a green marine seaweed distributed worldwide. It is involved in environmental concerning processes known as “green tides”; a phenomenon exacerbated by anthropogenic activities leading to water eutrophication [1]. *Ulva* is approved in Italy for the production of food supplements. *Ulva* contains unique polysaccharides called ulvan, which consist of disaccharides repeating units of rhamnose-3-sulfate (Rha3S) linked to *D*-glucuronic acid (GlcA), *L*-iduronic acid (IdoA) or *D*-xylose (Xyl). Ulvan has shown antihyperglycemic effect in aged diabetic mice [2] and anti-inflammatory and immunoregulatory activities in a murine model of DNBS-induced colitis [3]. However, the biological mechanisms involved in these activities and the influence on the human gut metabolome remain unexplored.
Thus, the aim of this study was to evaluate the prebiotic effects of purified ulvan through *in-vitro* colon fermentation and analysis of changes in gut microbial metabolome.

Experimental

Ulva lactuca was collected from an aquaculture tank belonging to Pescatori Orbetello cooperative, in the Orbetello lagoon, Tuscany, Italy. Ulvan extraction was conducted under acidic conditions for 105 minutes, followed by EtOH precipitation and freeze-drying. The dried crude extract was resuspended in water, dialyzed and freeze-dried again to obtain purified ulvan. The polysaccharide underwent *in vitro* enzymatic digestion following the INFOGEST 2.0 protocol. The undigested ulvan was then subjected to colonic microbial fermentation using an *in-vitro* static fermentation system [4]. The soluble fibre inulin and a growth control (no polysaccharide added) were included as positive and negative controls, respectively. Samples were collected at 0, 12, 24, 48 hours of fermentation. Short chain fatty acids (SCFA) and tryptophan-related metabolites were quantified using GC-FID and UHPLC-QQQ, respectively. Untargeted metabolomics was performed on methanolic extracts of fermented samples in both positive and negative ionization modes using an UHPLC-QTOF instrument.

Results

Overall, the production of SCFA, acetic, propionic and butyric acids, consistently increased over time (Figure 1a), with ulvan showing higher levels than the growth control and similar levels to inulin, except at the end of the fermentation (48h). The production of branched-chain fatty acids (BCFA), iso-butyric, valeric, iso-valeric acids, was comparable between ulvan and the growth control, but slightly lower for inulin (data not shown). SCFA are primary end-products of carbohydrate fermentation, suggesting that ulvan is metabolized by the colonic microbiota.
Among the 12 quantified tryptophan-related metabolites, a significant higher production of kynurenine was observed for ulvan over time (Figure 1b). Kynurenine pathway dysregulation is involved in immunoregulatory disfunction [5]. Interestingly, ulvan produced higher levels of indole-3-propionic acid (IPA) at 48h (mean values growth control/inulin/ulvan 0.07/0.03/0.12 µmol/L). IPA acts as an agonist on pregnane X receptor, enhancing gut epithelial barrier functions [6].

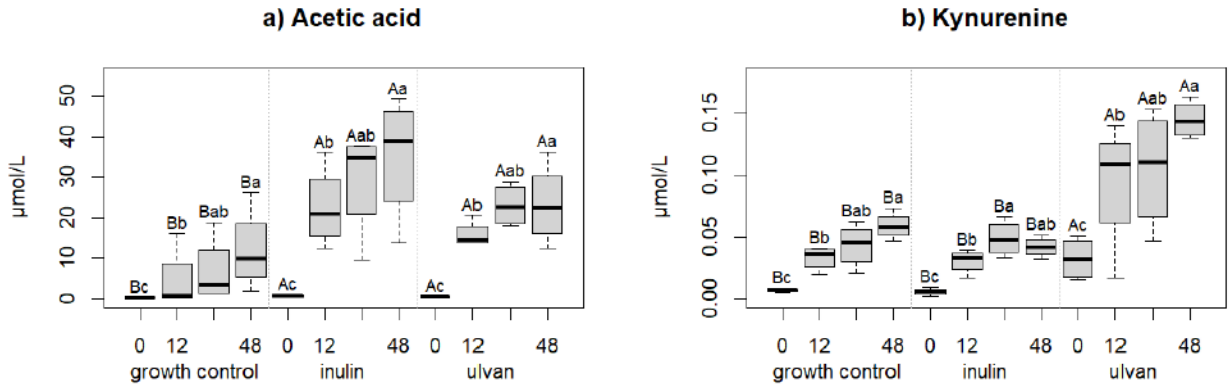

Figure 1. *Boxplots illustrating* ***a)*** *the sum of acetic, propionic and butyric acids production, and* ***b)*** *kynurenine production for the three treatments at the different timepoints. Significant differences over timepoints for each treatment and over treatments at the same timepoint are indicated by different lowercase and uppercase letters, respectively.*

Untargeted metabolomics analysis showed a distinct metabolite profile for ulvan samples, both in positive and negative ionization modes (Figure 2). Current on-going studies are investigating these metabolites differences and their potential roles to unravel the gut microbiota functions.

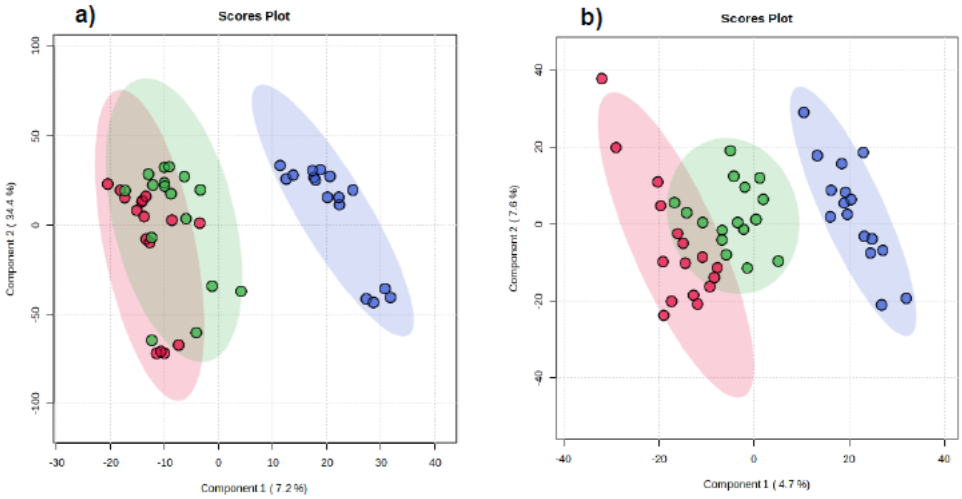

Figure 2. *Partial least squares-discriminant analysis (PLS-DA) scores plots of ulvan (blue), inulin (green) and growth control (red) samples using positive* ***(a)*** *and negative* ***(b)*** *ionization modes.*

Conclusions

This work represents the first comprehensive investigation of ulvan's impact on human gut metabolome. It provides insights into ulvan's potential applicability for upcycling *Ulva* biomass towards the development of functional food with prebiotic effects. Primary findings demonstrate ulvan's ability to modulate the gut microbiota, producing beneficial metabolites such as SCFAs and IPA. Further studies are necessary to explore the specific roles of these metabolites in the gut and their general implications for human health.

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P53

Development and validation of a method for the confirmation of six allergens in food by LC-QTOF

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Summary: *The protection of allergic consumers is fundamental for the public health and food industry. To detect unintentional contamination of food, a method was developed and validated for the quantification of six allergenic proteins by liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QTOF).*

Keywords: *Food allergens, peptides, LC-QTOF*

Introduction

Food allergy is a reaction of the immune system to some protein components, affecting about 5% of adults and 8% of children. The effects are mainly on the gastrointestinal, respiratory and skin systems, causing mild or even severe reactions such as anaphylactic shock. Allergy sufferers should avoid the offending foods in their diet and, in 2011, EU Regulation 1169/2011 highlighted 14 allergenic substances which must be necessarily declared in food labelling when deliberately used as ingredients [1]. However, during food production, possible unintentional cross contamination can happen and strict analytical controls are needed to protect vulnerable consumers. From many years, immunoenzymatic assays (ELISA) are the most used being fast and cheap; however, its main drawbacks are the possibility to give false positive results and the inability to analyze more than one allergenic ingredient. Accordingly, more recently, procedures based on liquid chromatography coupled to mass spectrometry (LC-MS) have been developed. Through the so-called bottom-up approach, LC-MS methods are able to indirectly determine allergenic proteins by detecting some of their characteristic (proteotypic) peptides. In this work, a confirmatory method was developed and validated to quantify milk, egg, peanut, almond, hazelnut and soy proteins in four main food commodity categories using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QTOF).

Experimental

The sample preparation protocol was that published by Planque et al. (2017) [2] with slight modifications. Analyses were carried out using a LC-QTOF system (SCIEX TripleTOF® 6600+) operating in positive ionization mode (ESI+). Data were acquired in MRM^{HR} mode. Chromatographic separation was achieved on a column ACQUITY UPLC® BEH C18 (2.1 mm x 150 mm, 1.7 µm, Waters) using acetonitrile and water both containing 0.1 % formic acid as mobile phases. The method was validated in the range 1-15 mg of allergen protein/kg performing seven replicates at each validation level (1, 5, 10 and 15) repeated on two separate occasions. Four commodity categories have been considered: "baked goods", "processed meat and meat-based products", "preserved vegetables" and "flours". Quantification was achieved applying the standard addition method [4].

Results

Candidate peptides were selected by means of *in silico* predictions and, then, confirmed by spiking experiments in food matrices representative of the four selected commodity categories. The quantification peptides for the 24 category-allergen combinations are listed in Table 1.

Table 1. *List of peptides used for quantification.*

Allergen	Quantification Peptide
Peanut	RPFYSNAPQEIFIQQGR
Milk	FFVAPFPEVFGK
Almond	GNLDFVQPPR
Hazelnut	INTVNSNTLPVLR
Soy	NNNPFSFLVPPQESQR
Egg	GGLEPINFQTAADQAR/ YPILPEYLQCVK[a]

[a]YPILPEYLQCVK was used as quantifier of egg proteins in "flours"

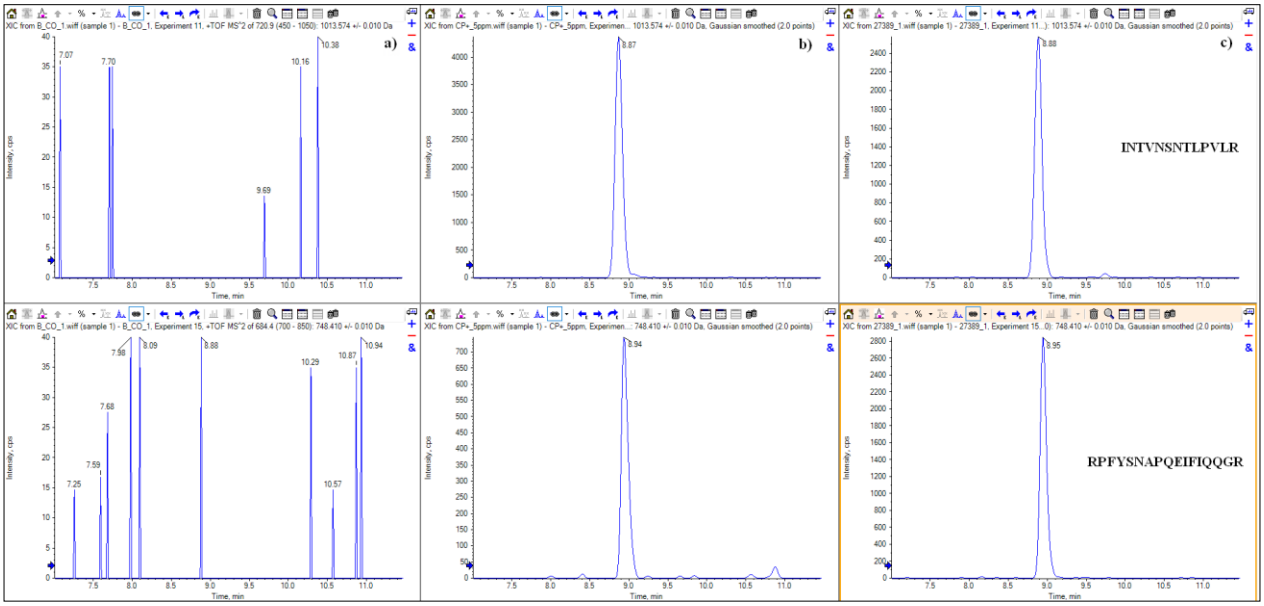

Figure 1. *LC-QTOF chromatograms of: a) "blank" cookie; b) cookie spiked with hazelnut and peanut (5 mg of allergen protein/kg); c) cookie analyzed within the Proficiency Test FAPAS 27389 (2024) containing proteins of hazelnut and peanut*

Method performances were assessed following AOAC SMPR 2016.002 guidelines [3]. Apparent recoveries were generally in the range 60-120 %, whereas coefficients of variation (CV%) evaluated in intra-laboratory reproducibility conditions were lower than 30 %. Estimated LODs and LOQs were between 0.6-3.1 mg of protein/kg and 1.1-10 mg of allergen protein/kg, respectively. In Figure 1 the chromatograms of hazelnut and peanut peptides detected in the test material (cookie) furnished by FAPAS are shown.

Conclusions

LC-MS methods for allergen detection can be very useful for confirming cases of food contamination detected by ELISA tests. Although several papers on this type of methods have been published in the last fifteen years, they are scarcely applied in official controls due to their cost and complexity. In an attempt to overcome this limitation, the procedure proposed here has been accredited according to ISO 17025 standard [4].

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P54

Food waste into bioactives: polyhydroxynaphtoquinones from different species of edible sea Urchins

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Summary: *under a circular economy perspective, in this work sea urchins' food waste was successfully valorized into high-value products – polyhyroxynapthoquiniones (PHNQ)- identified and characterized for their antioxidant activities. A comparative study between waste deriving from different edible species, showed species-specific differences in PHNQ identity, relative quantities and antioxidant activity.*

Keywords: *food waste valorization, polyhydroxynaphtoquinones, antioxidants*

Introduction

Approximately 63000 tons of sea urchins are consumed annually worldwide, with major consumers including Japan, Chile, New Zealand, Philippines, together with the European Mediterranean countries. Sea urchins are primarily marketed for their edible gonads, leaving however 70-90% of their mass as waste. As already demonstrated in previous works, this waste can be successfully valorized to extract valuable compounds such as polyhydroxynaphthoquinones (PHNQs) with significant biomedical applications. PHNQs in fact exhibit antioxidant, antimicrobial, and anti-inflammatory properties, and have shown promise in medical applications like antiallergic and anti-inflammatory treatments [1-4].
In this work, a comparative study between two edible species waste, *Paracentrotus lividus* and *Sphaerechinus granularis* was carried out to evaluate and compare their PHNQs content and antioxidant activity [5].

Experimental

PHNQ extraction: PHNQs were extracted using a previously optimised protocol [5] from two edible species waste, *Paracentrotus lividus* and *Sphaerechinus granularis* (white and violet), kindly donated by restaurants near the University of Milan. Briefly, lyophilised and powdered waste of different sea urchin species were treated with 6M formic acid solution. The reaction was kept under stirring conditions for 2 hours. The resulting solution was centrifuged at 6000 rpm for 5 minutes and the supernatant was further filtered using a Buchner funnel. To isolate the PHNQs from undesirable co-extracted compounds, liquid-liquid extraction was performed three times with ethyl acetate. The orange-pink organic phase was repeatedly washed with distilled water to remove inorganic salts. Anhydrous sodium sulphate was then added to completely remove water. The resulting solution was filtered and dried using a rotary evaporator (Buchi, Italia srl) and a mechanical vacuum pump.
PHNQ identification: A UPLC-PDA-ESI-HR-MS equipment was used to identify the PHNQs in the extracts. LC instrument: ACQUITY UPLC I-Class (Waters, Milford, MA, USA). Column: ACQUITY UPLC BEH C18 (100 × 2.1 mm, 1.7 µm Column temperature: 34°C. Eluents: A, water + 0.1% formic acid; B, acetonitrile + 0.1% formic acid. Mass spectrometer: Synapt G2-Si QToF (Waters, Milford, MA, USA). Ionisation source: ZsprayTM ESI-probe (Waters, Milford, MA, USA). Ionisation polarity: negative. Acquisition mass range: 50–600 m/z.
Antioxidant activity: The $ABTS^{\bullet+}$ assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) was performed to evaluate the antioxidant activity. Trolox® (EC_{50}: 0.0035 ± 0.0005 mg/mL) was used as a reference as it is a vitamin E analogue well known in the literature as a potent antioxidant.

Results

The yield of *S. granularis* (100-500 mg/kg biomass) was significantly lower than that of *P. lividus* (400-900 mg/kg biomass), suggesting that *P. lividus* waste might be a better antioxidant source in terms of extractable amount. The yield from *S. granularis* with white spines was lower than that with purple spines, likely due to the presence of less pigmentation in the white variant. In Figure 1 the chemical structure of the identified polyhydroxynaphtoquinones is displayed.

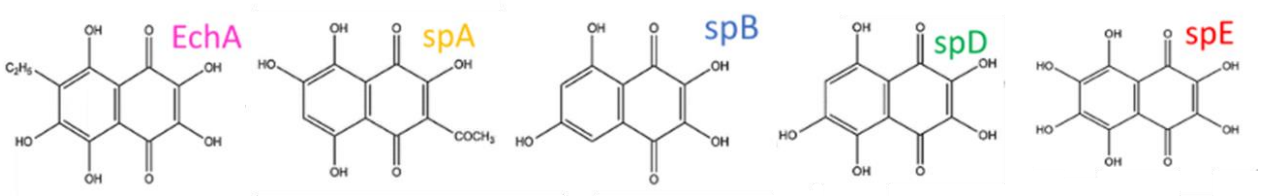

Figure 1.*Chemical structure of PHNQs (m/z:EchA=265.0350, SpA=263.0192, spB=221.0085, spD=237.0032, spE=252.9988).*

For example, in case of *P. lividus*, the EC_{50} decreases by an order of magnitude at high percentages of Spinochrome E. Accordingly, it has been shown that the antioxidant potential of PHNQs increases with an increasing number of double bonds, hydroxyl, and acetyl groups. Such chemical structures are more likely to be powerful antioxidants donating hydrogen atoms to radical agents. Thus, the antioxidant activity was well correlated with the number of hydroxyl groups present in each chemical structure: Spinochrome E has six hydroxyl groups compared to the five of Echinochrome A. Spinochrome A has four hydroxyl groups and one acetyl group, and Spinochrome B has only four hydroxyl groups. Similarly, Spinochrome D, which has five hydroxyl groups, should be slightly less antioxidant than Spinochrome E.

Conclusions

Despite variable extraction yields, partially species-specific mixtures of PHNQs were obtained. To specifically understand the origin of the variability in the PHNQ yields and relative ratio, further investigations would be necessary to correlate extraction data with different collection areas, depths, or seasonal periods. The PHNQ antioxidant activity was particularly dependent on the presence of Spinochrome E. Spinochrome E was frequently and consistently found in *S. granularis*, making it an optimal species for obtaining PHNQ mixtures with high antioxidant activity.

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P55

Dried olive mill wastewater: a rich source of phenols with promising anti-inflammatory and antiproliferative activities

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Summary: *The recovering of valuable molecules from oil industry by-products is a valid strategy to promote waste reuse, in line with circular economy. In this work, the phenolic content and biological activities, strictly linked to phenolic content, of dried olive oil mill wastewater, obtained by using spray drying technology, are presented.*

Keywords: *olive mill wastewater; phenols; biological activities*

Introduction

The processing of olives for oil production generates the most abundant agro-industrial by-products in the Mediterranean area [1]. Among these, olive mill wastewater (OMW) contains high quantities of sugars, tannins, phenolic compounds, polyalcohols and pectins [2]. Recently, the use of OMW has been successfully proposed for different applications, and many studies have focused on obtaining compounds with high added value, i.e., phenolic extracts, as phenols demonstrated to be excellent antioxidants with different biological and pharmacological activities [3-4]. In this study, spray drying technique [5] has been applied to obtain a powder from OMW. The resulting powder was analyzed by HPLC–MS/MS for its phenol content, and by antioxidant, anti-inflammatory and anti-proliferative essays for its biological potential activities. Furthermore, to evaluate the biological effect exerted by phenols belonging to other agri-food matrices, recognized for their beneficial effects on health, bergamot and orange juices were added to OMW and the related powders produced and analysed.

