Società Chimica Italiana
Divisione di Spettrometria di Massa

ISTITUTO NUTRIZIONALE CARAPELLI® FONDAZIONE ONLUS

MS Food Day 2022

Florence (Italy), October 5 - 7, 2022

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BOOK OF ABSTRACTS

Plenary, Keynotes, Orals

PROCEEDINGS OF THE 7th MS FOOD DAY

October 5-7, 2022

Florence - Italy

Scientific Committee

Gianluca Giorgi *(Chairman)*	*Università di Siena*
Giuseppe Avellone	*Università di Palermo*
Gianluca Bartolucci	*Università di Firenze*
Franco Biasioli	*Fondaz. Edmund Mach, S. Michele a/A (TN)*
Lucia Bonassisa	*Bonassisa Lab, Foggia*
Anna Cane	*Istituto Nutrizionale Carapelli, Firenze*
Donatella Caruso	*Università di Milano*
Chiara Dall'Asta	*Università di Parma*
Riccardo Flamini	*CREA-VE, Conegliano*
Emanuele Forte	*Ferrero, Alba*
Roberta Galarini	*IZS dell'Umbria e delle Marche, Perugia*
Renzo Galli	*Fileni, Cingoli (MC)*
Davide Garbini	*COOP Italia, Bologna*
Marzia Innocenti	*Università di Firenze*
Fulvio Magni	*Università di Milano Bicocca*
Nadia Mulinacci	*Università di Firenze*
Luciano Navarini	*illycaffè, Trieste*
Paola Pittia	*Università di Teramo*
Michele Suman	*Barilla, Parma*
Caudia Vatteroni	*Parmalat, Parma*
Sauro Vittori	*Università di Camerino*

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SCIENTIFIC PROGRAMME

Wednesday, October 5th, 2022

14:00 – 14:30 Registration and reception

14:30 – 15:00 **Welcome addresses**

Fulvio Magni
University of Milano Bicocca, President of the Division of Mass Spectrometry of the Italian Chemical Society

Roberto Sassoni
President of Istituto Nutrizionale Carapelli - Fondazione onlus - Firenze

Anna Cane
Board member of Istituto Nutrizionale Carapelli - Fondazione onlus Firenze

Gianluca Giorgi
University of Siena, 7 MS Food Day Scientific Committee, *Chair*

15:00 **1st Session: Food and human health, phytochemistry**

Chairs: ***Franco Biasioli*** *(Fond. E. Mach, S. Michele a/A),* ***Fulvio Magni*** *(Univ. of Milano Bicocca)*

15:00 – 15:40 **PL1: Advances in mass spectrometry to promote food safety and human health**

Richard Caprioli

School of Medicine, Vanderbilt University (USA)

15:40 – 15:55 **OR1: Nutrimetabolomics and consumption of polyphenols in elderly: how molecular markers can help the development of personalized diets to promote a healthy gut and a healthy aging**

Gregorio Peron, Tomás Meroño, Giorgio Gargari, Raul González-Domínguez, Antonio Miñarro, Esteban Vegas-Lozano, Nicole Hidalgo-Liberona, Cristian Del Bo', Stefano Bernardi, Paul Anthony Kroon, Antonio Cherubini, Simone Guglielmetti, Patrizia Riso, Cristina Andrés-Lacueva

Department of Nutrition, Food Sciences and Gastronomy, University of Barcelona, Barcelona (Spain)

15:55 – 16:10 **OR2: Discovering natural and healthy pigments: analysis of Anthocyanins on wheat by mass spectrometry**

Emanuela De Maio, Lucia Bonassisa, Stefano Sportelli, Luca Tommasi, Rosa Spaccavento

BonassisaLab S.r.l., Foggia (Italy)

16:10 – 16:25 **OR3: Determination of phyllobilins in the peels of apples cv. 'Gala' (*Malus* x *domestica* Borkh.) at five different ripening stages using high-resolution quadrupole-time-of-flight-mass spectrometry**

Luca Vestrucci, Lisa Marie Gorfer, Valentina Grigoletto, Valentina Lazazzara, Angelo Zanella, Peter Robatscher, Matteo Scampicchio, Michael Oberhuber

Faculty of Science and Technology, Free University of Bozen-Bolzano, Bolzano (Italy)

16:25 – 16:40 **OR4: Phytochemical investigation of seven unripe tomato cultivars (*Solanum Lycopersicum*)**

Vincenzo Piccolo, Elisabetta Schiano, Fortuna Iannuzzo, Maria Maisto, Ettore Novellino, Gian Carlo Tenore, Vincenzo Summa

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

16:40 – 17:15 Coffee break

Poster session (even number posters)

17:15 **2nd Session: Alkaloids, aminoacids, PFAS**

*Chairs: **Chiara Dall'Asta** (Univ. of Parma), **Emanuela Gregori** (ISS,* Roma)

17:15 – 17:30 **OR5: QuEChERS method combined to Liquid Chromatography and High-Resolution Mass Spectrometry for the accurate and sensitive simultaneous determination of pyrrolizidine and tropane alkaloids in cereals, spices & herbs**

Eleonora Rollo, Dante Catellani, Chiara Dall'Asta, Michele Suman

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

17:30 – 17:45 **OR6: Liquid chromatography/electrospray ionization with multistage mass spectrometry for L-Dopa determination in food matrices**

Carmen Tesoro, Maria Assunta Acquavia, Giuliana Bianco, Rossana Ciriello, Filomena Lelario, Angela Di Capua

Department of Sciences, University of Basilicata, Potenza (Italy)

17:45 – 18:00 **OR7: An analytical platform for the screening an identification of pyrrolizidine alkaloids in food matrices with high risk of contamination**

Serena Rizzo, Rita Celano, Anna Lisa Piccinelli, Luca Rastrelli

Department of Pharmacy, University of Salerno, Fisciano (SA, Italy)

18:00 – 18:15 **OR8: Incidence of perfluoroalkyl substances in marine and lake fish toward "One Health" perspective as key approach to consumer protection**

Maria Nobile, Sara Panseri, Francesco Arioli, Luca Chiesa

Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali, Lodi (Italy)

18:15 – 18:30 **OR9: Perfluoroalkyl substances (PFASs) analysis in chicken eggs from different poultry farms by a sensitive LC-MS/MS method in food**

Tommaso Stecconi, Tamara Tavoloni, Arianna Stramenga, Carolina Barola, Simone Moretti, Roberta Galarini, Gianni Sagratini, Arianna Piersanti

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

18:30 *End of session*

18:30 Welcome cocktail

Thursday, October 6th, 2022

9:00 **3rd Session: Microbiota, mycotoxins, meat**

*Chairs: **Roberta Galarini** (IZSUM, Perugia), **Michele Suman** (Barilla, Parma)*

9:00 – 9:40 **PL2: Going -omics to reveal the food-gut microbiota-host triangle**

Josep Rubert

Wageningen University (The Netherlands)

9:40 – 9:55 **OR10: Characterizing the food protein digestome by mass spectrometry: *in vitro* and *in vivo* perspectives**

Gianluca Picariello

Institute of Food Sciences - National Research Council (CNR), Avellino (Italy)

9:55 – 10:10 **OR11: Oleuropein-rich leaf extract affects intestinal microbiota and free fatty acids in Apc-mutant and wt rats**

Sofia Chioccioli, Jessica Ruzzolini, Silvia Urciuoli, Gianluca Bartolucci, Marco Pallecchi, Lido Calorini, Carlotta De Filippo, Francesco Vitali, Chiara Nediani, Francesca Bianchini, Giovanna Caderni

NEUROFARBA Department, Pharmacology and Toxicology Section, University of Florence, Florence (Italy)

10:10 – 10:35 **KN1: Mass spectrometry: the terminator of mycotoxin occurrence in foods**
Alberto Ritieni

Department of Pharmacy, University of Naples Federico II, Naples (Italy)

10:35 – 10:50 **OR12: Veterinary Drug Analysis for Meat Supply Chain Safety**

Claudia Ancillotti, Lisa Bonciani, Asia Gianni, Davide Passerini, Roberto Riccio, Gianna Salvatici, Giulia Scanavini, Jenny Vetralla