Experimental

Phenols of dried OMWs were quantitatively evaluated by ESI-MS/MS in negative ion mode using multiple reaction monitoring (MRM). The analytes were separated on a C8 column (5 µm particle size, 150 mm length and 4.6 mm i.d.) at a low rate of 300 µL/min with an injection volume of 10 µL. A binary mobile phase made up of 0.2% aqueous formic acid (A) and methanol (B) was gradient programmed to increase B from 10 to 100% in 10 min, hold for 5 min and ramp down to original composition (90% A and 10% B) in 10 min. The total elution time was 25 min per injection. The antioxidant capacity (AOC) of powders was determined by assessing their scavenging activity against DPPH and ABTS radicals in organic and aqueous environments, respectively. The ability of the powders to inhibit radicals was expressed in terms of IC50 (mg/ml). These measurements were performed to evaluate the existence of a linear relationship between the phenolic content and the antioxidant activity. The anti-inflammatory activity was performed on murine immune system cells (RAW 246.7 murine macrophages) stimulated by bacterial lipopolysaccharide (LPS) using the Griess assay. The antiproliferative activity was evaluated through the MTT test on human breast cancer cell lines MCF-7 and MDA-MB-231.

Results and Conclusions

The most effective extract in neutralizing DPPH and ABTS radicals was found to be the one deriving from OMW powder. The addition of citrus juices, bergamot and orange, to OMW, does not seem to benefit its antioxidant activity. However, all powders show a significant antioxidant capacity with an inhibition percentage directly proportional to their phenolic content, indicating a dose-dependent effect: higher concentrations of phenols, in OMW (8405 mg/kg), correspond to lower inhibitory concentrations (IC50) relating to DPPH and ABTS radicals (Figure 1 and 2). Among the most represented phenols, tyrosol, hydroxytyrosol, and oleuropein were found, as well as flavones such as apigenin, luteolin, and diosmetin.

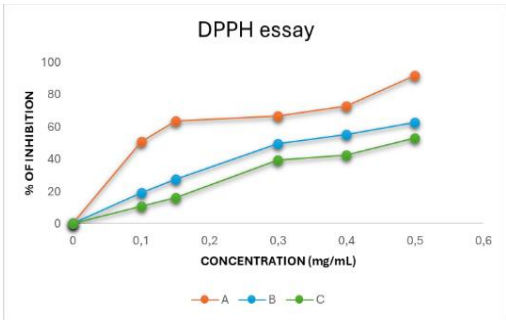

Figure 1. *Antioxidant activity by DPPH assay. (A), powders from OMW; (B), powder from OMW with the addition of bergamot juice; (C), powder from OMW with the addition of orange juice.*

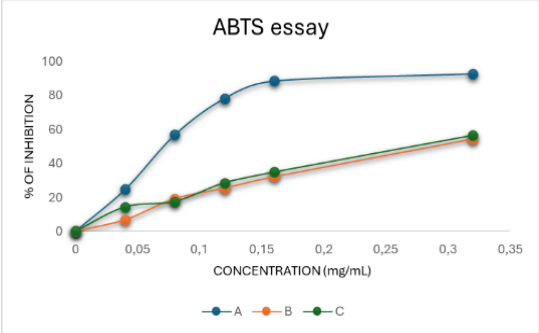

Figure 2. *Antioxidant activity by ABTS assay. (A), powders from OMW; (B), powder from OMW with the addition of bergamot juice; (C), powder from OMW with the addition of orange juice.*

All powders have anti-inflammatory activity: OMW and OMW with the addition of bergamot juice showed a similar trend compared to the one with the addition of orange juice, which resulted to have a slightly lower anti-inflammatory activity (Figure 3). The high biological value of these powders makes them suitable not only for experiments in the nutraceutical field, but also in the agronomic one; in fact, in vivo and in vitro tests are planned to evaluate their inhibitory activity on the growth of the olive pathogenic fungus *Verticillium dhaliae*.

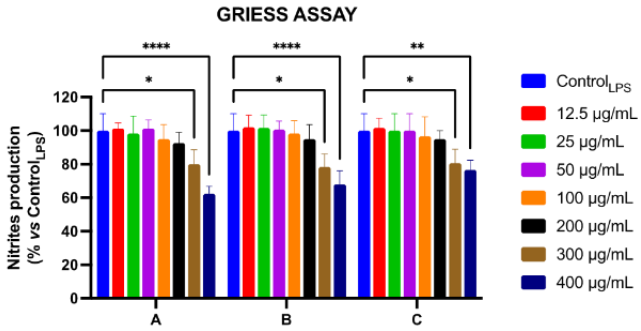

Figure 3. *Anti-inflammatory activity. (A), powders from OMW; (B), powder from OMW with the addition of bergamot juice; (C), powder from OMW with the addition of orange juice.*

Furthermore, only OMW with the addition of bergamot juice showed a weak, but significant anti-proliferative activity (approximately 20% inhibition of cell viability), at the highest concentration tested (Figure 4).

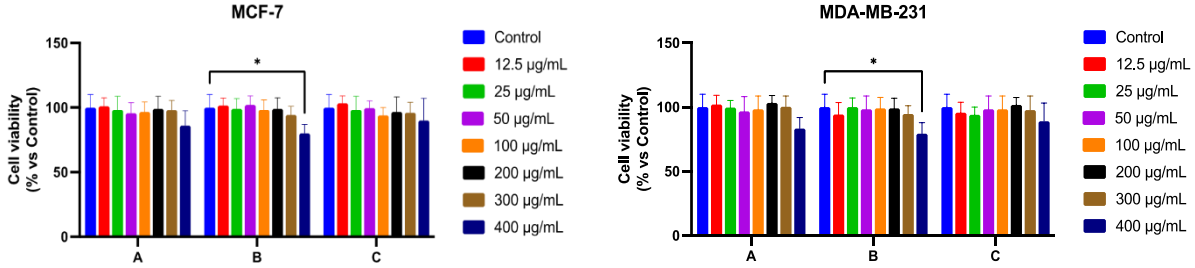

Figure 4. *Anti-proliferative activity on breast cancer cells viability. (A), powders from OMW; (B), powder from OMW with the addition of bergamot juice; (C), powder from OMW with the addition of orange juice.*

Acknoledgements
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P56

Integrated approach for the evaluation of food loss and waste of fresh spinach during its storage

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Summary: *This study investigated "BulkBox" (BB) container performance in preserving spinach quality during transport and storage. Mass spectrometry-based techniques were coupled to other instrumental and sensory methods to evaluate spinach quality in three different experiments. BB better preserved spinach freshness, reducing weight loss and some volatile organic compound emissions over 19 days of refrigerated storage.*

Keywords: *Proton Transfer Reaction Mass Spectrometry, Gas Chromatography Mass Spectrometry, volatile organic compounds*

Introduction

Food loss and waste (FLW) remains an environmental, economic, and social issue with about 131 kilograms of FLW pro capita generated in the EU in 2021 (Eurostat 2023). Following the Agenda 2030 SDGs, the EU aims to halve per capita FLW by 2030. As part of the EU project "SISTERS - Innovative systematic interventions for a sustainable reduction of food waste in Europe" (Horizon 2020, Grant Agreement No. 101037796 [1]), a smart container - the BulkBox (BB) – has been developed to reduce FLW and quality degradation during transportation and storage by exploiting passive modified atmosphere and the implementation of a sensor kit to monitor products' conditions in real time.
In this work spinach (*Spinacia oleracea L.*) which is a widely consumed leafy vegetable was selected as a case study to compare the BB and conventional approaches for bulk transportation. Different quality indicators, including volatile organic compounds (VOCs) were assessed to validate spinach freshness.

Experimental

Baby spinach of cultivars Lagiga and Meerkat was harvested in Andalusia region (Spain) and was transported to Fondazione Edmund Mach (FEM, Trentino, Italy) inside 2 BB and 2 standard bulk pallets (STD) during three different refrigerated shipments. Once the load arrived at FEM, the STD and BB were put at refrigerated temperature (2°C, 90-95% RH) and stored for up to 19 days. Headspace analysis of baby spinach leaves was performed by PTR-ToF-MS in a non-destructive way combined with measurements of CO_2 (LI-COR, USA) and Ethylene (Sensor Sense, The Netherlands) production. In this case 20 g of leaves were sampled and left at room temperature for 3 hours and then put in a 1300 mL jar and incubated at a room temperature for 30 min. VOCs profile of baby spinach was also evaluated in a destructive way by SPME-GC-MS (Agilent, USA) and SHS-PTR-ToF-MS (Ionicon, Austria). To evaluate spinach freshness weight loss, total soluble solids, color, texture and sensory analyses were also performed. Moreover, at the end of the experiment FLW for each treatment was measured.

Results

Despite the substantial inter-shipment variation observed, notable trends in different quality parameters emerged. Figure 1 shows a significant difference for the weight loss of baby spinach between STD and BB storage conditions where the BB had a better effect in preventing dehydration than STD storage conditions.
The production of CO_2 increased during the storage especially for the second and third shipments in agreement with More et al, 2022 [2]. Moreover, the CO_2 emissions were higher for STD samples rather than BB ones. The headspace analysis on the vials for both GC-MS and PTR-ToF-MS, highlighted an evolution of the spinach volatilome during the shelf-life experiments and differences between varieties.
The higher concentration of some mass peaks such as m/z 71.049 ($C_4H_6OH^+$ - tentatively identify (t.i) as butenal) and m/z 83.085 ($C_6H_{11}^+$ - t.i hexenol) were observed for STD samples especially as the shelf life progressed.
The same effect was observed for 2-methyl butanal, 3-methyl butanal and 1-hexanol measured by GC-MS. As showed in Figure 2, also ethanol (m/z 47.049), a well-known marker of fermentation [3] increased during shelf life reaching higher levels in the STD than in the BB. The increasing trend of some biomarkers connected to spinach spoilage such as dimethyl sulfide (Figure 2) and methional was observed for STD samples[2,3].

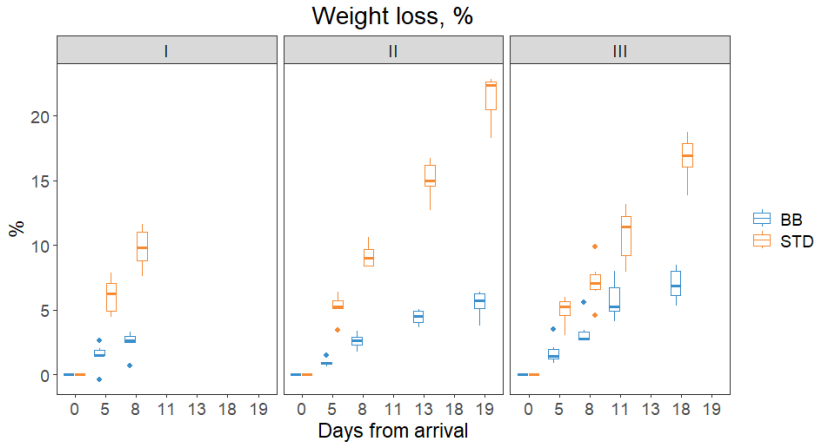

Figure1. *Weight loss of baby spinach during cold storage The plot is separated by three different transportation. The color represents the different storage conditions (BB: BulkBox, STD: Standard)*

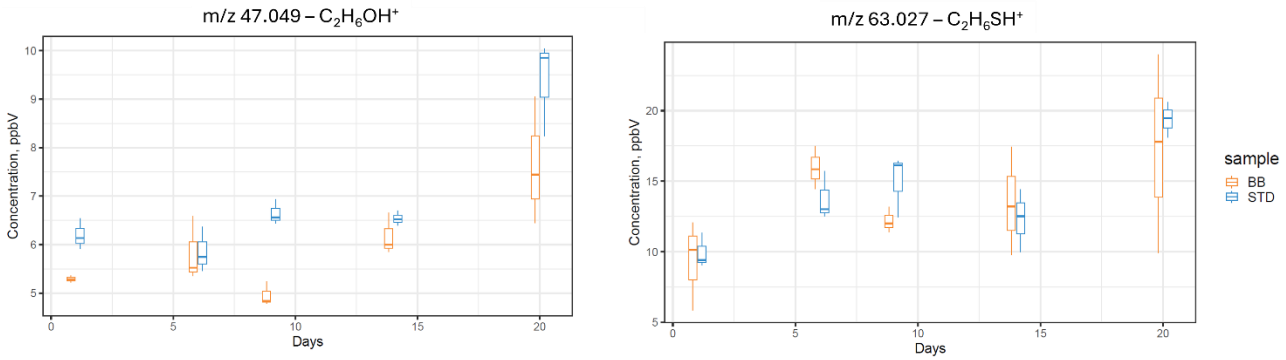

Figure 2. *Boxplot of m/z 47.049 – $C_2H_6OH^+$ (t.i. ethanol) and m/z 63.027 - $C_2H_6SH^+$ (t.i. dimethyl sulfide) during the storage of the second shipment of spinaches in BulkBox (BB) and standard (STD) conditions*

The positive impact of BB in preserving freshness during shelf life was confirmed by the sensory evaluation which highlighted better maintenance of freshness-related descriptors such as turgidity in the container.

Conclusions

The BB developed within the SISTERS project showed promising results for better preserving baby spinach quality during the post-harvest phases of transportation and storage. PTR-ToF-MS was successfully applied to monitor respiration rates and together with GC-MS VOCs emissions during the shelf life of baby spinach. While the correlation between PTR-ToF-MS VOCs profiling and other instrumental and sensory data requires further exploration, these initial findings underscore PTR-MS as a valuable tool for rapid and broad evaluation of the post-harvest quality of vegetables. The technique was also used to validate the BB performance which depends on the physiological characteristics of the fresh products, including respiration rates.

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P57

Storage quality of fresh-cut artichokes (Cynara scolymus L. Hayek) treated with dipping solution based on natural bioactive compounds

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Summary: *In this study, the effect of different dipping treatments (bergamot extract at 0,05 %), ethanol at 1% and water at 100 % (Ctrl)) on post-harvest quality parameters, volatile organic compounds (VOCs) profiles by HS-SPME/GC-MS and on the antimicrobial activity of fresh-cut artichokes cold stored for 8 days was evaluated.*

Keywords: *Artichokes (Cynara scolymus L. Hayek); Dipping solution; HS-SPME/GC-MS*

Introduction

Artichoke (Cynara scolymus L.) is a typical vegetable of the mediterranean area, widely cultivated around the world. It is mainly appreciated for its health-promoting properties, since it is a rich source of different bioactive compounds [1]. Artichoke is recognized as highly perishable crop and, when processed as a fresh-cut product, it suffers important degradative reactions, including the increase of respiration rate, browning of cut-surfaces, loss of the sensory and nutritional traits [2]. Therefore, the use of post-harvest technologies is mandatory to preserve the quality and extend the shelf-life of this perishable product. Dipping in an antioxidant solution obtained from plant extracts, is a useful method to prevent browning in fresh-cut fruit and vegetables [3]. In this context, bergamot peel extract obtained as waste by-product has demonstrated to contain valuable phenolic compounds with antioxidants and antimicrobial effects useful in the food industry [1]. The aim of this research was to evaluate the effect of three different dipping treatments with bergamot extract at 0.05 %, ethanol at 1 % and water at 100 % (Ctrl) on post-harvest quality parameters, on the volatile organic compounds (VOCs) profiles by HS-SPME/GC-MS and on the antimicrobial activity of fresh-cut artichokes cold stored for 8 days.

Experimental

Artichokes (Cynara cardunculus L. subsp. scolymus Hayek, cv Violetto) harvested at commercial maturity stage were hand-cut to remove leaves, stems and external bracts and washed in cold water (4 °C). The obtained samples were sliced in quarters and randomly dipped for 1 min in three different solutions: Bergamot extract (BE) at 0.05 %; Ethanol (ET_OH) at 1 % and water (Ctrl) 100 %
Then, fresh-cut artichokes were put in polyethylene bags (of about 150 g each) and stored in air at 5 °C for 8 days. After 3 days of storage, visual quality, unpleasant odour, respiration rate, antioxidant activity, total phenol content, o-quinones, enzymatic activity of polyphenoloxidases (PPO) and peroxidases (POD) were assessed. At each storage day (3, 6 and 8 days), VOCs, antioxidants compounds and microbial counts (Pseudomonas spp, Enterobacteriaceae, total aerobic mesophilic bacteria (TBC), total yeast and mould (Y/M)) were evaluated.

Results

Table 1 shows that, after 3 days of storage compared to fresh artichokes, the samples treated with BE increased the antioxidant activity, preserved the sensory parameters and limited the enzymatic browning, while ET_OH seemed to mainly worse the sensory traits.
Overall, 53 VOCs were detected in the fresh and different treated samples of fresh-cut artichokes across storage. Most detected volatiles have been previously reported in literature [2]. A Principal Component Analysis (PCA), performed on the HS- SPME/GC-MS data, revealed that the samples differently treated are well discriminated in the plot (Figure 1). Moreover, the BE samples appeared to be characterized by a higher volatiles content and statistically correlated to the so called “green- leaves” volatiles, including 1-hexanol, 2-hexen-1-ol, 3-hexen-1-ol hexanal, 2-hexenal, which are correlated with the sensory attributes of freshness (Herbaceous smell and Herbaceous Aroma) and directly associated to positive aroma sensory descriptors,

likely preserving the organoleptic properties of this crop [4]. Regarding the antimicrobial activity, starting from 3rd day of storage, samples treated with BE reduced Pseudomonas spp load by a 1 log CFU/g in comparison to control samples (Figure 1). Counts from other microbial populations were not affercted by treatments.

Table 1. *Mean effects of dipping treatments (BE, ET_OH, Ctrl) 3 days after treatment compared to fresh artichokes on sensory, physiological and chemical parameters*

Treatments	Visual quality	Unpleasant odour	Respiration rate (mL CO2/kg h)	Antioxidant activity (mg Trolox/100 g fw)	Total phenols (mg chlorogenic acid/100 g fw)	*o*-quinones (OD437nm /5g)	PPO (uPPO/min g fw)	POD (uPOD/min g fw)
Fresh artichokes	5.0 a	5.0 a	79.2 c	897.3 b	951.8 a	0.2 c	929.0 b	73.5 a
BE	4.2 b	4.7 a	103.1 b	1006.1 a	721.8 b	0.5 b	882.5 b	40.3 b
ET_OH	3.5 c	3.2 b	112.7 a	850.6 b	739.4 b	0.7 a	1112.9 a	77.1 a
Ctrl	4.0 b	4.7 a	102.7 b	875.1 b	784.3 b	0.2 c	1150.7 a	35.6 b

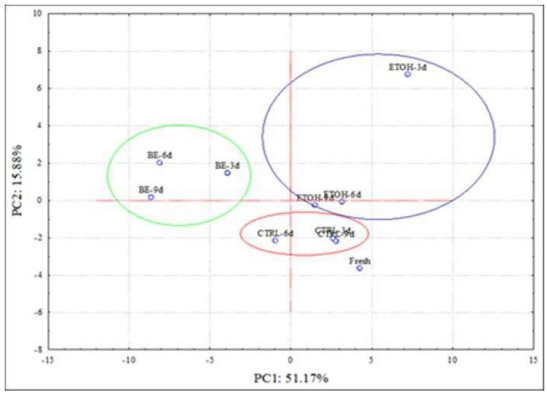

Figure 1*. Principal Component Analysis (PCA), performed on the HS-SPME/GC-MS data overall obtained from fresh and different (BE, ET_OH, Ctrl) samples of fresh-cut artichokes across storage time.*

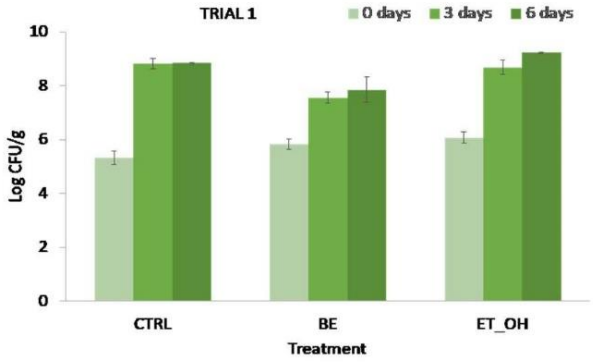

Figure 2. *Pseudomonas spp counts in treated and untreated sample over storage for 6 days at 5°C.*

Conclusions

An improvement of quality attributes of fresh-cut artichoke dipped in solutions containing BE extract rich in natural bioactive compounds was observed in terms of quality parameters, antimicrobial activity and sensory traits. Repurposing of waste streams for bioactive components from bergamot by-products as a protective agent in dipping solutions is an innovative aspect of this research, which can promote sustainable resource use, and encouraging eco-conscious practices.

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P58

Prickly Pear (*Opuntia ficus-indica*) callus culture: exploration of bioactive compounds and future biotechnological prospects

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Summary: *This study explores the use of prickly pear cactus (Opuntia ficus-indica) callus culture for producing bioactive compounds. By optimizing culture conditions, callus biomass was generated from mature prickly pear pulp and subsequently analyzed. Metabolomic analysis revealed significant shifts in chemical classes from the original tissues to the callus, with an enrichment of polyunsaturated fatty acids and flavonoid aglycones.*

Keywords: *Opuntia ficus-indica, callus culture, LC-MS*

Introduction

The prickly pear cactus (Opuntia ficus-indica) has recently garnered attention for its potential, and callus culture has emerged as a promising field in biotechnology. Callus, derived from various plant tissues such as cotyledons, leaves, and even fruit flesh, could be a valuable source for obtaining bioactive compounds.This method offers advantages over traditional cultivation by providing a controlled environment free from seasonal constraints, pests and environmental contaminants. By optimising the production of callus biomass from prickly pear pulp, its secondary metabolites can be harnessed, potentially unlocking valuable functional properties for various health applications.

Experimental

The production of callus from the mature pulp of the prickly pear (*Opuntia ficus-indica*) was investigated using various hormonal treatments. Pulp explant cultures were prepared and incubated in controlled conditions. Callus biomass was measured by recording the fresh weight (FW) and dry weight (DW) after two months, allowing for the identification of optimal culture conditions for callus production. These conditions were maintained for subsequent monthly subcultures. [2]
The harvested callus was stored, freeze-dried, and prepared for chemical analysis. Additionally, mature prickly pear pulp and peel were freeze-dried and stored for comparative metabolite analysis. Methanolic extracts of the callus, pulp, and peel were prepared using ultrasound-assisted extraction and LC-MS experiment was performed. The chromatographic separation was obtained with Accela (Thermo Fisher Scientific. Milan. Italy) HPLC interfaced through an ESI source to a linear ion trap coupled to a high-resolution mass analyzer (LTQ-Orbitrap XL. Thermo Fisher Scientific. Milan. Italy) operating in negative- and positive-ion modes. [3]

Results

The metabolite profile revealed a significant shift in chemical classes from the original plant tissues to the callus. The pulp and peel were abundant in polyphenolic compounds, including phenolic acids and flavonoids, with various glycosylation patterns. Several metabolites such as hydroxycoumarin-O-hexoside, which exhibited a neutral loss of 162 for the sugar moiety, were identified. O-glycosylated flavonoids, including isorhamnetin, quercetin, and luteolin, were also detected. Conversely, the callus was significantly enriched in polyunsaturated fatty acids, particularly derivatives of linoleic and linolenic acids, with up to three different isomers identified. Moreover, the callus exhibited a high concentration of flavonoid aglycones and polar compounds such as betalains, which were scarcely present in the pulp and peel.

Conclusions

This study highlights the differences in secondary metabolites produced during callus formation from fruit pulp and highlights the potential of callus cultures for the targeted production of bioactive compounds. The identification and use of bioreactors designed for the production of specific primary and specialised metabolites is a promising future direction. This could improve the production of high value compounds and increase their availability for pharmaceutical, nutraceutical and biotechnological applications. Using these biotechnological products, it is possible to obtain matrices rich in bioactive compounds or of health interest under controlled conditions and without sacrificing the source plant or tissue. In the present case of prickly pear callus, a potential future use could be linked to the presence of linoleic and linoleic acid, polyunsaturated fatty acids that are central in the prevention of cardiovascular diseases such as the formation of atherosclerotic plaques. [4]

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P59

The added value of natural flavourings: characterization and comparison of Calabrian chili pepper flavourings obtained with different extraction techniques

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Summary: *flavourings from Calabrian chili peppers were extracted through 3 different techniques using hydroalcoholic solvents. The volatile molecules of all these extracts were analysed through HS-SPME-GC-MS. Their important bioactive compounds were quantified through HPLC-DAD-FLD and UHPLC-MS/MS. All the results are compared through an accurate statistical analysis.*

Keywords: *sustainability; extraction; statistical comparison*

Introduction

The term "natural flavourings" specifically refers to flavouring preparations of which at least 95% is obtained from a material of plant, animal or microbiological origin, by appropriate physical, enzymatic or microbiological processes, as described by the EU Regulation 1334/2008. In particular, the aim of the present work is to compare natural flavourings obtained from Calabrian chili peppers by three extractive techniques, both in terms of their volatile molecules, linked to specific sensorial properties, and through the characterization of their relevant bioactive compounds1. This research is part of a broader project aimed not just at the valorisation of natural flavourings from Calabrian chili pepper variety, considering the initiated procedure to obtain the PGI mark, but also at the potential reuse of chili peppers, unsuitable for a direct sale, to obtain flavouring extracts, with a view to a circular economy. In fact, a characterization fully dedicated to this chili variety is also still missing in the scientific literature.

Experimental

Dry extracts obtained from pre-dried and grinded Calabrian chili peppers through Soxhlet, Ultrasound Assisted Extraction (UAE) and Supercritical CO2 Extraction (SC-CO2 with hydroalcoholic cosolvents) were compared. Thus, the usage of hydroalcoholic solvents with different percentages of ethanol (50, 70 or 96 % v/v) was explored, since they are sustainable, they are permitted in food flavouring industry and they haven't been already compared in different extraction techniques, as for chili pepper. All the extracts were then analysed in terms of pungency, related to the concentration of the two main capsaicinoids (capsaicin and dihydrocapsaicin), vitamin C, vitamin E, polyphenols, and carotenoids2. Analyses were performed using different methods and instruments, such as spectrophotometer, HPLC-DAD and UHPLC-MS/MS. The volatile molecules were analysed by HS-SPME-GC-MS (scan mode) and compared to those of the original matrix.

Results

The most impactful parameter turned out to be the ethanol/water percentage of the extraction solvent, since, for example, capsaicinoids and vitamin E were more present in chili extracts from ethanol 96%, unlike vitamin C and antioxidant activity. Surprisingly, especially the level of total flavonoids was significantly two-fold higher in extracts obtained from the little more hydrophobic ethanol 96% through UAE or SC-CO2, compared to Soxhlet or solvents with a higher percentage of water.

Conclusions

From the results, ethanol 96% in combination with UAE or SC-CO2 represents the best option to valorise the most important bioactive compounds analysed, which are also characteristic in chili pepper and they seem to be less hydrophilic, compared to other matrices. Conversely, vitamin C and the antioxidant activity both decrease with the reduction of the percentage of the water in the extraction solvent, for instance: they seem to be more correlated. Anyway, in all the cases high levels of beneficial molecules were obtained, compared also with other chili pepper varieties reported in the literature4. However, an accurate statistical analysis is being soon applied to compare all the obtained data through Principal Component and Pearson correlation analyses.

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P60

Comparison of HS-SPME, Vac-HS-SPME and HiSorb pre-concentration techniques for profiling of volatile compounds in monovarietal extra virgin olive oil using GC×GC-MS

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Summary: *Traditional solid phase microextraction (Regular-SPME) was compared with vacuum microextraction (Vac-SPME) and HiSorb techniques to analyze volatile organic compounds (VOCs) in 56 monovarietal olive oil samples from Tuscany, Apulia, and Sicily. Using GC×GC-MS and chemometrics, Vac-SPME and HiSorb improved the sensitivity detection of minor VOCs, aiding in authentication and classification.*

Keywords: *SPME, virgin olive oil, chemometrics*

Introduction

Extra virgin olive oil is a prized food and a fundamental element of the Mediterranean diet, known for its health benefits and distinctive sensory qualities. The term "extra virgin" indicates a superior quality product. According to European regulation No. 2104/2022, virgin olive oil is classified into three categories based on chemical and sensory evaluation, the latter performed with highly standardized procedures by the International Olive Council (IOC).

Understanding the content and composition of volatile organic compounds in monovarietal VOO is crucial. This knowledge is useful for authenticating varietal VOO and predicting or confirming its quality category [1, 2]. Therefore, monovarietal and geographical characterization of VOO based on the composition of volatile aroma compounds remains a topic of strong interest.

The most used technique for VOCs sampling and pre-concentration is SPME, often applied in the headspace and coupled with gas chromatography and mass spectrometry. SPME is an economical, solvent-free, and versatile technique, especially suitable for untargeted metabolomic studies when coupled with comprehensive GC×GC-MS [3]. This combination provides highly specific chromatographic fingerprints, enabling detailed information extraction through dedicated data management producing clearer mass spectra and resolving more chromatographic peaks than conventional 1D GC-MS [6].

However, SPME operates under equilibrium conditions, and the time to reach equilibrium can vary from a few minutes to several hours. Additionally, prolonged extraction times may lead to competition phenomena, hindering the extraction of some compounds. Alternative methods have also been proposed, including the use of vacuum microextraction (Vac) [4] and the recent HiSorb device. Only one study has focused on the volatile profile of virgin olive oil using this latter method [5].

HiSorb is a technique designed to extend the use of thermal desorption, based on the same fundamental principles as SPME with a higher sensitivity. The objective of the study was to compare the performance of the three extraction techniques to evaluate differences in sensitivity and ability to detect compounds that SPME traditional method might miss. The data obtained were statistically analyzed through cross-sample analysis to extract information at various levels, including commercial classification and geographical origin.

Experimental

In the present study, 56 samples of Italian monovarietal virgin olive oil were collected from three Italian regions –(i.e., Tuscany, Apulia, Sicily) during the 2022-2023 season. The samples

were evaluated by a professional panel-test focusing particularly on defects identification. All samples were analyzed using the three techniques under investigation. For the Vac–HS–SPME technique, the air inside the sampling device was evacuated before introducing the oil sample (0.1 g). The extractions for the DVB/CAR/PDMS SPME fiber were set at 45 °C with a range time of 30-45 min. For the GC×GC analysis, the first dimension was performed with a BPX5 (20 m × 0.18 mm × 0.18 µm) column while a BPX50 ms (0.25mm × 0.25 µm, 3 m) was used in the second dimension. A Shimadzu GCMS-TQ8050 NX (Kyoto, Japan) was used for all analyses. The ChromeSpace and ChromeCompare+ softwares were used to aligned, compare and identify the most discriminating volatile analytes. PCA and others multi statistical approaches were applied to

elaborate the data.

Results

Sensory analysis enabled the identification and classification of the sensory characteristics of all samples. By chemical analysis, ≈ 170 VOCs were detected, belonging to esters, alcohols, aldehydes, ketones, carboxylic acids, terpenes. The outcomes of this study include an increase in the sensitivity of both techniques compared to the traditional SPME method. It led to an improved capability to measure minor volatiles, such as 1-octen-3-ol, 2-octanone, and ethyl esters. Furthermore, the comparative approach of traditional SPME with Vac-HS-SPME and the use of HiSorb probes aided in selecting the most suitable method for determining volatiles in VOO. The combination of GC×GC-MS fingerprinting and statistics allowed gaining new insights into the performance of the three compared techniques and the volatile profile of monovarietal VOOs. After preliminary data exploration, samples were categorized based on the geographical origin and commercial classification (EVOO, non-EVOO). The samples that were perceived with a defect ranging 0-1 were used to verify whether the approach was also able to correctly classify also the critical “borderline” samples.

Conclusions

The different sampling methods of the VOCs and the GC×GC-MS analysis allowed improving the knowledge of the composition of the complex volatile fraction of VOOs and was usefully combined to the results of the sensorial analysis.

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Fatty acid profiles and biological activities of the vegetable oils of *Argania spinosa, Pinus halepensis* and *Pistacia atlantica* grown in Tunisia

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Summary: *The chemical composition and some biological properties of the vegetable oils from Argania spinosa, Pinus halepensis and Pistacia altantica were studied. Linoleic acid was the main component of the samples. P. atlantica oil showed significant inhibitory activity against the cholinesterases, α-amylase and α-glucosidase. The oils showed antioxidant, anti-inflammatory, antibiofilm activities.*

Keywords: *Fatty acids; anti-enzymatic activity; nutraceutical field*

Introduction

Recently, the interest in plant products for the prevention and treatment of several pathologies has increased. The vegetable oils are especially used in the pharmaceutical, cosmetic and food industries and could be a good alternative to classic drugs [1]. The biological activities and nutritional values of vegetable oils have been attributed mainly to the presence of fatty acids, even if other compounds like phytosterols are involved in metabolic regulation. In fact, they are capable of inhibiting cholesterol absorption [1,2]. Fatty acids may neutralize free radicals, not allowing their propagation and protecting cells from oxidative damage to DNA [3]. Furthermore, they can act on inflammatory processes, reduce inflammatory cytokines [4], and play an essential role in the physiological functions of the brain [5]. This research was focused on the study of the fatty acid composition of the oils of *A. spinosa*, *P. halepensis*, and *P. atlantica* and on the evaluation of their possible antioxidant, anti-cholinesterase, anti-α-amylase, anti-α-glucosidase, antimicrobial, and anti-inflammatory activities: some of these activities are unlike anything ever studied for three oils.

Experimental

- *Oil extraction and determination of fatty acids*

Ten g of nuts of *A. spinosa* and seeds of *P. halepensis* and *P. atlantica* were extracted by a Soxhlet apparatus with *n*-hexane for six hours. Fatty acid methyl esters (FAMEs) were obtained as previously described [6] and analyzed by GC -MS. The chromatographic separation was performed using an HP-5MS capillary column.

- *Antioxidant activities*

The antioxidant activities were evaluated by DPPH, FRAP, ABTS assays [6].

- *Anti-enzymatic activities*

The following enzymes were evaluated: acetyl- and butyrylcholinesterase (AChE and BChE), α-amylase, α-glucosidase [7] through spectrophotometric methods.

- *Anti-inflammatory activity*

The anti-inflammatory activity was evaluated by measuring the inhibition of BSA (bovine serum albumin denaturation) [6].

- *Antibacterial activity*

Resazurin microtiter plate assay determined the MIC. Subsequently, the ability of the oils to influence a mature bacterial biofilm was evaluated and the metabolic activity of the sessile cells within the bacterial biofilm was evaluated after 24 hours through the MTT colorimetric method [6].

Results

The yields of oils from *A. spinosa* nuts and *P. atlantica* and *P. halepensis* seeds were 57.2%, 36.9%, and 30.8%, respectively. High percentages of linoleic acid were identified in all oils. *P. halpensis* oil contained a significantly higher percentage of this acid (66.6%), compared to *A. spinosa* and *P. atlantica* oils (58.9% and 58.1%, respectively). *P. atlantica* oil contained the highest percentage of oleic acid (21.2%), followed by *A. spinosa oil* (16.6%). Palmitic acid was the saturated fatty acid most present in oils, mainly in the oils of *P. atlantica* and *A. spinosa*. Linolenic acid, however, was mostly present in *A. spinosa* oil (7.8%). Long-chain unsaturated fatty acids were also detected in the oils; arachidic acid was present in all oils, with a percentage between 1.1 and 4.0%, while gadoleic acid was detected in *A. spinosa* and *P. halepensis* oils and arachidonic acid was identified only in *P. halepensis* oil.

The antioxidant activity of the oils was evaluated by DPPH, FRAP and ABTS assays. In the DPPH assay, the efficacy of the oils was the same (EC_{50}=0.35 µg/mL). FRAP values ranged from 2.46 to 4.93 ± 0.28 mM TE (Trolox eq.)/g; *P. atlantica* showed the highest activity in reducing ferric iron. *P. atlantica* oil showed the highest TEAC value (2.84 mM TE/g) in the ABTS test.
P. atlantica oil exhibited the highest activity against AChE with an EC_{50} value of 4.81 µg/mL..*P. atlantica* oil was also the most active against BchE, with an EC_{50} value of 11.38 µg/mL, lower than galantamine. The three oils showed a similar inhibition of α-amylase, with EC_{50} values ranging from 310 to 370 µg/mL. The oil of *P. atlantica* was the most active against α glucosidase, with an EC_{50} value of 10.23 µg/mL and more active than acarbose, used as a positive control (EC_{50} = 80.13 µg/mL). In the anti-inflammatory test, the three oils were active, with EC_{50} values lower than 2 µg/mL, significantly lower than the control, diclofenac sodium, which showed an EC_{50} of 5.47 µg/mL.
The antibacterial activities were performed on the bacterial strains, *Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa, Listeria monocytogenes* and *Staphylococcus aureus* subsp. *aureus*. In the test on mature biofilms, the three oils exhibited quite different behavior from each other. The only common feature was their ineffectiveness against *S. aureus*, with inhibition percentages between 0 and 1.29%. The three oils showed inhibitory effects up to 50.30% (oil of *A. spinosa vs*. *A. baumannii*) and in several cases exceeded 40% (45.25 and 42.15% inhibition exerted by *P. halepensis* oil *vs. P. aeruginosa* and *vs L. monocytogenes*, respectively).

Conclusions
The presence of mono- and polyunsaturated fatty acids highlighted the use of these vegetable oils in the nutraceutical and health fields. These oils could represent a natural resource against the onset of infections caused by some pathogens; in particular, *A. spinosa* oil was active against mature biofilm. Furthermore, the anti-inflammatory activity of the three oils suggests their possible presence in a dietary plan as natural anti-inflammatories. However, the use of these oils would not be limited to the nutraceutical sector alone; in fact, the reported *P. atlantica* oil anticholinesterase activity may suggest its possible use in preventing neurodegenerative diseases. Furthermore, the *P. atlantica* oil was also the most active toward α-amylase and α-glucosidase enzymes, which could also be used to avoid the onset of pathologies linked to diabetes.

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Software assisted approach to identify authenticity and adulteration markers for saffron quality control

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Summary: *A software-based approach to identify potential markers able to discriminate saffron pure from saffron adulterated with safflower and turmeric was herein describes. Candidate molecules were in Full-MS traces validated and tentative calibration curves aimed at exploring the minimum levels of adulteration detectable were built up.*

Keywords: *saffron, authenticity, safflower, turmeric, marker compounds, software for small molecule*

Introduction

Saffron is one of the most expensive agricultural products in the world and as such, the most common adulterated spice. Common adulterants include plant-based surrogates or synthetic components. A number of analytical methods based on targeted or untargeted approach have been developed for saffron authenticity with mass spectrometry (MS) representing one of the most promising techniques for quality control of saffron [1]. In the current study a software based approach to discover authentication marker compounds for targeted MS analysis of saffron adulterated with safflower and turmeric was described. The study was accomplished according to five steps: 1) analysis of saffron, safflower and turmeric in the pure form via LC-High Resolution Mass Spectrometry (LC-HRMS); 2) processing of the respective MS spectra by dedicated software for molecule identification; 3) selection of candidate authentication markers specific for safflower or turmeric on the base of ANOVA tests; 4) validation of the selected molecules in Full MS - XIC of pure samples; 5) building-up of tentative calibration curve for each selected marker by analysing samples of saffron adulterated at three different levels (5-10-20%) with both adulterants.