Biochemie Lab, Campi Bisenzio (FI, Italy)

10:50 – 11:25 Coffee break

Poster session (odd number posters)

11:25 **4th Session: Oil, authenticity**

*Chairs: **Anna Cane** (Istituto Nutrizionale Carapelli, Firenze)*, ***Marzia Innocenti** (Univ. of Florence)*

11:25 – 11:50 **KN2: Mineral oils in vegetable oils: background, analysis and the role of MS**

Sabrina Moret, *Luca Menegoz Ursol*

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

11:50 – 12:05 **OR13: Artificial Intelligence strategies based on GC×GC-MS/FID patterns capture extra-virgin olive oil aroma blueprint and unique identity**

Chiara Cordero, Simone Squara, Federico Stilo, Andrea Caratti, Erica Liberto, Carlo Bicchi, Stephen E. Reichenbach, Luis Cuadros-Rodriguez, Humberto Bizzo

Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Torino (Italy)

12:05 – 12:20 **OR14: Analysis of 3,5-Stigmastadiene in Extra virgin Olive Oil by GC-MS**

Andrea Serani, Matteo Serani

COTECA Srl, Pisa (Italy)

12:20 – 12:35 **OR15: Response Surface Methodology optimization of HS-SPME-GC-MS method for the analysis of pentene dimers and terpenes in extra virgin olive oil**

Lorenzo Cecchi, Serena Orlandini, Diletta Balli, Marzia Migliorini, Elisa Giambanelli, Stefano Catola, Sandra Furlanetto, Nadia Mulinacci

Department of NEUROFARBA, University of Florence, Sesto F.no (FI, Italy)

12:35 – 12:50 **OR16: Potential of Trapped Ion Mobility combined with LC-HRMS in food authenticity studies**

Giuseppe F. Labella, Sofia K. Drakopoulou, Dimitrios E. Damalas, Carsten Baessmann, Nikolaos S. Thomaidis

Bruker Italia, Macerata (Italy)

13:00 – 14:30 Buffet lunch

14:30 **5th Session: Authenticity, metabolomics, drugs**

*Chairs: **Sauro Vittori** (Univ.of Camerino), **Donatella Caruso** (Univ. of Milan)*

14:30 – 15:10 **PL3: Applications of high-resolution MS metabolomics in the traceability of the agri-food products**

Luigi Lucini

Università Cattolica del Sacro Cuore, Piacenza (Italy)

15:10 – 15:25 **OR17: Integrating TD-(+/-)DART-HRMS, data fusion and LASSO method for rapid authentication of grounded black pepper**

Alessandra Tata, Carmela Zacometti, Andrea Massaro, Tommaso di Gioia, Stephane Lefevre, Jean-Louis Lafeuille, Ingrid Fiordaliso Candalino, Michele Suman, Roberto Piro

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

15:25 – 15:40 **OR18: Assessing chicken meat authenticity within divergent farming systems (organic *versus* antibiotic-free) using SWATH-MS-based proteomic analysis and chemometrics multivariate tools**

Laura Alessandroni, Gianni Sagratini, Renzo Galli, Mohammed Gagaoua

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

15:40 – 15:55 **OR19: Ultra-high sensitivity quantification of veterinary drug residues in animal by-products**

Marco Biglietto

AB Sciex, Milano (Italy)

15:55 – 16:30 Coffee break

Poster session (even number posters)

16:30 **6th Session: Cannabis, hemp, contaminants**

*Chairs: **Nadia Mulinacci** (Univ. of Florence), **Gianluca Bartolucci** (Univ. of Florence)*

16:30 – 17:10 **PL4: High resolution mass spectrometry as an efficient tool in cannabis research**

Jana Hajslova, Matěj Malý

Institute of Chemical Technology, Prague (Czech Republic)

17:10 – 17:25 **OR20: Rheological and nutritional profile of spaghetti and bread fortified with hemp flours**

Vita Di Stefano, Carla Buzzanca, Fabiola Sciacca, Nino Virzì, Sonia Bonacci, Maria Grazia Melilli

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo (Italy)

17:25 – 17:40 **OR21: The challenging identification of isomers by HR-MS/MS: a case study from pre-cannabinoids**

Simona Piccolella, Marialuisa Formato, Severina Pacifico

Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Caserta (Italy)

17:40 – 17:55 **OR22: Advancing MOSH/MOAH analysis towards speciation and contaminants identification**

Daniela Peroni, Andrea Carretta, Luca Menegoz Ursol, Sabrina Moret

SRA Instruments S.p.A., Cernusco sul Naviglio (MI, Italy))

17:55 – 18:10 **OR23: Autenticity and Fraud: regulatory and analytical point of view by IRMS and HRMS**

Giancarlo Quaglia

Lifeanalytics Srl, Carleveri (CN)

18:15 End of session

20:30 Social dinner

Friday, October 7th, 2022

9:00 **7th Session: Coffee & surroundings**

*Chairs: **Luciano Navarini**, illycaffè, Trieste (Italy), **Gianni Sagratini** (Univ. of Camerino)*

9:00 – 9:40 **PL5: Chemistry and analysis of chlorogenic acids from coffee**

Nikolai Kuhnert

Jacobs University, Bremen (Germany)

9:40 – 9:55 **OR24: Identification and quantification of sinapoylquinic acid isomers in green coffee (*Coffea arabica* L. and *C. canephora* Pierre ex Froehner) extracts**

Silvia Colomban, Elena Guercia, Elisabetta De Angelis, Luciano Navarini

Aromalab illycaffè S.p.A., Trieste (Italy)

9:55 – 10:10 **OR25: Phenotyping Green and Roasted Beans of Nicaraguan Coffea Arabica Varieties Processed with Different Post-Harvest Practices**

Gaia Meoni, Claudio Luchinat, Enrico Gotti, Alejandro Cadena, Leonardo Tenori

Magnetic Resonance Center (CERM), University of Florence, Sesto Fiorentino (Italy)

10:10 – 10:25 **OR26: Quantification of glyphosate in milled and brown rice in LC-ICP-MS/MS**

Paolo Scardina, Andrea Carcano, Gian Maria Beone, Maria Chiara Fontanella, Agnese Salvatico

Agilent Technologies Italia S.p.A., Cernusco Sul Naviglio (MI, Italy)

10:25 – 11:00 Coffee break

Poster session (odd number posters)

11:00 **8th Session: Milk, fermentation, authenticity, adulteration**

*Chairs: **Riccardo Flamini** (CREA-VE, Conegliano), **Tiziana Nardin** (Fondaz. E. Mach, S. Michele a/A)*

11:00 – 11:15 **OR27: On-line mass spectrometry-based high-throughput analysis of volatile aging markers in long-life milk**

Jonathan Beauchamp, Antonia Krempl, Bettina Handwerker, Andrea Strube, Klaus Rieblinger

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

11:15 – 11:30 **OR28: Understanding the generation of volatile organic compounds by yeast during beer fermentation**

Rebecca Roberts, Franco Biasioli, Iuliia Khomenko, Graham Eyres, Phil Bremer, Pat Silcock

Department of Food Science, University of Otago, (New Zealand)

11:30 – 11:45 **OR29: Green analytical approach meets sustainable food processing: PTR-ToF-MS applications for VOCs monitoring during food fermentation**

Mariagiovanna Fragasso, Antonia Corvino, Iuliia Khomenko, Franco Biasioli, Vittorio Capozzi

National Research Council of Italy - Institute of Sciences of Food Production (ISPA) c/o CS-DAT, Foggia (Italy)

11:45 – 12:00 **OR30: RADIAN™ASAP: Ambient Mass Spectrometry for food authenticity and adulteration**

Andrea Perissi

Waters Italia, Sesto San Giovanni (MI, Italy)

12:00 – 12:15 **OR31: Characterization of phenolic and aromatic profiles of Radler beers by HPLC-ESI-MS/MS and GC-MS techniques**

Paola Di Matteo, Martina Bortolami, Ludovica Di Virgilio, Rita Petrucci

Dept. of Basic and Applied Sciences for Engineering (SBAI), Sapienza University of Rome, Rome (Italy)

12:15 – 12:30 **OR32: HRMS profiling of grape glycosidic aroma precursors finalized to selection of Glera crossings resistant to the main vine diseases and suitable for Prosecco wine production**