Experimental

Saffron, safflower and turmeric powder in pure form (n=2 each) along with saffron samples singly adulterated with safflower or turmeric at three different inclusion level (5-10-20%, n=2 each) were firstly submitted to metabolite extraction according to a protocol previously optimized in our lab [2]. Samples were then analyzed by untargeted HRMS/MS analysis on a hybrid quadrupole-Orbitrap™ mass spectrometer Q- Exactive Plus coupled with a UHPLC pump system (Thermo Fisher Scientific). The chromatographic separation was accomplished with an Acclaim™ 120, C18 analytical column (3 µm, 120 Å, 2.1 × 150 mm, Thermo Fisher Scientific, Bremen). MS spectra were acquired in positive ion mode by running the instrument in Full-MS/dd-MS^2 and Full-MS/AIF mode according to optimized conditions. The raw data referred to pure samples were finally processed by Compound Discoverer v.3.3.1 (Thermo Fisher Scientific) for molecule identification and statistical analysis. The values of the critical parameters for feature extractions were the following: precursor ion deviation 3 ppm; maximum retention time shift 1 min; minimum base peak height for a peak to be retained 300,000 Arbitrary Unit. Unknown compound identification and prediction of the elemental composition were accomplished by activating the mzCloud node search which allowed to identify molecules in accordance with the criteria “level IIa” (probable structure) set out by the Metabolomic Standard Initiative [3]. For more reliable identification, mzCloud results were visually inspected by the operator, and only features showing ≥2 most abundant fragments that were correctly recognized were considered. Successively, ANOVA tests between the groups pure safflower/ pure saffron and pure turmeric/pure saffron were performed and only the molecules fulfilling the criteria: p-value thresholds ≤1.0e-8 and area ratio ≥50 were retained. Validation of the candidate marker were performed in pure spices acquired in Full-MS /AIF ion mode by analysing the extracted ion chromatogram (XIC) of each compound along with the respective fragments retrieved by the mzClouds search. Saffron samples adulterated with safflower or turmeric at 5-10-20% inclusion levels were finally evaluated to obtain tentative calibration curves for the selected markers.

Results

Metabolite extracts of saffron, safflower and turmeric powder in pure form (n=2 each) were analyzed by LC-HRMS equipment in Full-MS/dd- MS^2 and FullMS/AIF positive ion mode to produce characteristic MS spectra to be processed by Compound Discoverer v. 3.3.1 software for molecule identification based on mzCloud approach. In particular, 469 molecules fulfilling the internal criteria “≥ 2 most abundant fragments correctly

recognized" were selected and after performing ANOVA test, 21 and 11 compounds featured by a p-value thresholds ≤1.0e-8 and an area ratio ≥ 50 were sorted out. Successively, TIC traces referred to saffron, safflower and turmeric in pure form analyzed in Full-MS/AIF acquisition mode were filtered (XIC) for each selected candidate marker precursor ion along with the respective fragments retrieved by mzCloud search thus to confirm the presence/identity of the molecule in the samples. In Figure 1 overlays of the XIC traces which were filtered on the accurate mass (Δmass=3 ppm) of the precursor ion of Quercetin (safflower) and Caryophyllene oxide (turmeric) along with their most abundant fragments, are displayed.

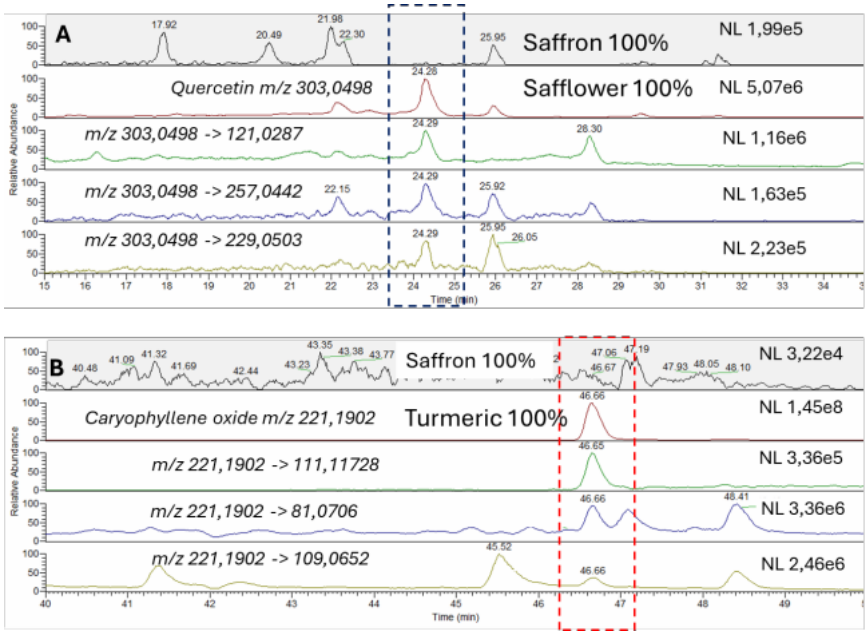

Figure 1. *Comparison of XIC chromatograms referred to Quercetin (A) and Caryophyllene oxide (B) in pure saffron /safflower/turmeric along with the respective fragments recorded in FullMS-AIF traces.*

Following the visual inspection of each safflower/turmeric candidate marker, the list was further refined and 5 molecules tracing safflower or turmeric presence in saffron were finally identified. Marker ions shortlist for both adulterating substances, is schematized in Table 1. All ions were detected as $[M+H]^+$ adduct with a Δmass comprised between -0.5 and 1.5. Finally, tentative calibration curves were obtained by plotting peak areas of XIC traces for each marker obtained from Full-MS runs of saffron singly adulterated with safflower and turmeric at three different levels (5-10-20%) versus the three percentage of adulterations.. Good linearity (R=0.99) was obtained for at least two markers selected for both safflower and turmeric, suggesting their efficacy to detect any potential adulteration by these spices of lower economical value at the lowest adulteration level of 5%.

Table 1. *Selected markers peculiar for safflower and turmeric adulterants to be used for saffron authentication. Most relevant parameters are also reported.*

Adulterant	Name	Formula	m/z	Area Ratio	P-value
Safflower	Quercetin	C15 H10 O7	303,0498	306,491	1E-15
	Kaempferol-3-O-rutinoside	C27 H30 O15	595,166	301,852	1E-15
	Chlorogenic acid	C16 H18 O9	355,1021	110,781	1E-15
	7-Hydroxycoumarine	C9 H6 O3	163,0389	108,294	7E-11
	Proline	C5 H9 N O2	116,0709	89,333	1E-15
Turmeric	Caryophyllene oxide	C15 H24 O	221,1901	2164,126	1E-08
	Curcumin	C21 H20 O6	369,1336	743,913	1E-15
	ar-Turmerone	C15 H20 O	217,1588	467,466	5E-08
	Flavanone	C15 H12 O2	225,0909	89,099	1E-12
	2,4-Dimethylbenzaldehyde	C9 H10 O	135,0806	53,475	1E-15

In Figure 2 calibration curves referred to Quercetin (marker for safflower) and Caryophyllene oxide (marker for turmeric) are displayed. Our results show the potentiality of the software based approach herein described to select reliable and sensitive molecules for saffron authentication. These molecules could also

represent a good starting point to develop targeted methods for saffron quality control based on MS platforms.

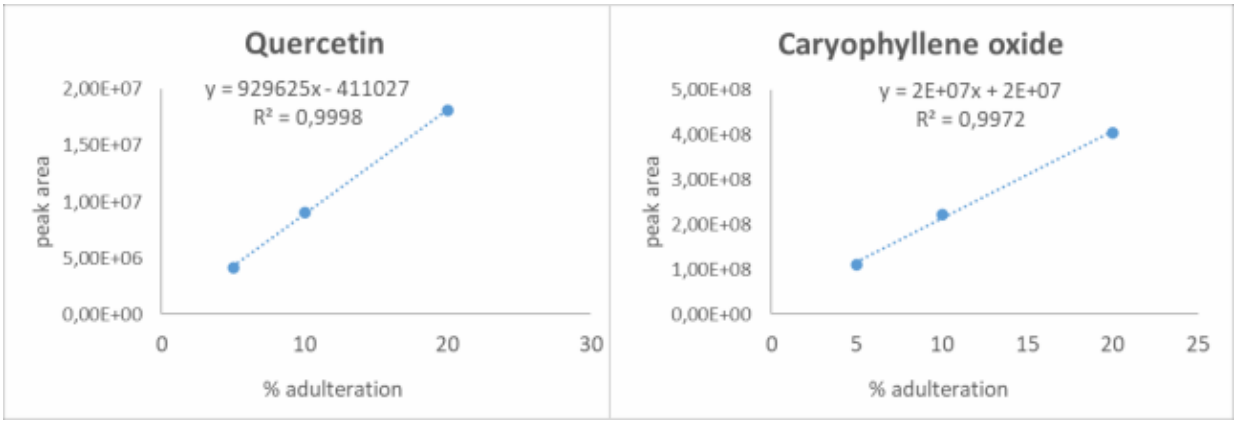

Figure 2. *Curve calibrations referred to Quercetin (marker of safflower) and Caryophyllene oxide (marker of turmeric) along with the respective relevant parameters.*

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Direct analysis in real-time with high resolution mass spectrometry: a rapid tool for black truffle authentication

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Keywords: *Food Authenticity & Food Fraud*

Food fraud leads to financial losses for food processors. Particularly expensive or luxury products like Truffles (prices up to 2000 €/kg for Périgord truffle *Tuber melanosporum* Vittad) are prone to adulteration. Other black truffle species like the Asian truffle (*T. indicum* Cooke et Massee) are morphologically similar but much cheaper. We developed a rapid, comprehensive and highly selective workflow for food authenticity screening through Direct Analysis in Real-Time (DART) ionization coupled to QTOF MS.
Ten samples (T. melanosporum VITTAD, aestivum VITTAD and T indicum COOKE ET MASSEE) were cut into pieces, lyophilized and grounded. After extraction 3 µL aliquots were applied onto QuickStrip™ cards. An Impact II VIP QTOF (Bruker) was operated in negative ionization mode for data-dependent MS/MS experiments with a scheduled precursor list. Data processing was done in MetaboScape (Bruker), including feature extraction, statistical analysis and tentative unknown annotation based on accurate mass, isotopic pattern and MS/MS spectra.
Samples from the same Tuber species are clearly clustered in the statistical PCA. Based on the MS/MS spectra, the marker compounds with the largest contribution to the discrimination were identified either by a spectral library search or by a semi-automated annotation workflow comprising elemental composition prediction, structure assignment and *in silico* fragmentation. Annotated marker compounds included mevalonic acid, lactic acid, uracil and sugar alcohols. Compared to chromatographic methods, DART-QTOF offers significantly shorter analysis times of 15 s per sample and reduced solvent consumption. DART-QTOF paired with chemometrics presents a fast, robust, and resource-saving method to counteract adulteration of black truffles.

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A rapid solution for tomato purees authentication by flow injection and high-resolution mass spectrometry detection

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Summary: *A rapid analytical tool for Datterino purees authentication by FIA-HRMS was developed and validated on commercial samples. Unsupervised (NIPALS-PCA) and supervised (LDA) statistical approaches were applied to describe the data system and build a reliable predictive model based on metabolites fingerprinting. Markers discovery by multivariate exploratory ROC analysis was accomplished and candidate compounds identified by MS/MS.*

Keywords: *tomato puree authenticity, high resolution mass spectrometry, rapid method*

Introduction

Among fruits and vegetables, tomatoes (Solanum esculentum) are commonly valued for their sensorial and nutritional properties due to the high content in antioxidants and bioactive compounds.[1] Over the last decade, several analytical approaches have been proposed to investigate tomato varieties accounting for specific classes of compounds, e.g. antioxidants,[2] phenols [3] and only few of them pursued authenticity/traceability purposes.[4]
The aim of this study was to develop a rapid and green analytical approach for Datterino purees authentication by flow injection analysis based on high resolution mass spectrometry (FIA-HRMS).

Experimental

Commercial samples of tomato purees were purchased from local market equally distributed into two groups: (A) labelled as 100% Datterino, (B) alternative purees with no varietal specifications. Extraction with water, ethanol and ethyl acetate were tested and compared to methanol for typical metabolic profiles (solvent to sample ratio 1:10). Three independent extracts were prepared for each puree sample under optimized conditions (day 1, 2, 3).
FIA-HRMS acquisitions were performed in Full scan modes by switching positive/negative polarities with high resolving power (70000@200m/z) on a hybrid quadrupole/orbitrap MS (Q-Exactive Plus, Thermo Fisher Scientific). HRMS collected spectra were averaged over the whole acquired peak (fixed time range of 30 s) and subtracted of spectral background by Xcalibur software (Thermo Fisher Scientific). The positive and negative spectra were merged and exported into .csv files to compile sample lists of accurate m/z ratios and peak intensities. The lists were processed by MetaboAnalyst 6.0 [5] for data mining: (i) peak matching with a mass tolerance of 0,001 m/z, (ii) removal of features with more than 50% of missing value, (ii) imputation of the remaining missing values by LoDs, (iii) data filtering upon variance (interquartile range) and abundance (compared to mean intensity value) and normalization (normalization by sum, $\log_{10}$ transformation and auto-scaling).
Statistical analysis was carried out by MetaboAnalyst 6.0 and Statistica v.7 software (StatSoft Inc., now TIBCO Software Inc., Palo Alto, CA, USA).

Results

The FIA-HRMS analytical method was developed on a set of commercial tomato puree samples with different declared composition.
First an optimization of the sample preparation protocol was performed focusing on a green and rapid workflow from sampling to data acquisition. Indeed, only three main steps were taken into consideration: (i) homogenization, (ii) extraction and (iii) filtration. High resolution Full MS spectra were acquired upon flow injection by switching between positive and negative polarity modes in the same injection. The comparison of four extraction solvents at fixed solvent to sample ratio proved that ethanol represents a valid green alternative to the well-established methanol-based extraction protocols.[5] The total run time, including sample preparation and detection, was below 30 min. Three independent extracts processed in consecutive days were prepared for each commercial puree sample and freshly analysed. Full data set was subjected to an accurate pre-process to build a proper data matrix for multivariate statistical analysis.
Principal Component Analysis (PCA) by Non-linear Iterative Partial Least Squares (NIPALS) algorithm with a V-fold cross-validation (V=7) was used as unsupervised approach to exclude the presence of outliers and

report on the significant PCs, describing the multivariate system. These latter provided a total explained variance of 96,2% ($R^2X(Cum)$) and predicted variance of 96,1% (Q2). Afterwards, such PCs were used as input for supervised pattern recognition by Linear Discriminant Analysis (LDA). The total number of acquisition samples was randomly divided into analysis (2/3) and validation sets (1/3). The LDA model achieved a prediction ability of 100% for the analysis set and 95% for the testing set in both groups A (datterino) and B (other), see Figure 1.

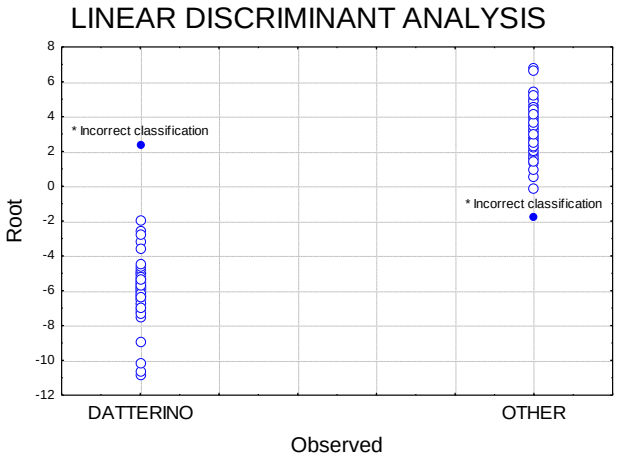

Figure 1. *Predicted classification achieved by Linear Discriminant Analysis*

Further statistical investigation was carried out to discover reliable discriminant markers by multivariate exploratory ROC (Receiver operating characteristic) analysis. More conservative filters were applied to the original data set to exclude non-informative and low intensities features (variance ≥40% of the interquartile range and intensity ≤20% of the mean intensity value). The multivariate ROC curves were automatically generated based on support vector machines (SVM) algorithm, by Monte-Carlo cross-validation. The classification method provided the best modelling with 100 important features, with a predictive accuracy of 96,5%, an area under the curve (AUC) of 0,986 and a 95% confidence interval (CI) of 0,895-1.

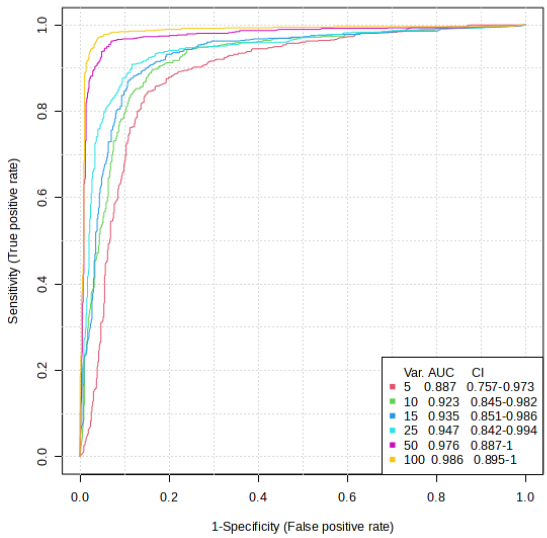

Figure 2. *Multivariate exploratory ROC analysis by SVM classification algorithm.*

The important features were browsed to compile a list of candidate markers among the top30 ranked features, both as selected frequency and average importance. Classical univariate ROC analysis was calculated for each candidate feature to compute optimal cutoffs, and to estimate performances. Identification by MS/MS is ongoing.

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P65

Validation of an analytical method for authentication of mint essential oils: a combination of GC-IRMS and GC-MS/MS

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Summary: *The natural mint essential oils contain mainly menthol and menthone. Their antibacterial and antioxidant activities are of great interest in the food and pharmaceutical industries. However, frauds and adulterations have been public concerns for long and therefore it is important to authenticate the products and to trace their origins.*

Keywords: *mint essential oils, authentication, stable isotopes*

Introduction

It is a long history for humans to use plants for foods, beverages and pharmacies and currently, mint is also used in toothpaste, candies, perfumes, chewing gums and tobaccos (now banned in some countries like the US) [1,2]. Among the plants, mint (also known as mentha) is one of the most widely used. In the Lamiaceae family, the most important genus is Mentha which, according to recent studies, can be classified into 42 species, 15 hybrids and hundreds of subspecies, varieties and cultivars [1] which are spread nearly all over the world. One of the most common and popular mint is M. × piperita L. (Peppermint) [3]. The natural mint essential oils are extracted from herbs and flowers. The principal components of mint essential oils are menthol and menthone. The global market size of mint essential oils of the year 2022 was 925.3 million U.S dollars and is anticipated to grow at a compound annual growth rate (CAGR) of 9.8% from 2023 to 2030 [4]. Fraudulent items and adulterants enter into the market as the global demand for mint and mint essential oils grow without having been tested. As a result, their adverse effects are not monitored during consumption. It becomes important to identify the adulterants for the authentication of mint essential oils in order to guarantee consumers' protection [5]. This work aims to develop and validate a new analytical method for verifying the genuineness of mint essential oils by means of GC-IRMS using stable isotope ratios as possible markers of oil' genuineness as already demonstrated in previous studies [6].