Mirko De Rosso, Annarita Panighel, Daniele Migliaro, Tyrone Possamai, Fabiola De Marchi, Riccardo Velasco, Riccardo Flamini

Council for Agricultural Research and Economics – Viticulture & Oenology (CREA-VE), Conegliano (TV, Italy)

12:30 – 12:45 **OR33: Mycotoxins comprehensive panel analysis**

Emanuele Ceccon

Restek S.r.l., Cernusco S/N (MI, Italy)

12:45 – 13:00 Closing ceremony

POSTER COMMUNICATIONS

P1 Phenolic and sugar evaluation of *Carolea*, *Nocellara Messinese* and *Leccino* olives before and after their debittering with the Spanish-style method to enhance them as table olives

Cinzia Benincasa, Rosa Nicoletti, Massimiliano Pellegrino, Enzo Perri, Flora Valeria Romeo

Council for Agricultural Research and Economics (CREA), Research Centre for Olive, Fruit and Citrus Crops, Rende (CS, Italy)

P2 Volatile compounds evolution in vegetable oils subjected to mild thermal stress

Cesare Ravagli, Federica Pasini, Silvia Marzocchi, Maria Fiorenza Caboni

Department of Food-science and Biotechnology, University of Bologna, Goidanich plaza 60, 47521 Cesena (FC, Italy)

P3 Evaluation of the polyphenol content in Sicilian extra virgin olive oils: chemical characterization by LC/MS

Claudia Lino, Rosa Pitonzo, David Bongiorno, Giuseppe Avellone

ATeN Center, Università di Palermo, Palermo (Italy)

P4 A new 3-alkyl-isocoumarin derivative in extra-virgin olive oil: tentative structural assignment by high resolution-mass spectrometry

Francesco Siano, Ermanno Vasca, Gianluca Picariello

Institute of Food Sciences, National Research Council (CNR) Avellino (Italy)

P5 Study of the high quality extra-virgin olive oils volatilome: potentiality of "comprehensive" two-dimensional gas chromatography for the discrimination of olive cultivation methodologies

Andrea Caratti, Simone Squara, Erica Liberto, Carlo Bicchi, Stephen E. Reichenbach, Raquel Maria Callejon Fernandez, Luis Cuadros Rodriguez, Chiara Cordero

Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Torino (Italy)

P6 Effects of storage in biophenolic profile of monovarietal olive oils obtained from mills of Calabria region

Marialaura Frisina, Sonia Bonacci, Giuseppe Iriti, Antonio Procopio

Department of Health Science, University Magna Græcia of Catanzaro, Catanzaro (Italy)

P7 Validation of an optimized method for determination of pesticides in vegetable oils using liquid and gas chromatography tandem mass spectrometry

Gabriella Cancemi, Giuseppina Castrezzati, Emanuela Muratori, Mara Gasparini

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

P8 Oleuropein-rich leaf extract modulates the plasma metabolome in an Apc-mutant rat model

Gabriele Rocchetti, Sofia Chioccioli, Jessica Ruzzolini, Silvia Urciuoli, Giovanna Caderni, Chiara Nediani, Luigi Lucini

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

P9 Spectrometric and spectroscopic metabolomic profile of *Coffea arabica* leaves

Lorenzo Cangeloni, Claudia Bonechi, Marco Consumi, Flavia Bisozzi, Agnese Magnani, Claudio Rossi, Gabriella Tamasi

Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena (Italy)

P10 The chemistry of the temporal evolution of coffee flavor quality

Giulia Strocchi, Manuela R. Ruosi, Giulia Ravaioli, Francesca Trapani, Gloria Pellegrino, Carlo Bicchi, Erica Liberto

Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Turin (Italy)

P11 UHPLC-ESI-QqTOF-MS/MS characterization of minor di-acyl quinic acid isomers in green *Coffea canephora* Pierre ex Froehner (Robusta coffee)

Mirko De Rosso, Silvia Colomban, Riccardo Flamini, Luciano Navarini

illycaffè S.p. A., Trieste (Italy)