Experimental

A complete validation process was followed to test and check the performance of a GC-IRMS approach for the determination of $\delta^{13}C$ and $\delta^{2}H$ compound specific stable isotope analysis directly on specific aroma compounds like menthol and menthone.

Results and conclusions

Linearity, intraday precision and interday precision were evaluated for both elements using three different solvents (acetone, hexane, ethyl acetate). Bias, standard deviation, and relative standard deviation were determined for intraday precision and pooled standard deviation and pooled relative standard deviation were determined for interday precision. The results showed that acetone is a better choice than the other two solvents for the purpose in this work (see Figure 1 as an example).

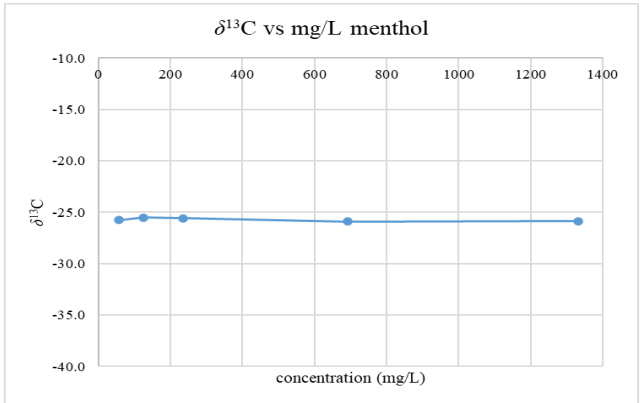

Figure 1. *$\delta^{13}C$ values of menthol in acetone at different concentration levels.*

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Production of the Italian 'Tarallo Pugliese' (TAP) fortified with lentils hulls and its chemical characterization by LC-HRMS/MS analysis

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Summary: *An innovative and nutritionally valued snack product "tarallo pugliesi" were produced at laboratory scale and are described in this note as a good example of innovative functional food product obtained by mixing wheat flours with lentil hulls at different inclusion levels. Protein and chemical analyses of tarallo samples blank and fortified with lentil hulls were carried out by untargeted LC-HR tandem mass spectrometry and final collected data were processed by using an integrated advanced software for high throughput compound identification by interrogating databases online available.*

Keywords: *nutritionally valued food products, functional foods, tarallo, high resolution mass spectrometry*

Introduction

The market of innovative and functional foods has progressively expanded in the last decades for the wealth of beneficial compounds contained. Pulses and legumes play a vital role in metabolic and physiological processes due to the presence of various bioactive compounds, and the majority of them are phenolic acids, flavonoids, and tannins. As a result, foods fortified with legumes or pulses are important in the human diet.
Some food processes of pulses lead to loss valuable by-products rich in phenolics and fibres [1]; therefore, the current concerns of recycling bio-products disposed from food industries play an important role in the circular economy
contributing to valorise and re-utilize end products that would be otherwise discarded. In parallel, the improvement of baked products can be carried out by using new cereals such as Tridordeum® (×*Tritordeum martinii* A. Pujadas, nothosp. nov.) which is known to have a reduced amount of gluten allergens, but is rich in minerals, fibres and phenolic acids [2]. Consequently, development of innovative and nutritionally valued food based on bio-products re-utilization is on the rise.

Experimental

Lentil hulls provided by a local retailer were first ground and sieved before their used.
The traditional recipe of 'Tarallo Pugliese' was: 125 g of flour blend mixed with EVO oil (28 g), white wine (43 g) water (11 g) and salt (2.5g). The dough was kneaded and into cords sealing into rings, boiled in water, drained in the air and baked until golden brown (Table 1 and Figure 1)
Flour blend was composed by various % of wheat flour, alternatively replaced (as weight basis) with 0 or 10 and 20% of Tritorduem ® flour (Sample A, B and C, respectively) or 10 and 20% of lentil hull flour (35 mesh; Sample D and E). Taralli produced with flour blend with 20% of both Tritorduem ® and lentil hull flour were also produced (Sample F; Table 1)
Two independent LC-HRMS analyses were run for each sample. Metabolites were separated on an Acclaim™ 120, C18 analytical column (3 µm, 120 Å, 2.1 × 150 mm, Thermo Fisher Scientific) and MS analysis performed on a hybrid quadrupole-Orbitrap™ mass spectrometer Q- Exactive Plus coupled with a UHPLC pump system (Thermo Fisher Scientific). MS spectra were acquired in positive and negative ion mode by running the instrument in Full-MS/dd-MS^2 mode according to optimized conditions. Compound Discoverer software v. 3.3.1 (Thermo Fisher Scientific) based on the activation of mzCloud and ChemSpider nodes search was used for chemical identification. The intrinsic characteristics of these two identification tools llowed identification compounds according to the "level IIa" (probable structure = more advanced) and "level III" (putatively characterized = medium) criteria which were set out by Metabolomic Standard Initiative [3]. The list of features retrieved by the software were further refined by fulfilling some stringent criteria internally defined [4].

Results

In Figure 1 are depicted the main steps of fortified Tarallo production using different blend of lentil hulls and Tritordeum® according to what reported in Table 1.
In order to identify beneficial compounds characterizing the lentil hulls and contributing to accrue the nutritional

value of the final fortified bakery product, the five samples produced and extracted according to the method already mentioned, were analysed in Full-MS/dd-MS^2 mode on an Orbitrap based mass spectrometry platform and compound identification of the detected ions, along with statistical analysis, were accomplished by Compound Discoverer v. 3.3.1 software by the aid of Chem Spider and mzCloud on line database.

Table 1. *Flour blends used to fortify several types of the 'taralli' produced.*

Sample	Spring Wheat	Tridordeum	Lentil hulls
A	125	0	0
B	112.5	12.5 *(10%)*	0
C	100	25 *(20%)*	0
D	112.5	0	12.5 *(10%)*
E	100	0	25 *(20%)*
F	75	25 *(20%)*	25 *(20%)*

Figure 1. *Scheme of the different steps of tarallo production*

Volcano plots showing the results of ANOVA test between E and A samples (Figure 2), highlighted the compounds discriminating the presence of lentil hulls in tarallo product. They are circled in red line in the upper right corner of the inset (Figure 2). Results obtained upon software elaboration produced a list of total 70 compounds mainly characterizing the samples fortified with lentil hulls although the list of discriminant ions was further reduced to 47 total ions upon visual inspection of the generated MS spectra.
By pinpointing the compounds spinning in the list and showing to be highly abundant in the fortified tarallo samples, a final shorter list of discriminating compounds was obtained.

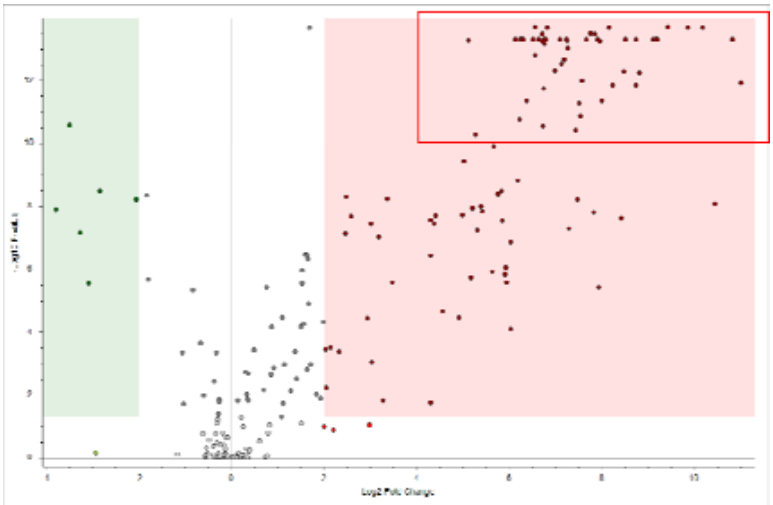

Figure 2. *Volcano plot of tarallo samples produced with 80% wheat flour 20% of lentil hulls versus tarallo with 100% wheat and no lentil hulls added. (P-value= 0.05, Log fold change=2).*

Among them, specific compounds belonging to the polyphenol category known for their beneficial properties on humans were found such as catechin, gallocatechin and procyanidin proving to have an MS intensity of

several hundred times higher compared to what detected in tarallo without any fortification. The nutritionally valued and innovative tarallo product will be further investigated for technological and sensorial analysis and a deep study of the compounds detected by HR MS/MS analysis will be performed in future for their structural elucidation. These results confirm the importance of the choice to valorise the lentil coats currently disposed since they have the majority of the phenolic compounds and flavonoids (mainly catechins and procyanidins which are considered beneficial for human health as shown by Singh et al., (2017). Our preliminary results are consistent with those previously obtained on lentil coats by other authors [5].

Fundings
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Comparative study of carotenoid and fat-soluble vitamin levels in alpine *vs.* industrial dairy products

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Summary: *Carotenoids and fat-soluble vitamins are vital nutrients obtained through diet, with dairy products being a key source. This study developed a method to analyze these compounds in cheeses, butters, and creams. Comparing alpine and industrial products revealed higher levels of several vitamins and carotenoids in alpine cheeses and butters, with minimal differences in creams.*

Keywords: *carotenoids, fat-soluble vitamins, dairy products, Orbitrap.*

Introduction

Carotenoids and fat-soluble vitamins are bioactive compounds essential for human beings and are involved in numerous processes necessary for maintaining health [1]. Except for vitamin D, which can be metabolized through exposure to UV rays, all other compounds must necessarily be obtained through the diet. Dairy products are an important source of micronutrients, including carotenoids and fat-soluble vitamins and the feeding of cows, based on grazing, strongly influences the content of these compounds [2], which could represent important markers in the traceability of mountain products. Carotenoids and fat-soluble vitamins have different polarity and solubility, which makes extracting and quantifying them simultaneously extremely difficult.

Experimental

In this work, a method was developed for the simultaneous analysis of these analytes in cheeses, butters, and creams, focusing on the extraction and analytical aspects. Subsequently, various products derived from mountain pastures (N=26) were compared with industrially produced ones (N=9). The developed method was able to analyse the contents of lutein, β-carotene, retinol, α-tocopherol, β/γ-tocopherol, δ-tocopherol, vitamin K1, and vitamins D2 and D3 using high-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS) with APCI source. From the analysis conducted, the content of α-tocopherol, β/γ-tocopherol, retinol, lutein, vitamin K1, and vitamin D2 was found to be higher in mountain cheeses (KW test), while for butter samples, a higher content of lutein, β-carotene, vitamins D2 and D3, and δ-tocopherol was also observed in the alpine products. Overall, creams did not show significant differences except for the lutein content, although in general, concentrations tended to be higher in mountain products.

Results

From the analysis conducted, the content of α-tocopherol, β/γ-tocopherol, retinol, lutein, vitamin K1, and vitamin D2 was found to be higher in mountain cheeses (KW test), while for butter samples, a higher content of lutein, β-carotene, vitamins D2 and D3, and δ-tocopherol was also observed in the alpine products. Overall, creams did not show significant differences except for the lutein content, although in general, concentrations tended to be higher in mountain products. A PCA per matrix was performed to explore the variability of the different dairy samples (example cheese, Figure 1) and, excluding creams, the clusterisation of alps sample respect to industrial samples is evident.

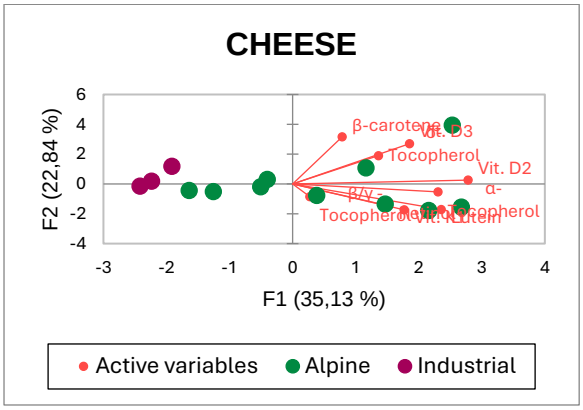

Figure1. *PCA based on the content of fat-soluble vitamins and carotenoids in cheese*

Conclusions

The high-resolution mass spectrometry technique allows for the sensitive, selective, and simultaneous quantification of carotenoids and fat-soluble vitamins, primarily due to the use of an APCI source. The validated method enabled the study of various alpine and industrial products, revealing nutritionally interesting contents, especially for retinol and α-tocopherol in alpine products. Statistical tests indicated the potential use of some of these compounds as traceability markers for cheese and butter.

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Levels of 3-monochloropropane-1,2-diol (3-MCPD as sum of free and ester form) and glycidyl esters (GE) in oils and fats: results of ten-year monitoring

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Summary: *The GC-MS method for the detection of food contaminants process 3-MCPD (sum of free and ester form expressed as 3-MCPD) and GE (glyciyl esters expressed as glycidol) in edible oils and fats was introduced in Lombardia and Emilia Romagna in 2014. Here, a first general overview of the results obtained since the method by GC-MS was introduced is given, from the end of 2014 until the first semester of 2024 and various types of matrices available on the Italian market were compared.*

Keywords: *food processing contaminants, 3-MCPD, gycidyl esters.*

Introduction

3-Monochloropropane-1,2-diol (3-MCPD) and glycidyl esters (GE) may be formed as process contaminants during refining of edible fats and oils. 3-MCPD can be present in the free form or in form of esters with different fatty acids (3-MCPD esters); 2,3-Epoxi-1-propanol (free glycidol) is highly reactive and it can be present only as esters form as glycidyl fatty acid esters (GE) [1,2]. GE are believed to be fully transformed into free glycidol, a compound that is probably carcinogenic to humans (Group 2A). Equally, MCPD esters are hydrolysed within the gastro-intestinal tract. Free 3-MCPD is listed as possibly carcinogenic to humans (Group 2B) [3, 4]. The European Commission has set maximum levels (MLs) for MCPD (sum of free and bound form) and GE in edible fats and oils, as well as infant formula and baby food [5] and has established the performance criteria for the analytical methods [6]. The National Monitoring Plan has carried out sampling based on the indications and numbers provides from Ministry of health [7]. Here, the results of this monitoring plan from at the end of 2014 were summarized, since the method for determination of 3-MCPD (sum of free and bound form) and glycidyl esters by GC-MS was introduced in routine analysis and we compared the various types of edible oils and fats available on the Italian market.

Experimental

Sampling criteria

According to the National Monitoring Plan, official control personnel of the veterinary services sampled edible oils and fats at supermarkets and wholesale companies in some Italian regions (Lombardia, Emilia-Romagna, Piemonte, Liguria, Toscana, Marche, Campania, Calabria and Sicilia). The sampling was unexpected and carried out in any day of the week during all the year. Each sample was constituted of at least 100 mL/100g.

3-MCPD and GE analysis

The determination of 3-MCPD (sum of free and bound forms) and GE was performed according AOCS Official Method Cd 29c-13 2013.

Sample preparation consisted in a liquid-liquid extraction with hexane-terbutylmethylether mixture of oil or liquefied fat (100 mg) into two independent essays (A and B). Ester cleavage step by basic hydrolysis and stop cleavage step by addition of acidified sodium chloride solution for glycidol transformation in 3-MCPD (A) and acidified chloride-free salt solution (B) follow the extraction. Finally, after a matrix clean-up with 2-Methylpentane, a derivatization step with phenylboronic acid (PBA) saturated in diethyl ether solution followed. Analyses were performed by GC-MS equipment described in Table 1.

3-MCPD and GE quantification

Quantification of the sum of 3-MCPD (sum of free and bound forms) and glycidyl esters was based on single-point calibration, multiply the ratio of signal areas of the analyte and the isotopic labelled standard (3-MCPD-d5) based on corresponding ion traces with the spiking level of the isotopic labelled standard in Assay A; for sum of 3-MCPD (sum of free and bound forms) multiply the ratio of signal areas of the analyte and the isotopic labelled standard (3-MCPD-d5) from Assay B; the content of glycidol is the difference of the result obtained in Assay A and Assay B.

Results

From the end of 2014 to the first semester of 2024, fourteen types of edible oils and fats were analysed for a total of 172 samples. The majority of samples consisted of sunflower oils (47 samples), followed by extra-virgin olive oils (45 samples) and maize oils (21 samples). 3-MCPD were detected in all palm oils, soybean oils, rice oils, mixed oils, sesame oil, olive pomace oils and coconut oils; it was detected above 80% in maize oils, peanut oils and olive oils; it was detected in approximately in 30% of the fish oils. GE were detected in all

maize oils, peanut oils, palm oils, soybean oils, coconut oils, mixed oils, rice oils, sesame oil and pomace oils; it was detected above 80% in olive oils; it was detected in 50% of fish oils. 3-MCPD and GE were not detected in any samples of extra-virgin oils and salted butter.

Table 1. *GC-MS equipment*

Gas chromatography	6890N (Agilent Technologies)
Column	DB-17MS, 30 m x 0.25 mm x 0.25 μm, 50% Phenyl, 50% Dimethylpolysiloxane
Carrier gas	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
Injection temperature	250 °C
Injection mode and volume	Splitless, 1 μL
Purge flow	100 ml/min for 0.5 min
MS parameters	
Ionisation	Electronic Ionisation
Operation mode	Selected Ion Monitoring (SIM)
Monitors Ions	For d-5-3-MCPD-PBA: 150, 201 and 203 For 3-MCPD-PBA: 147, 196 and 198

Regarding 3-MCPD, one sunflower oil sample exceed ML of 1250 μg/kg; one pomace oil sample and 2 palm oil samples exceed ML of 2500 μg/kg. Regarding GE, one palm oil sample exceed ML of 1000 μg/kg (the same sample that exceeded the ML for 3-MCPD). Results are summarized in Figures 1,2,3,4,5,6,7,8.

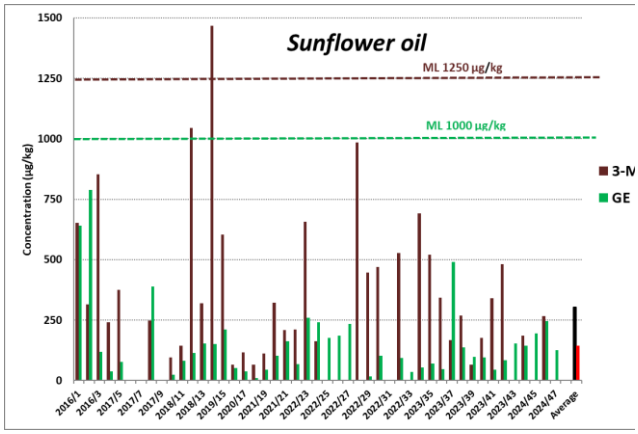

Figure 1. *Concentration (μg/kg) of 3-MCPD (brown bar) and GE (green bar) in 47 sunflowers oil samples. Average value for each graph are shown as a black bar (3-MCPD) and red bar (GE).*

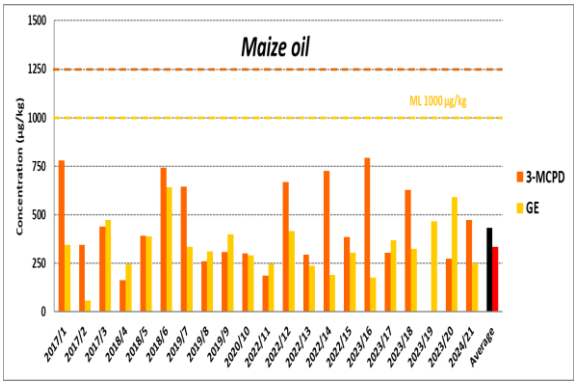

Figure 2. *Concentration (μg/kg) of 3-MCPD (orange bar) and GE (yellow bar) in 21 maize oil samples. Average value for each graph are shown as a black bar (3-MCPD) and red bar (GE).*

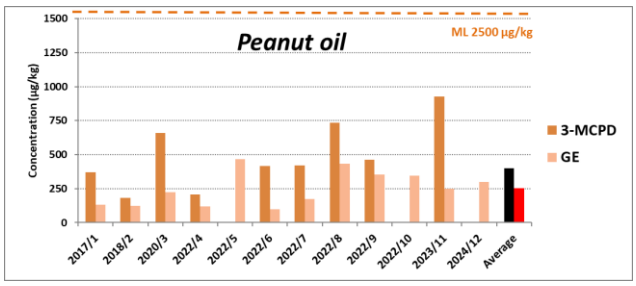

Figure 3. *Concentration (μg/kg) of 3-MCPD (brown bar) and GE (pink bar) in 12 peanut oil samples. Average value for each graph are shown as a black bar (3-MCPD) and red bar (GE).*

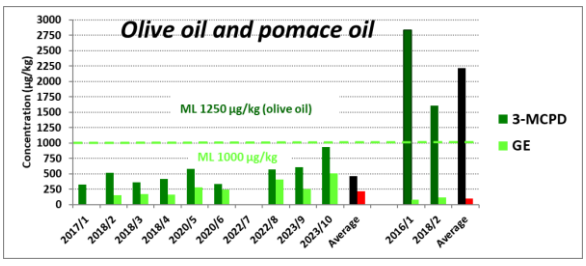

Figure 4. *Concentration (μg/kg) of 3-MCPD (dark green bar) and GE (green bar) in 10 olive oil samples and 2 pomace oil samples. Average value for each graph are shown as a black bar (3-MCPD) and red bar (GE).*

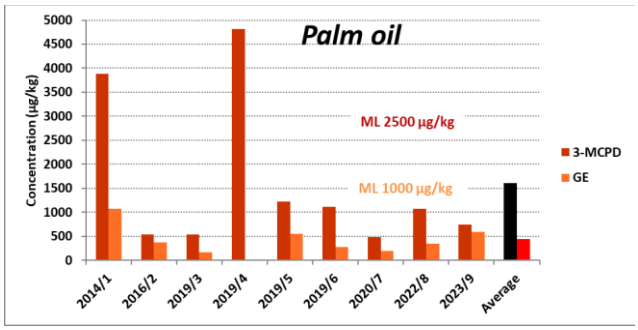

Figure 5. *Concentration (µg/kg) of 3-MCPD (brown bar) and GE (orange bar) in 9 palm oil samples. Average value for each graph are shown as a black bar (3-MCPD) and red bar (GE).*

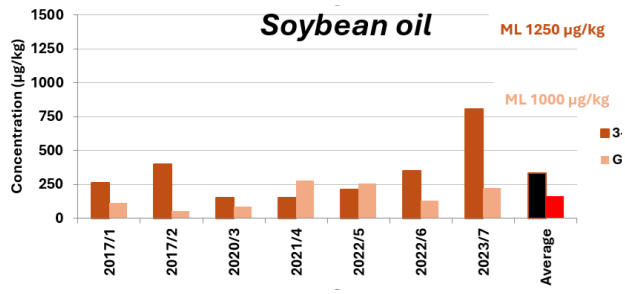

Figure 6. *Concentration (µg/kg) of 3-MCPD (brown bar) and GE (pink bar) in 7 soybean oil samples. Average value for each graph are shown as a black bar (3-MCPD) and red bar (GE).*

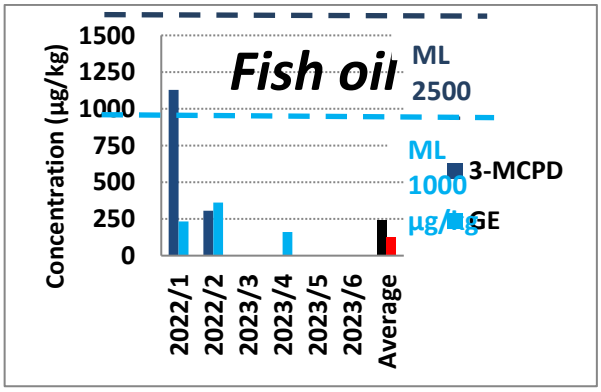

Figure 7. *Concentration (µg/kg) of 3-MCPD (dark blue bar) and GE (blue bar) in 6 fish oil samples. Average value for each graph are shown as a black bar (3-MCPD) and red bar (GE).*

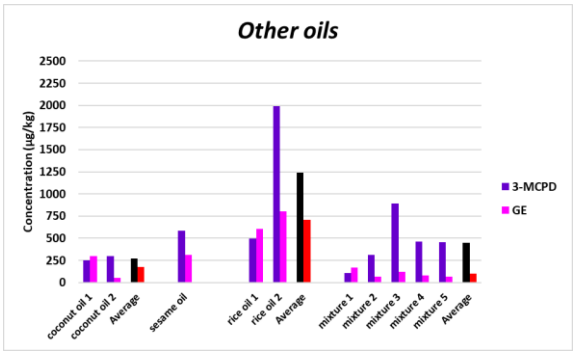

Figure 8. *Concentration (µg/kg) of 3-MCPD (purple bar) and GE (pink bar) in 2 coconut ois, 1 sesame oil, 2 rice oils and 5 mixed oils. Average value for each graph are shown as a blck bar (3-MCPD) and red bar (GE).*

Conclusions

Fourteen types of edible oils and fats were analysed for determination of 3-MCPD (sum of free and bound form) and GE (glycidyl esters) for a total of one hundred and seventy-two samples. In fifty-four samples (forty-five extra-virgin oils, three salted butters, two sunflower oils, one olive oil, three fish oils), corresponding to 31% of the total, 3-MCPD and GE were not detected (< LOQ 80 µg/kg). Four samples, corresponding to 2% of the total, were not compliant and exceed the MLs established.

Acknowledgment

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P69

"From field to barrel": carbon and oxygen isotope analysis in the production chain of Aceto Balsamico Tradizionale di Modena (ABTM)

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Summary: *The product-territory link and the impact of the production process of Aceto Balsamico Tradizionale di Modena (ABTM) were analyzed by highly specific analytical techniques, employed to monitor isotopic changes in the $^{13}C/^{12}C$ ratio for glucose and fructose. The production of cooked musts, alcoholic fermentation, acetic bio-oxidation, and the transformation of vinegar in barrel batteries were analyzed.*

Keywords: *HPLC-IRMS; food traceability; ABTM*

Introduction

Italy leads the EU in quality-certified products, with Emilia Romagna ranking highest for the economic impact of food products with geographical indications. Modena significantly contributes to these ranking, particularly in the production of Aceto Balsamico Tradizionale di Modena (ABTM), which has a major economic impact. Understanding and verifying the entire ABTM production process, from the origin of raw materials to the final product, is crucial for ensuring product authenticity, especially for PDO (Protected Designation of Origin) and PGI (Protected Geographical Indication) food products, like ABTM.

This study focused on analysing the ABTM production chain to verify its product-territory link and assess the impact of various production stages, including the cooking of musts, fermentation, and vinegar transformation in barrel batteries, on the final product's characteristics. Using advanced analytical techniques, the study monitored changes in the isotopic ratios of carbon ($^{13}C/^{12}C$) in glucose and fructose. These indicators were tracked across all stages of production, from raw materials to finished products, to ensure traceability and authenticity of ABTM.

Experimental

Samples were collected from three vinegar producers in the region of Modena, designated as "Producer I", "Producer II", and "Producer III". The study involved the analysis of 18 grape samples at two ripening stages, 27 must samples during cooking, and 27 vinegar samples from four different ABTM production batteries. Carbon isotope ratios were determined using Isotope Ratio Mass Spectrometry (IRMS), with the system comprising an HPLC unit and a gas-phase sample introduction system known as "Liquiface". The CO_2 analyte, ionized by electron impact, was generated through the chemical oxidation of organic molecules. This conversion occurred via a redox process using potassium persulfate in an acidic environment maintained at 100 °C. Chromatographic separation of glucose and fructose is hindered by organic acids, which reduce signal resolution during HPLC elution. To address this, samples were treated with solid-phase extraction (SPE) using an amino column (NH_2) to remove the interfering acids. After appropriate dilution, all vinegar samples, from acidified musts to ABTM, underwent SPE-NH_2 treatment, and the sugars were then separated on an HPLC column with styrene-divinylbenzene and Ca functionalization.

Results

The carbon isotopic ratio $^{13}C/^{12}C$ reveals the metabolic pathways plants use to synthesize organic compounds, primarily sugars. This ratio differentiates plants with various photosynthetic pathways: C3, C4, or CAM. Each pathway has a specific range of $^{13}C/^{12}C$ values that can vary depending on the climatic conditions (temperature, water, and CO_2) and soil characteristics. *Vitis vinifera* follows the C3 photosynthetic pathway [1]. Globally, grape sugars typically have $\delta^{13}C$ values ranging from -20 ‰ to -36 ‰ V-PDB. In Emilia Romagna, where ABTM grapes grow, this range narrows to -31 ‰ and -23 ‰ V-PDBE, making the $^{13}C/^{12}C$ ratio a more precise indicator for this specific area.

Analysis of grape sugars showed $\delta^{13}C$ values consistent with C3 plants, with a narrower variability than the global averages, reflecting local meteorological conditions. However, $\delta^{13}C$ data for glucose and fructose revealed distinct ratio values during the maturation phase, with convergence observed as the grape reached full ripeness. These disparities may be attributed to the specific vegetative cycles inherent to the different grape varieties. Must sugars also fell within the expected range of C3 plants. Among the three musts analysed, those from Lambrusco Salamino grapes (Producers I and II) had similar values, whereas the must from Lambrusco Grasparossa (Producer III) had notably higher values. This suggests that $\delta^{13}C$ values may be influenced by grape variety, geographic area, or weather conditions. Prolonged heat treatment of the must did not cause

significant isotopic fractionation, although fructose generally had a slightly more positive $^{13}C/^{12}C$ value than glucose. Although degradation reactions might alter carbon isotopic ratios during cooking, studies on different processing methods have not yielded conclusive results. Typically, concentration processes under reduced pressure and low temperatures do not significantly affect $\delta^{13}C$ values [2]. Direct flame heating at atmospheric pressure is more extreme, and extended exposure to high temperatures produced results that differ from those reported in the literature. This is supported by the fact that glucose and fructose have different thermal stabilities, with fructose being less stable than glucose. Analysis of ABTM samples confirmed that the measured $^{13}C/^{12}C$ values fell within the typical range for C3 plants, verifying the grape origin and absence of adulteration. Notably, the isotopic ratios for glucose and fructose showed a consistent pattern, with fructose generally having a slightly more positive $^{13}C/^{12}C$ ratio than glucose. However, an unusual trend was observed in samples from the batteries of Producers I and II. For Producer I's battery, the $^{13}C/^{12}C$ values exhibited a unique pattern: by moving from the larger barrel (containing younger vinegar) to the smaller barrel (containing older vinegar intended for sale as ABTM), the $^{13}C/^{12}C$ values shifted to more positive values. This trend is difficult to explain without considering that the product may have been mixed with vinegar containing sugars with a more negative $\delta^{13}C$ value, suggesting that the rebalancing of the battery might be incomplete. The topping-up process starts with the smallest, terminal barrel and moves backward to the largest barrel, which is replenished with acidified must from the previous year. This situation could occur if there are changes in raw material suppliers, if acidified must is consistently purchased from different sources, or if production specifications are not followed, leading to the introduction of musts or vinegars from different geographic origins. When raw materials come from grapes grown in Modena and the reduced cooked must is acidified according to the specifications, the refining and aging processes should not significantly alter the carbon isotopic ratios of the sugars. This is evident in the $\delta^{13}C$ values for glucose and fructose in samples from Producer II's battery, which follows a "from field to barrel" approach, using acidified must from their own grapes that undergo the specified fermentation processes. The trends shown in Figure 1 indicate that the $\delta^{13}C$ values related to the ABTM production chain for producer II and the carbon isotopic ratio of the sugars remained relatively consistent throughout the ABTM production process, including grape maturation, must cooking, microbiological transformations, and aging in barrels. A gradual increase in the carbon isotopic ratio was observed during the transition from must to vinegar, with must showing more negative $\delta^{13}C$ (VPDB) values compared to vinegar. Additionally, glucose consistently had a more negative $\delta^{13}C$ value than fructose throughout the ABTM production process.

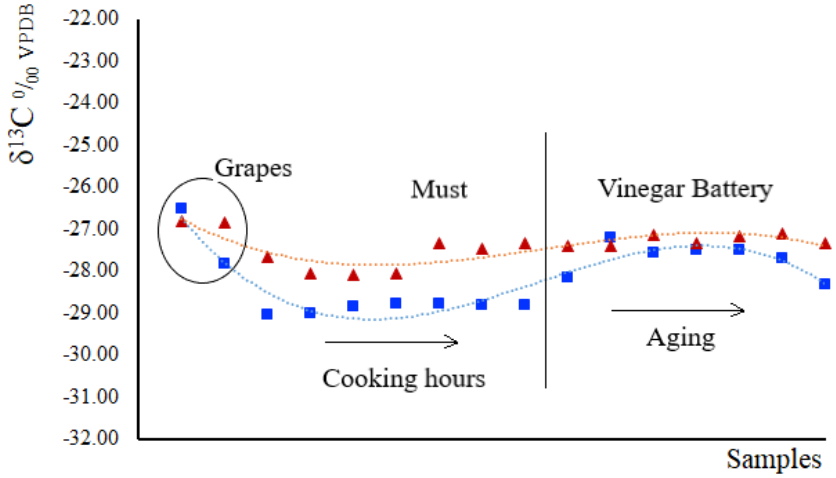

Figure 1. *$\delta^{13}C$ ‰ V-PDB values for grapes, must, and vinegar in the battery related to the ABTM production chain for producer II. (■) values measured for glucose; (▲) values measured for fructose.*

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P70

Comparison and differentiation of flavor profiles in vegan food and their non-vegan precursors by a novel GC-HRMS technology

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Keywords: *authentication, vegan, non-target, GC-HRMS, EI, CI, food quality*

An increased awareness to health, environment and animal welfare has led to a growing public interest in vegan products, and the market for meat- and dairy-free alternatives is growing rapidly.
One market strategy is the imitation of popular non-vegan products with the advantage that customers are already familiar with a product and may want to switch to a vegan alternative with similar taste and affection. Characterization of flavor and aroma profiles is of particular interest during the development of these types of products. Since a large part of flavor and aroma perception arises from volatile compounds, GC-HRMS is often used for the analysis. However, in non-target studies conventional EI (electron ionization) combined with NIST library searches suffers from an ambiguous identification of unknowns due to less specific fragmentation, missing molecular ion signals or absence in reference libraries. Presented will be a novel GC-HRMS that uses simultaneously both chemical ionization (CI) for the exact molecular mass assignment and EI for NIST searchable fragment spectra in a single GC run.
10 g of a vegan cheese from New Roots AG as well as its supposed non-vegan equivalent (Appenzeller Classic) were incubated for 10 mins in a headspace vial. Each cheese was sampled in triplicate at 60°C for 30 mins using a Restek Polyacrylate SPME fiber (BGB Analytik, Switzerland). The GC-HRMS consists of a high resolution TOF analyzer operating simultaneously a standard 70 eV EI source and a medium pressure CI source with an automated selection of different CI reagents ions (NH_4^+, N_2H^+, H_3O^+. It enables the adjustment of reactant selectivity and the degree of fragmentation for various compound classes of interest.
Using both pieces of information, EI and CI, in a single run enables a highly confident compound identification and quantification in complex matrices. Between the two types of cheese a clear
difference between common flavor compounds such as butanoic acid and 2/3- methyl butanoic acid can be observed. Several peaks of interest for both the vegan and the non-vegan equivalent were selected at different retentions times to evaluate profile differences. Statistical tools like volcano plots revealed potential markers for both samples. The value of additional CI information due to the selection of different CI reagents ions results in increased compound identification yields and certainties. Especially when EI library hits are only accounted as “fair” with low corresponding probability, the additional chromatographic and CI information can be used to increase compound identification confidence. False positives from an EI-only approaches can easily be identified and often correctly annotated using the additional information generated by CI. Furthermore, compounds not listed at all in libraries have a high uncertainty for identification using an EI-only approach. Using the accurate mass information on the molecular ion provided by CI, sum formula for these unknowns can be derived. Combining this molecular information with the structural information generated by EI, tentative structure elucidation becomes feasible in many cases.

P71

Fatty acids and macro elements evaluation in wild boar meat supplemented with hazelnuts

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Summary: *The target of this work is to evaluate fatty acids composition of wild boar meat and to compare it with wild boar supplemented with hazelnuts. It also aims to give a general composition of macro and micro elements.*

Keywords: *Mass spectrometry; wild boar; fatty acids*

Introduction
Pig intensive farming has a negative impact on environment and there are many problems related to its sustainability. Wild boar meat is increasingly consumed as alternative to pork meat, both for the increasing natural wild boar population and for the organoleptic characteristics, very attractive for consumers. In an increasingly nutrient-conscious world, knowing the composition of the food we consume every day becomes increasingly important. In this work we will determine the fatty acid composition, of meat derived from wild boar having classical feeding compared with wild boar supplemented with hazelnuts.

Experimental
Fatty acid analysis
The meat was subjected to acid hydrolysis and then divided into two aliquots, for the determination of short and long chain fatty acids after derivatization with butanol and methanol, respectively. The analyses were performed by GC-MS. Macro and micro elements characterization was carried out by ICP-MS after wet digestion.

Results
GC-MS after derivatization, allowed to determine the total content of SCFA and LCFA and to give a trend of the composition of wild boar meat after supplementation with hazelnuts. It was possible to observe a rich and complex composition of FA probably derived from alimentation.

Conclusions
GC-MS and ICP-MS analyses resulted to be valid tools to investigate molecular composition of such a complex matrix in order to highlight a possible link to the alimentation. Moreover, the fatty acids content detected in wild boar indicate this food as a possible valid alternative to pork meat.

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P72

UHPLC-Q-Orbitrap-MS-based lipidomic study of swine sausage produced by adding natural ingredients to replace nitrite and nitrate addition

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Summary: *The M.A.R.I. project aims at developing a novel approach of meat products stabilization based on the use of specific natural ingredients coupled to food irradiation. In this report, the results related to lipid profiles of swine sausage samples produced using extra virgin olive oil, rosemary essential oil and lemon albedo in the place of nitrite and nitrate are discussed under a lipidomic approach based on Orbitrap high-resolution mass spectrometry analysis.*

Keywords: *Food safety, meat science, lipidomics*

Introduction

Nitrites and nitrates are used as food preservatives especially in meat curing. Their wide use is due to the ability of stabilising red colour of meat, prolonging the shelf-life. However, the most significant reason of nitrite/nitrate addition in meat products is the stabilisation against *Clostridium botulinum* growth.
During years, different approaches have been proposed for replacing nitrite/nitrate addition in meat, since it is well-known that high intake of these additives is linked to methemoglobinemia and gastric cancer.
However, none of these studies assessed the effects of these novel technologies on the growth/sporulation and toxin production of *C. botulinum*. For this reason, the M.A.R.I. project proposes food irradiation as proven treatment commonly used for enhancing the safety of meat.
In this contest, the main objective of this project is the evaluation of a novel stabilisation techniques of meat products, based on the possible synergistic effect of food irradiation coupled to natural ingredients addition, without using nitrite and nitrate.
The first step of this research is the optimization of a meat sausage formulation obtained by adding ingredients such as extra virgin olive oil, rosemary essential oil and lemon albedo, since their effect in food stabilisation has already been proven [1]. The effects of these formulations, compared to the use of traditional additives, were evaluated by means of lipidomic approach, to highlight the quali-quantitative variations of lipids in seasoned swine meat samples under study.