P12 Non-targeted fingerprinting of green arabica coffee volatile organic compounds (VOCs): HS-GC-IMS versus GCxGC-MS

Matteo Bordiga, Marcello Manfredi, Elettra Barberis, Emilio Marengo, Luciano Navarini, Valentina Lonzarich, Cesare Rossini, Marco Arlorio

illycaffè spa, Trieste (Italy) & Aromalab illycaffè spa, Trieste (Italy)

P13 Exploring the influence of coffee extraction parameters on aroma compounds using with proton transfer reaction-mass spectrometry

Nina Buck, Andreas Stenzel, Jonathan Beauchamp

Fraunhofer Institute for Process Engineering and Packaging IVV, Giggenhauser Str. 35, 85354 Freising (Germany)

P14 Determining the phytochemicals composition and bioavailability of whole coffee cherry fruit extract by DAD-ESI-LC/MS/MS

Boris Nemzer, Nebiyu Abshiru, Zb Pietrzkowski

VDF FutureCeuticals, Momence, IL (USA)

P15 Authentication of coffee: target screening of markers

Jana Kvirencova, Klara Navratilova, Vojtech Hrbek, Jana Hajslova

Department of Food Analysis and Nutrition, University of Chemistry and Technology Prague (Czech Republic)

P16 Effect of coffee variety, post-harvesting treatments and different roasting degrees on the concentration of acrylamide and furanic compounds in ground coffee

Laura Acquaticci, Simone Angeloni, Nazarena Cela, Nicola Condelli, Fernanda Galgano, Sauro Vittori, Giovanni Caprioli

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

P17 A comprehensive comparative study of the newly developed Pure Brew method with classical ones for filter coffee production

Agnese Santanatoglia, Giovanni Caprioli, Lauro Fioretti, Sauro Vittori

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

P18 Identification of potential aroma markers of coffee oxidized note

Giulia Strocchi, Eloisa Bagnulo, Manuela R. Ruosi, Giulia Ravaioli, Francesca Trapani, Carlo Bicchi, Gloria Pellegrino, Erica Liberto

Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Turin (Italy)

P19 A new innovative approach for the analysis of residual solvents in coffee and tea using modified QuEChERS and GC-MS/MS

Stefano Ruben Di Tofano, Lucia Bonassisa, Stefano Sportelli, Luca Tommasi

BonassisaLab S.r.l., Foggia (Italy)

P20 Metabolic profile of *Agropyron repens* (L.) P. Beauv. rhizome herbal tea by tandem-mass spectrometry

Martina Bortolami, Paola Di Matteo, Marta Feroci, Daniele Rocco, Rita Petrucci

Department of Basic and Applied Sciences for Engineering, Sapienza University of Rome, Rome (Italy)

P21 Mepiquat natural formation in cocoa commercial products

Tiziana Nardin, Roberto Larcher

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

P22 Valorization of cocoa shell by-product as a source of methylxanthines by pressurized hot water extraction

Stefania Pagliari, Rita Celano, Luca Rastrelli, Elena Sacco, Federico Arlati, Luca Campone

Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan (Italy)

P23 Mineral content in plant-based drinks

Vincenzo Nava, Angela Briguglio, Angela Giorgia Potortì, Vincenzo Lo Turco, Giovanni Bartolomeo, Giuseppa Di Bella

BIOMORF Department, University of Messina (Italy)

P24 Determination of phytochemical compounds residues in raw cow milk

Federico Cozzi, Andrea Urbanella, Isabel Mueller, Tyler Turner, Silvia Wein, Georg Weingart

DSM - BIOMIN Research Center, Tulln (Austria)

P25 Authenticity of hay milk vs milk from maize or grass silage by lipid analysis using HRMS

Ksenia Morozova, Sebastian Imperiale, Matteo Scampicchio

Free University of Bozen-Bolzano, Faculty of Science and Technology Bolzano (Italy)

P26 Traceability of pasture milk using alkaloid profile

Roberto Larcher, Tiziana Nardin

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

P27 Occurrence of polyphenols and their metabolites in Pecorino cheese

Danilo Giusepponi, Carolina Barola, Simone Moretti, Fabiola Paoletti, Francesco Agnetti, Raffaella Branciari, Rossana Roila, Roberta Galarini

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

P28 QuEChERS extraction and simple clean-up procedure for the GC-MS/MS quantification of polycyclic aromatic hydrocarbons (PAHs) in cheese

Mauro Paolini, Loris Tonidandel, Roberto Larcher

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

P29 Effect of different amino acids on the volatile organic compound (VOC) profile produced by *Lactobacillus brevis* during fermentation

Sarathadevi Rajendran, Phil Bremer, Pat Silcock

Department of Food Science, University of Otago, Dunedin (New Zealand)

P30 Functional compounds in experimental Provola Ragusan cheese

Federica Litrenta, Luigi Liotta, Arianna Bionda, Angela Giorgia Potortì, Vincenzo Lo Turco, Giuseppa Di Bella

Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali (Biomorf), Messina (Italy)

P31 Trentingrana production monitored by SPME/GC-MS: application of ASCA to reveal factors affecting Volatile Organic Compounds in ripened cheese

Michele Ricci, Flavia Gasperi, Leonardo Menghi, Isabella Endrizzi, Danny Cliceri, Pietro Franceschi, Eugenio Aprea

University of Trento - Center Agriculture Food Environment, San Michele all'Adige (TN, Italy)

P32 HS-SPME/GC–MS and chemometric approach for the study of volatile profile in X-ray irradiated mozzarella cheese

Rosalia Zianni, Annalisa Mentana, Michele Tomaiuolo, Maria Campaniello, Marco Iammarino, Diego Centonze, Carmen Palermo

Università di Foggia, Dipartimento di Medicina Clinica e Sperimentale, Foggia (Italy)

P33 Comparison of three extraction techniques for lipid profile characterization of mozzarella cheese by UHPLC-Q-Orbitrap-MS

Annalisa Mentana, Maria Campaniello, Rosalia Zianni, Michele Tomaiuolo, Oto Miedico, Marco Iammarino, Valeria Nardelli

Laboratorio Nazionale di Riferimento per il trattamento degli alimenti e dei loro ingredienti con radiazioni ionizzanti, Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia (Italy)

P34 Identification of LC/QTOF markers to reveal the use of not-allowed grape varieties in the production of Pinot grigio wine

Annarita Panighel, Mirko De Rosso, Antonio Raffaele Mazzei, Michele Fugaro, Fabiola De Marchi, Riccardo Flamini

Council for Agricultural Research and Economics – Viticulture & Oenology (CREA-VE), Conegliano (TV, Italy)

P35 Metabolomics based on mass spectrometry for the evaluation of the impact of autochthonous yeast strains on the volatolomic and chemical profiles of sparkling wines

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ABSTRACTS

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PL1

Advances in mass spectrometry to promote food safety and human health

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The field of mass spectrometry continues to grow at an astounding rate, bringing new capabilities that were non-existent only a few years ago. These advances include multi-omics analyses, high mass resolution measurements with high dynamic range, ultra-fast scanning instruments, rapid ion separations by ion mobility, and spatial information through imaging mass spectrometry. The primary driver of these innovations is improving human health – including fundamental biological research, molecular descriptions of disease and their metabolic causes, drug metabolism and disposition, nutrition, food safety, clinical lab analyses, and direct patient care.

This presentation will focus on the use of these new tools to help unfold the molecular complexity of the effect of nutrients and contaminants on human cells and the concomitant alterations created in molecular pathways that support life. Applications of these advances include analyses of oxidative damage of lipids and proteins from prolonged high sugar levels, such as occurs in diabetes, using targeted high mass resolution measurements of kidney, the effect of ingested zinc levels from food and industrial contamination, nutrient derived activation of bacterial spores in the gut, imaging mass spectrometry applied to plant and food analysis, as well as others. The presentation will also address the process of rapidly determining the effect of xenobiotics that can be found as contaminants, adulterants, and drugs in the food chain. Here, the multi-omics approach is of immense importance in combining proteomics, transcriptomics, lipidomics and metabolomics to obtain a comprehensive view of the molecular status of cells. The use of multi-omics will be illustrated with a specific example in terms of the benefits it brings in assessing molecular pathway alterations on exposure of human cells to a xenobiotic.