Experimental

In this study, 12 different formulations were tested to prepare swine meat sausage samples. These products were prepared with and without using nitrite and nitrate, and by adding different percentages of extra virgin olive oil (from 0% to 1.5%), rosemary essential oil (from 0% to 0.15%) and dried lemon albedo (from 0% to 7%) using a Central Composite design (CCD). These products were then seasoned following a traditional procedure lasting 24 days at controlled temperature and humidity. The lipid extraction was obtained by using the well-established Folch's extraction procedure. This method is based on biphasic solvent system consisting of chloroform/methanol/water in a volumetric ratio of 8:4:3 (v/v/v). The extracts were analysed by an Ultimate 3000 UHPLC system with a binary pump (Thermo Fisher Scientific, Waltham, MA, USA), combined to heated electro-spray ionization (HESI) Q-Exactive Focus Orbitrap Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The lipid identification from raw data was performed by Thermo Fisher LipidSearch™ 5.0.63.8 software. The MS and software parameters followed a previously published work [2].

Results

In this work, diacylglycerols (DG) and oxidized triacylglycerols (OX_TG), were useful for understanding the stabilizing effects of natural ingredients under investigation added to meat products. Comparing sausages samples prepared at central conditions (CC) of experimental design (2.5% dried lemon albedo, 0.05% rosemary essential oil and 0.5% extra virgin olive oil) to samples prepared with (NN) and without (NO-NN) nitrite and nitrate, DG showed a decreasing in NO-NN ones (Figure 1). Contrariwise, several OX_TG increased in non-treaded samples (Figure 1), due to the oxidation phenomena naturally occurring in cured meat. Note that the amounts of DG and OX_TG in each run of experimental design depend on type and percentage of natural antioxidant employed.

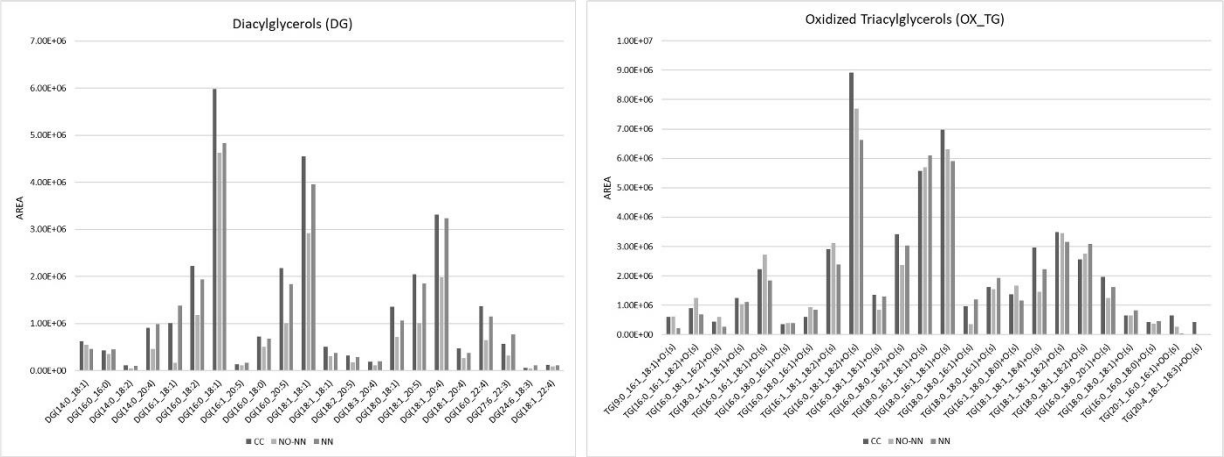

Figure 1. *Diacylglycerols and oxidized triacylglycerols amounts comparing sausage samples prepared at central conditions (CC), with nitrite and nitrate (NN) and without nitrite and nitrate (NO-NN)*

Conclusions

The results highlighted the stabilizing effect of natural antioxidants in seasoned sausages on lipid oxidation and degradation. Applied to this study, lipidomic approach prove to be a powerful tool useful for evaluation of new food preservation techniques. The optimised formulation will be used in the following tests of the M.A.R.I. project, coupled to food irradiation, in order to evaluate the possible inhibition of *C. botulinum* growth [1].

This study was financed by the Italian Ministry of Health (Rome, Italy), project code IZSPB 04/22 RC.

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P73

Identification of phyllobilin biomarkers in apple leaves (*Malus* x *domestica* Borkh.) affected by fungal diseases using high-resolution QTOF-MS

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Summary: *Fungal pathogens cause severe symptoms on plant leaves, such as necrotic spots and chlorotic areas. These visible anomalies are associated with chlorophyll breakdown. The products of autumnal chlorophyll degradation, known as phyllobilins, were investigated as potential biomarkers to monitor plant health.*

Keywords: *Chlorophyll breakdown, plant biomarkers, fungal pathogens*

Introduction

In plants, fungal infections trigger several phytohormones including ethylene and jasmonic acid [1]. Similarly, these molecules also control genes which encode enzymes in the pheophorbide a oxygenase/phyllobilin PaO/PB pathway, a biochemical process that degrades chlorophyll into PBs during senescence [2]. In this study, we investigated chlorophyll catabolites in healthy, diseased, and senescent leaves of three apple cultivars to test their potential as biomarkers for fungal infection in agriculture.

Experimental

Information on the collected samples is reported in Table 1.

Table 1. *Detailed description of the leaf samples used for analysis. g = green, d = diseased, s = senescent*

Cultivar	Fungal disease	Leaves collection date	Symptoms on leaves
Golden Delicious	Apple scab	14.07.2022 (g, d) 25.11.2022(s)	Olive-green spots
Gala	Powdery mildew	29.07.2022 (g, d) 25.11.2022(s)	Chlorotic spots
Golden Delicious	Alternaria Leaf blotch	29.07.2022 (g, d) 25.11.2022(s)	Brown spots
Red Delicious	Marssonina apple blotch	27.09.2022 (g, d) 07.12.2022 (s)	Brown spots

PBs analysis was performed using UHPLC-QTOF-MS with the following conditions: mobile phase A, ammonium acetate 4 mM pH 7; mobile phase B, ACN. The solvent gradient was: 0−3 min, 20 % B; 6 min, 30 % B; 7.65 min, 70 % B; 8.3 min, 95 % B; 16 min, 95 % B; 19 min, 10 % B; 21 min, 10 % B. ESI source was set with positive ion polarity, 500 V for end plate offset, 4500 V for capillary, 3.0 bar for nebulizer, 12.0 L min-1 for dry gas and 230 °C for dry temperature. Acquisition was performed from 50 *m/z* to 1500 *m/z*, 0.5 Hz spectra rate, with data-dependent method based on a “Precursor Ions List” containing all known and hypothesized pseudo-molecular ions of PBs (in total 76) with a mass width tolerance of 0.50 *m/z*. All data were analysed with Software Bruker Compass DataAnalysis 4.2. PBs were identified using molecular ions (< 5 ppm mass accuracy) and mass fragments (minimum two fragments per PB with mass accuracy < 5 ppm.

Results

The majority of PBs were found in senescent leaves, with a maximum of 29 catabolites identified in the apple cultivar Golden Delicious. All diseased leaves had fewer PBs, except in Marssonina apple blotch, where 20 compounds were found. Notably, the PB DNCC-632 was detected in 60% of healthy leaves of Alternaria Leaf blotch samples. This suggests the enzyme CYP89A9, which synthesizes this catabolite, could be affected by fungal diseases. Furthermore, a novel 5-methoxyPB was found in senescent apple leaves (see Figure 1).

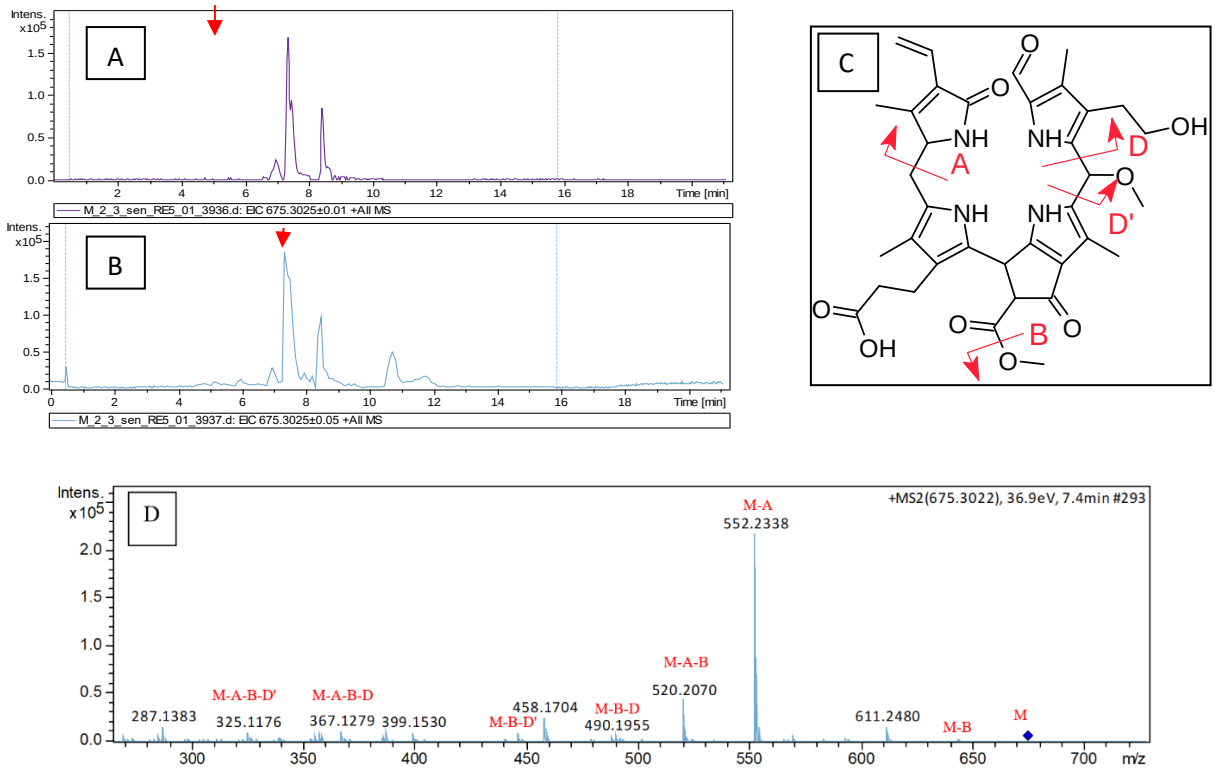

***Figure 1**. Extracted ion chromatogram of phyllobilin NCC-674 acquired in full scan mode (A) and in MS² mode (B), with tentative constitutional formula and fragmentation sites of putative novel 5-methoxyPB (C). Additionally, the fragmentation pattern of the molecule is reported (D).*

Conclusion

Chlorophyll degradation is a complex biochemical pathway that occurs in response to various stimuli [2]. The number of PBs detected in apple leaves differs from the pathology. Marssonina apple blotch induces senescence in plants and causes pronounced chlorosis. Thus, PBs can serve as biomarkers for pathologies associated with this visible process.

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P74

Identification and quantification of coenzyme Q10 in innovative *Brassica* vegetables by liquid chromatography/mass spectrometry coupled with atmospheric pressure chemical ionization

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Summary: *A detailed fragmentation pattern of Coenzyme Q10 was defined using a combined approach of RPLC coupled with high/low resolution/accuracy mass spectrometry by APCI in negative ion mode. Additionally, the quantification of CoQ10 was performed on both innovative and common vegetables, following method validation*

Keywords: *Coenzyme Q10, mass spectrometry, innovative vegetables*

Introduction

Innovative vegetables, including microgreens, micro leaves, and baby leaves of *Brassica rapa* and *Brassica oleracea*, offer an alternative to their mature counterparts. Microgreens are defined as young, tender vegetables typically harvested within 10-15 days of seedling emergence. Micro leaves are slightly more mature, harvested within 15-20 days, and baby leaves are harvested after 20-40 days once up to eight young leaves have fully developed.[1] These innovative vegetables may serve as substitutes for their mature forms, which are commonly consumed. A significant difference between these forms and mature vegetables lies in their preparation: unlike mature vegetables, that are often cooked, these microforms are generally consumed raw, such as in salads. In recent years, numerous studies have focused on determining the nutraceutical compounds of these vegetables to evaluate their potential as future food alternatives.[2] In this presentation, we focus on the identification of coenzyme Q10 (CoQ10), a vital vitamin-like substance that plays an essential role in mitochondrial electron transfer and ATP production. CoQ10 consists of a benzoquinone head linked to ten isoprenoid units. While various studies have used reversed-phase liquid chromatography (RPLC) coupled with mass spectrometry (MS) by electrospray ionization (ESI) and/or atmospheric pressure chemical ionization (APCI) to characterize CoQ10 structurally,[3] detailed studies on the fragmentation patterns achieved in negative ion mode are scarce. Typically, the analysis of CoQ10 in positive ion mode, using either ESI or APCI, results in a protonated molecule or an alkaline adduct, depending on the mobile phase composition. However, tandem MS spectra obtained for those positive ions are not very informative. Additionally, generating deprotonated CoQ10 using ESI in negative ion mode is not efficient. The best results are obtained with APCI in negative ion mode, which detects CoQ10 as a negative radical ion ($[M]^{-\bullet}$) at *m/z* 862.684. Tandem mass spectra acquired on this ion generate numerous product ions associated with different fragmentation pathways, which have not yet been explained in the literature and were addressed in the present work. We developed a quantitative method for CoQ10 in different forms of *Brassica rapa* and *Brassica oleracea* vegetables, evaluating its linearity, limit of detection (LOD), limit of quantification (LOQ), matrix effect, repeatability, and reproducibility. The complete fragmentation pattern scheme of CoQ10 and the results from the quantification are presented in this communication.

Experimental

A CoQ10 stock solution was prepared at a concentration of 500 mg/L in 2-propanol and subsequently diluted for further analysis. For extraction from all the vegetables under study, 0.5 mL of 0.15 M NaCl, 0.8 mL of ethanol, and 2 mL of hexane were added to 200 mg of lyophilized material. The samples were shaken and then centrifuged at 5000 rpm for 15 minutes; the hexane layer was collected. This extraction process was repeated twice by adding 0.5 mL of ethanol and 2 mL of hexane each time, and all hexane aliquots were combined and dried under nitrogen. The samples were then resuspended in 200 µL of 2-propanol for RPLC-MS analysis.

Chromatographic separation was performed using an Ascentis Express C18 column (150 × 2.1 mm ID, 2.7 µm particle size) equipped with an Ascentis Express C18 pre-column (5 × 2.1 mm ID). A 40-minute elution gradient was employed, with methanol (phase A) and 2-propanol (phase B), at a flow rate of 200 µL/min. The APCI source was used in negative ion mode. Two different facilities were utilized: the Q-Exactive for high-resolution/accuracy acquisition and the VelosPro for MS^n acquisitions.

Results

As previously mentioned, the RPLC-APCI-FTMS in negative ion mode enabled the detection of CoQ10 as a radical ion $[M]^{-\bullet}$ at *m/z* 862.684. The RPLC-APCI-FTMS/MS analysis revealed two ions at *m/z* 847.656 and 832.634, corresponding to two consecutive loss of methyl groups from the methoxy groups on the benzoquinone ring of CoQ10. This fragmentation process highlights the principal series of CoQ10 product ions, generated upon the loss of oligo isoprene radicals from the ion at *m/z* 832.634. These fragmentation pathways generate the base peak at *m/z* 219.066, associated with a species containing one isoprene unit linked to the head. The potential for cyclization in a six-membered ring provides this species with high stability, making it the most abundant product ion in the spectrum. Other minor fragment series are produced during MS/MS acquisition, resulting from the loss of water, a radical methyl group, and CO. This product ion alongside the ion at *m/z* 287.129 (fragment with two residual isoprene units), were diagnostic for identifying other CoQn-related species in the samples using the all-ion fragmentation (AIF) mode. This approach revealed species such as CoQ9, CoQ8, and CoQ7 in both innovative and mature vegetables.

Furthermore, the quantification of CoQ10 was performed on all vegetable samples. For innovative vegetables, different growing conditions with varied irradiation (sunlight and/or LED irradiation in red/blue) were considered to establish their potential effect on CoQ10 levels. For mature vegetables, different cooking processes were evaluated to ascertain their impact on CoQ10 content. This comprehensive analysis not only mapped the fragmentation pathways of CoQ10 but also provided insights into how various cultivation and preparation methods influence its levels in different vegetables.

Conclusions

Using RPLC-APCI coupled with both high and low resolution/accuracy MS in negative ion mode, we have, for the first time, detailed the fragmentation pattern of coenzyme Q10 and related species (CoQn). Following method validation, we quantified this important nutraceutical compound in both innovative and mature forms of *Brassica rapa* and *Brassica oleracea*. For the innovative vegetables, we considered different growing conditions with varying light irradiation to see how they affect CoQ10 levels. For the mature vegetables, we evaluated the impact of various cooking processes on their CoQ10 content.

Acknowledgements

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P75

Essential fatty acids and mineral elements in baby food products

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Summary: *In this study, 27 samples of baby food purchased in supermarkets in Messina (Italy) were examined. The first objective was to evaluate the levels of mineral elements by ICP-MS to assess the potential toxicological risk for infant from daily consumption of these products, and the second objective was to evaluate the content of essential fatty acids by HRGC-FID to identify the daily energy intake to improve infant's health. The results showed that some products contained almost no or too high a daily intake of essential fatty acids and an amount of toxic and potentially toxic elements (especially As, Cd, Pb and Hg) above reference levels in almost all samples.*

Keywords: *baby food products, food safety, mineral elements, fatty acids*

Introduction

Nutrition during infancy plays a crucial role in the healthy and complete development of the infant, influencing not only physical growth, but also cognitive development and psychological well-being. The World Health Organization (WHO) has stated that infants should be exclusively breastfed for the first six months of life, and then be introduced to foods that meet their energy and nutritional needs for complete and optimal growth. Baby food products are formulated to meet the specific needs of infants, contributing to a balanced and nutritious diet. These products include baby food, snacks, milk powder and homogenized foods, ideally made with high quality ingredients and free of harmful additives. Scientific research pays particular attention to infant's health through regular and careful analyses of infant-specific products as these can have a lasting impact on future food preferences and susceptibility to metabolic disorders. Therefore, the analysis of infant food products is not only necessary to ensure safety and proper nutrition, but also crucial in the context of a healthy and sustainable diet for the next generation.[1] In particular, the content of essential fatty acids (ω3 and ω6) and mineral elements is of paramount importance, as they promote brain development in infant and support the immune system and the various metabolic processes essential for healthy and complete growth.

Experimental

In this context, the following study had two aims: to evaluate the mineral and essential fatty acid content of 27 samples of baby food (different types of baby food and powdered milk) purchased in supermarkets in Messina (Italy). More precisely, the content of Li, Be, Na, Mg, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Cd, Sb, Pb, Sn and Hg was assessed by ICP-MS and DMA-80, evaluating the potential toxicological risk for infant after consumption of these products; On the other hand, for the evaluation of the essential fatty acid content (ω3/ω6), the various products were initially extracted and esterified to be analyzed by HRGC-FID, quantifying the daily intake for infant's health.

Results

In this study, based on 27 baby food products, potassium was the most abundant mineral in almost all the samples (range: 1179.67±22.72 - 6024.34±26.57 mg/kg), except in homogenized processed cheese, which had a higher calcium content (average: 3784.40±26.66 mg/kg). Among the essential trace elements, iron and zinc were the most abundant in all the samples analyzed. About toxic and potentially toxic elements, European Regulation 915/2023 sets maximum limits for As, Cd and Pb in some baby products. In general, the infant formula samples analyzed in this study exceeded the permitted levels of arsenic (0.020 mg/kg), cadmium (0.010 mg/kg) and lead (0.020 mg/kg). In addition, most of the infant formulae analyzed in this study had an arsenic content above the maximum permitted level. For the toxicological risk assessment, a range of consumption between 40 g/day and 250 g/day was considered as the most common amounts of tinned infant formulae, with reference to the EFSA guidelines, and an average weight of 9 kg for infants aged 6-11 months[3,4]. Consumption of these baby foods resulted in a complete intake (exceeding the recommended intakes) of Cr for homogenized veal, Mo for homogenized zucchini and potatoes, Na, Ca and Co for homogenized processed cheese and Ca, Co, and Se for powdered milk. However, consumption of most of the baby products analyzed resulted in intakes of toxic and potentially toxic elements (particularly As, Cd, Pb and Hg) exceeding the reference levels in almost all samples. With regard to the content of essential fatty acids, ω3 and ω6, which can promote the absorption of fat-soluble vitamins and increase the energy density of foods, are particularly high in some rice and fruit products and low in other meat and fish products. The results also show the ω3/ω6

ratio and all of the products analyzed fall outside the range established by EFSA with a recommended intake of 3-9 E% for infant aged 6-11 months.[5] In fact, the products analyzed have a particularly low ratio compared to the established range.

Conclusion

In the light of the results obtained, it is therefore of paramount importance to continue monitoring the mineral and essential fatty acid content of these products to protect infant's health.

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Monitoring the volatilome of kefir and kefir-like cereal-based beverages during fermentation by PTR-Tof-MS

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Summary: *Enhancing fermentative bioprocesses is a needed step towards higher environmental, social, and economic sustainability standards. Functional beverages, milk kefir in particular, stand out as a rapidly expanding segment for their health benefits. This study demonstrates the feasibility of green real-time monitoring of volatile profile kefir-like beverages during fermentation.*

Keywords: *omics, lactic acid bacteria, functional food*

Introduction

Functional beverages stand out as a rapidly expanding segment within the realm of emerging food categories. Milk kefir, an ancient traditional fermented beverage, holds a place within the domain of functional foods, acknowledged for its established edonistic and health-enhancing properties. Milk kefir is commonly produced using a combination of lactic acid and alcoholic fermentation by microbial flora, conferring a small amount of ethanol content and a unique characteristic flavour to this product. The traditional method of making milk kefir draws in kefir grains that are mixed cultures consisting of various lactic acid bacteria yeasts, and acetic acid bacteria that co-exist in a symbiotic association and are responsible for an acid alcoholic fermentation [1,2].
Different formulations and biotechnological innovations were recently proposed to develop kefir and kefir-like products with improved sensory, nutritional, and functional features [3]. For instance, kefir grains were used to ferment non-dairy raw materials, such as vegetables and cereals to produce functional beverages enriched in vitamins, bioactive compounds or able to vehicle potential health-promoting bacteria.
In this contest, volatile organic compounds (VOCs) serve as valuable indicators to monitor bioprocesses, offer insight into the quality of the matrices and can be considered as promising biomarkers in terms of sensory properties. Proton-transfer-reaction, coupled with Time-of-Flight Mass Spectrometer (PTR-ToF-MS), represents a green, rapid and non-invasive analytical solution to screen microbial volatilome and was widely applied in the field of fermentation monitoring [4].
This work aimed to evaluate the dynamic changes of volatile profile of products obtained during the fermentation by PTR-ToF-MS coupled with a multipurpose GC automatic sampler.
In the present research different matrices were selected as two case studies: raw milk and UHT milk (case study 1) and oat, maize and barley flours (case study 2).

Experimental

Case study 1: raw milk and UHT milk were fermented with a commercial starter (milk kefir preparation). VOCs produced during fermentation were sampled automatically every 3 hours for 48 hours at 26°C by static headspace module of multipurpose GC automatic sampler (Gerstel GmbH, Mulheim am Ruhr, Germany) into a PTR-ToF-MS 8000 device (Ionicon Analytik GmbH, Innsbruck, Austria).
Case study 2: standard milk–based kefir (as control) and three cereal-based kefir-like beverages, obtained from oat, maize and barley flours, were fermented with commercial starters: water kefir preparation and milk kefir preparation with or without the addition of *Lactoplantibacillus plantarum* strain, according to the method previously reported by Yépez et al. [3]. VOCs produced during fermentation were sampled automatically every 2 hours for 48 hours at 26°C by a dynamic headspace module of multipurpose GC automatic sampler into a PTR-ToF-MS 8000 device.
All PTR-ToF-MS data collected were processed and analyzed using the procedure described by Cappellin et al. [5].

Results

Changes of variability in the time of the inoculated samples were observed in more than 300 mass peaks at significant concentrations. The differences in the matrix formulation, starter and additional inoculation within the kefir beverage types under investigation affected their VOCs profiling in all samples. The formation of

volatile higher alcohols and corresponding esters during kefir fermentation was influenced by the composition of the starter and inoculation with Lactoplantibacillus plantarum. Considering different matrices, the inoculation has a higher influence on the VOCs profiling of corn and oat kefir than on of barley and milk beverages. The evidence helps to underline the role of volatiles in the study of the development of protechnological and spoilage microbes (raw milk trial). The dominance of the starter over the evolution of the psychrotrophic microflora has also been assessed.

Conclusions

The evidence gathered in the present study indicated that PTR-ToF-MS measurements effectively assess and monitor VOCs during fermentation processes, allowing for the tracking of their evolution and kinetics. By detecting significant VOCs produced by microbial metabolism, such as higher alcohols, esters, volatile fatty acids, and sulfur compounds, our ability to customize food flavor is enhanced. This includes gaining a better understanding of flavor-active molecules and developing new methods for reducing off-flavors. Simultaneously, VOC monitoring can aid in leveraging other pro-technological and beneficial aspects of microbial physiology, thereby improving the functionality and safety of the product. Additionally, the observed differences in VOC profiles among kefir beverage types are expected to result in varying sensory properties, particularly influencing consumer overall liking. The potential of the technique for monitoring undesired microbial growth was also explored, highlighting possible uses for safety monitoring, limiting spoilage phenomena, and for rapid information on microbial starter dominance.

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P77

Stable isotope ratio analysis to detect biosynthetic citric acid addition to Italian tomato sauce

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Summary: *An innovative LC-co-IRMS (liquid chromatography-isotope ratio mass spectrometry) method to detect the addition of biosynthetic citric acid to tomato sauce was optimized and validated. Threshold carbon isotopic values for authentic tomato citric acid were established and used to test a group of commercial samples available on the market.*

Keywords: *stable isotopes, liquid chromatography – isotope ratio mass spectrometry, Italian tomato sauce*

Introduction

Tomato sauce is an important product in Italian market and economy. Citric acid (E330) is naturally present in tomatoes and, according to EU laws, it can be added to tomato sauce with no restrictions about its amount and origin [1]. Unlike consumer perception, the citric acid most added to the tomato sauce does note derive from citrus fruits [2], but it is biosynthetically obtained from *Aspergillus Niger* fungus fed on cheap starting materials such as corn and cane. As consumers are willing to pay an extra for products labelled as “100% natural” or “no additives”, a method to detect the addition of biosynthetic citric acid to food and, in this case, to Italian tomato sauce, is required.
Tomato belongs to the group of C3 plants, meaning that inside the plant carbon dioxide (CO2) is fixed via Calvin cycle during the photosynthesis. On the other hand, cheap sources like cane and corn, provided to *Aspergillus Niger* for citric acid synthesis, are classified as C4 plants. These plants adapted to environmental conditions choosing an alternative photosynthetic pathway. This results in statistically different carbon isotopic values ($\delta^{13}C$) for C3 and C4 plants [3], making it possible for the stable isotope ratio analysis (SIRA) to discriminate between different sources.
The AIJN (Association of the Industry of Juices and Nectars) provided reference values for the citric acid of other types of fruits (lemon, orange, grapefruit) but no values are given for tomato citric acid. Reference $\delta^{13}C$ are reported for tomato bulk sugar [4], but to the best of our knowledge, no information about single sugars like glucose and fructose are available.
This work had therefore multiple goals. First, we wanted to validate a method that could provide $\delta^{13}C$ values for tomato citric acid ($\delta^{13}CCA$) and apply it on a wide dataset of authentic Italian tomato sauce samples to establish reference values. Once assessed reference $\delta^{13}CCA$ values, we wanted to apply them to commercial samples available on the market to check whether they were added with biosynthetic citric acid or not. Moreover, since it has been reported that in some cases the ratios between the isotopic values of acids and sugars were more effective than the $\delta^{13}C$ itself to detect additions to the samples [5], ratios between the $\delta^{13}CCA$ and the $\delta^{13}C$ of tomato glucose (RCA/G) and fructose (RCA/F) were considered.

Experimental

A 300 samples dataset has been considered, mainly represented by Italian authentic tomato sauces, sampled by ICQRF (Central Inspectorate for Fraud Repression and Quality Protection of the Agri-Food Products and Foodstuffs), and an additional group of tomato sauce samples available on the market.
The analyses were carried out through a liquid chromatographer coupled with an isotope ratio mass spectrometer (LC-co-IRMS) (Thermo Scientific, Bremen). The column (Rezex ROA- Organic Acid H+ (P/N 00H-0138-K0, Phenomenex, Torrance, USA) selected for citric acid isolation of made it possible to simultaneously measure the $\delta^{13}C$ of citric acid ($\delta^{13}C_{CA}$), glucose ($\delta^{13}C_{G}$) and fructose ($\delta^{13}C_{F}$).
Samples preparative included the centrifugation of the tomato sauce sample and their dilution 1 to 10 with milliQ water. The analytical protocol followed for the analysis and a deeper description of the apparatus selected are described in the literature [6].

Results

To validate the LC-co-IRMS method, the within- and between-days repeatability of both a standard and an authentic tomato sauce sample were measured. The tomato sample within-day repeatability gave standard deviations of 1.5‰, 1.5‰ and 1.2‰ for $\delta^{13}C_{CA}$, $\delta 13C_{G}$ and $\delta^{13}C_{F}$, respectively. The between-days repeatability

gave standard deviations of 1.1‰, 0.8‰ and 0.9‰ for $\delta^{13}C_{CA}$, $\delta^{13}C_G$ and $\delta^{13}C_F$, respectively.
The $\delta^{13}C_G$ and $\delta^{13}C_F$ of Italian tomato sauce samples were in line with the values provided by the AIJN for bulk tomato sugar. The two parameters resulted linearly correlated (Pearsons's coefficient = 0.84), in agreement with previous results on grape glucose and fructose [6].
The $\delta^{13}C_{CA}$ of authentic Italian tomato samples averaged -26.5±1.3‰, ranging from a minimum value of -31.3‰ to a maximum value of -23.6‰ (Figure 1). Considering the standard deviation and the analytical uncertainty, a threshold value for the $\delta^{13}C_{CA}$ for authentic samples was established. As previously mentioned, when evaluating the authenticity of the commercial samples, the $\delta^{13}C_{CA}$ values were used together with the ratios between citric acid values and glucose/fructose ones (RCA/G, RCA/F). Applying the threshold values to the commercial samples, it turned out that 5.2% of them was added with biosynthetic citric acid.

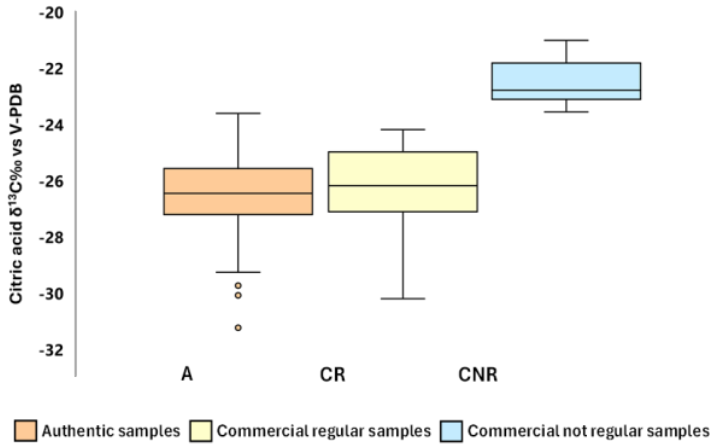

Figure 1. *Citric acid $\delta^{13}C$ of authentic Italian tomato samples and for regular/not regular commercial samples*

Conclusions
In the present study, a wide dataset made up of more than 300 authentic Italian tomato sauce samples was analysed through LC-co-IRMS to simultaneously measure the $\delta^{13}C$ of citric acid, glucose and fructose. For the first time, reference $\delta^{13}C$ values for tomato citric acid were established, giving the opportunity to test a group of commercial tomato sauce samples. The 5.2% of the test group turned out to be added with biosynthetic citric acid. For a broader applicability of the method, authentic tomato sauces having different geographical origins should also be analysed.

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Rapid detection of safflower as adulerant in saffron: development of a targeted MS/MS method on DART EVOQ platform

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Summary: *A method based on target DART-MS/MS analysis for the detection of safflower in adulterated saffron has been developed on a newly launched triple quadrupole platform interfaced with a DART system. The method proved to be a promising tool for future applications in the rapid analysis of analytes contaminating/adulterating food products. Total analysis time for running 10 samples was around 5 minutes with no sample prep required.*

Keywords: *DART-MS, targeted analysis, saffron, authenticity, safflower, metabolite analysis*

Introduction

Direct analysis in real time-mass spectrometry (DART-MS) has evolved over the years proving to be an effective analytical technique for the rapid and accurate analysis of food samples [1]. Unlike traditional analytical methods that usually require time-consuming sample treatment steps, DART-MS provides rapid detection readouts, considerably decreasing the whole analysis time.
Numerous applications of DART-MS in the field of forensic and food control/safety have been published in the last 5 years mostly applied to food authentication and traceability [2,3] and to the detection of contaminants in food products. DART-MS is an essential tool in food safety, which can detect contaminants such as pesticides, mycotoxins, and veterinary drug residues in various food products.
Saffron is a precious and highly appreciated spice obtained in different Mediterranean regions by drying the stigmas of Crocus sativus L. (family Iridaceae). Nowadays, saffron quality is certificated in the international trade market on the basis of its aroma, flavor, and color strength using the ISO 3632-1: 2011 method, which combines spectrophotometric measurements of picrocrocin and safranal, and chromatographic profiles of pigments (crocins) and apolar dyes that can be toxic (as Sudan dyes) [4, 5].
It has been demonstrated that saffron adulterants (safflower, marigold, or turmeric) up to 20% (w/w) were not detected by the ISO normative [6]. Therefore, development of fast, simple, and robust screening methods suited for identifying saffron adulteration are needed. A promising alternative strategy to detect saffron adulteration is offered by metabolomics approach by untargeted HRMS analyses integrated with multivariate statistical analysis for data treatment as described in previous papers of our group [7].
In this note an overview of the DART-MS based methods applied to the analysis of saffron will be provided and pre-identified candidate markers will be assessed for their feasibility to build up a MRM targeted method for saffron authentication on the newly launched DART-EVOQ triple quadrupole Mass Spectrometer. The identification of specific markers tracing safflower presence in saffron by targeted DART-MS method will be presented along with their capability to quantify tiny percentage of safflower added to authentic saffron at the lowest 5% level.
An overview of the methods so far developed for saffron authentication with special regard to fast and high throughput methods will be also provided in this note.

Experimental

Saffron and safflower powder in pure form (n=2 each) along with saffron samples singly adulterated with safflower at three different inclusion levels (5-10-20%, n=2 each) were extracted according to a quick extraction protocol (50 mg of saffron powder extracted with 5 ml of EtOH/water, 70/30 under shaking) for massive metabolites extraction and directly analysed by DART-MS upon 1/1 dilution, skipping any sample prep. Calibration curves in real matrix were produced by diluting the saffron extract with safflower extract to reach the following inclusion levels 10%, 20%, 30%, 50%, 70% of safflower. A total of 4 repetitions for DART-MS analysis were recorded for each sample and analysis of blank samples were run along the batch to verify the absence of any carry over. Samples were analyzed in Full scan MS and Product Ion Scan acquisition mode in order to pinpoint potential markers and to choose the highest MS/MS fragments. Once the markers had been identified, MRM experiments on the most sensitive ions along with specific maker/transitions were carried out on the DART- EVOQ triple quadrupole mass spectrometer (Bruker) at set DART and MS conditions. In order to improve linearity of the calibration curve and decrease variability of the whole analysis, suitability of caffeine analyte added to the samples as internal standard was also investigated throughout the analysis.

Results

Metabolite extracts of saffron and safflower together with the progressively diluted solutions prepared (4 replicated analysis each) were analyzed by Full Scan DART-MS mode and product ion scan mode once the precursor ions had been identified. After optimization of the collision energy, the same samples were acquired in MRM mode and the following precursor ions/transition were monitored as potential authenticity markers for saffron adulteration: 116.2/70.1 ; 446.4/116.2.

The workflow used in the following investigation aiming at developing a quick and high-throughput method for the detection of saffron adulterated with safflower in depicted in Figure 1.

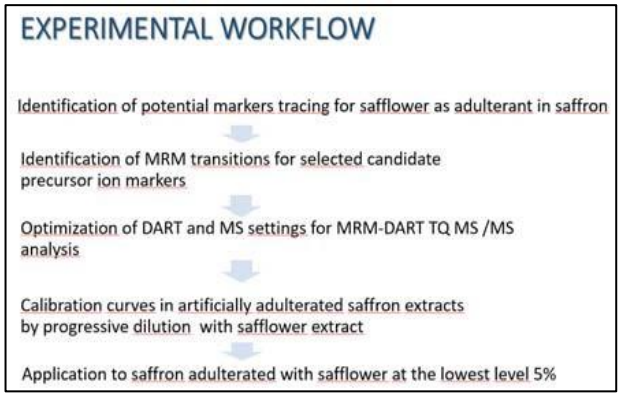

Figure 1. *Experimental workflow of a targeted DART MS/MS method for the detection of safflower as adulterant in saffron.*

By running full scan MS analysis of safflower extract and authentic saffron extract, two potential markers emerged from the MS spectra recorded and thoroughly analysed by the analyst by visual inspection, namely 116.2 and 446.2 m/z. The detection of the same pre-candidate markers was then ascertained in the extract mixture at the different fortification levels (from 10 to 70%) to assess their suitability to detect safflower in saffron adulterated samples. Once the collision energy of both precursor ions had been optimized by ad hoc product ion scan experiments, the chosen precursor/fragment transitions were selected and monitored in the following analysis. Calibration curve obtained by MRM analysis tracing two safflower markers in saffron extract fortified at increasing inclusion levels (from 10% to 70% of safflower extract) proved to correlate satisfactorily and calibration curve equation obtained with addition of the internal standard in all samples was the following: y = 62,061x - 1,9498, R^2 = 0,9755 as reported in Figure 2.

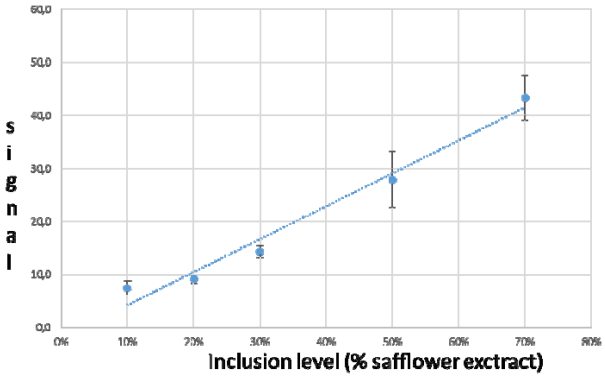

Figure 2. *Calibration curve obtained by MRM analysis monitoring the sum of following transitions 116.2/70.1; 446.4/116.2.*

Conclusions

The developed DART MRM triple Quadrupole MS method and the preliminary results herein summarised and presented proved to be a promising approach for fast detection and quantification of safflower present as adulterant in saffron.

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