OR1

Nutrimetabolomics and consumption of polyphenols in elderly: how molecular markers can help the development of personalized diets to promote a healthy gut and a healthy aging

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Summary: *Dietary polyphenols can reduce the intestinal permeability (IP) in older subjects through the induction of beneficial metabolites in serum and changing the composition of the gut microbiota. These effects depend on variable responses of subjects to the diet (metabotypes), and on their physiological characteristics such as renal function and IP.*

Keywords: *Polyphenols; metabolomics; intestinal permeability*

Introduction

Increased intestinal permeability (IP), a condition also known as “leaky gut”, has been proposed as a potential contributor to inflamm-aging and a wide range of intestinal disorders such as inflammatory bowel disease, coeliac disease, and Crohn's disease, as well as several chronic diseases such as cardio-renal-metabolic diseases. Increased IP is characterized by a low-grade systemic inflammation triggered by the diffusion of toxins or bacterial factors to the bloodstream [1]. Age has been reported as an independent risk factor for altered IP, and some studies have shown an increased IP over the age of 50 [2]. Moreover, gut microbiota (GM) is another regulator of IP implicated in the renovation of the intestinal epithelial cells and in maintaining the integrity of tight junctions. Indeed, a detrimental age-related modification of the microbial community structure in the gut (dysbiosis) can lead to a loss of immune tolerance and to the development of a gut inflammatory environment coupled with increased IP. The hypothesis of the MaPLE study is that dietary polyphenols can promote health in older people affected by increased IP by acting locally on the intestinal wall and on GM, and promoting the production of bioactive metabolites

with beneficial local and systemic effects.

Experimental

The MaPLE study was a randomized, controlled, crossover trial involving adults ≥60 y.o. (n = 51) living in a residential care facility during an 8-week polyphenol-rich (PR)-diet. The volunteers were characterized by an increased IP, assessed by monitoring serum zonulin, i.e. a protein released by enterocytes able to promote the activation of the signalling transduction pathway that cause tight junction protein disassembly [3]. The PR dietary pattern was designed by the substitution of some low-polyphenol products in the control diet with other comparable products but high in polyphenols (berries and related products, blood orange and juice, pomegranate juice, green tea, Renetta apple and purée, and dark chocolate), while maintaining as much as possible the overall energy and nutrient composition. Mean total polyphenol intake was 1391 mg/day in the PR-diet vs. 812 mg/day in the control diet. During the trial, serum, faeces and urine samples were collected, which were then evaluated for their contents in metabolites (metabolomics), bacteria (metataxonomics), and clinical markers of inflammation and IP. Semi-targeted metabolomics analyses of serum and urine were performed by a validated UHPLC-QTRAP-MS/MS method, comprising more than 1000 metabolites [4]. The bacterial community structure of faecal samples was assessed by 16S rRNA gene profiling, using an Illumina MiSeq sequencer and computational tools. IP was evaluated by quantifying serum zonulin concentrations at the beginning and the end of each intervention period with a specific ELISA kit. Vascular function markers (DNA damage, VCAM-1, ICAM-1), and inflammatory markers (CRP, TNF-α, IL-6) were measured at enrolment and at each time-point, as well as metabolic and functional parameters (i.e. glucose, insulin, lipid profile, liver and renal function), by using enzymatic assays, specific ELISA kits, and an automatic biochemical analyser, respectively. Data were interpreted by multivariate statistical analyses, performed using the R platform.

Results

The PR-diet could significantly reduce serum zonulin levels, indicating a positive effect on the IP. The efficacy of the dietary intervention was greater in subjects with higher serum zonulin at baseline, who showed more pronounced alterations in the markers under study. Furthermore, zonulin reduction was also stronger among subjects with higher body mass index and with insulin resistance at baseline, thus demonstrating the close interplay between IP and metabolic features [1]. These effects were correlated to a PR-diet-driven induction of specific molecular markers in serum, which were identified mainly as phenolic compounds formed by degradation of dietary polyphenols by the GM, and theobromine and methylxanthines derived from cocoa and tea [5]. These latter were positively correlated with butyrate-producing bacteria (e.g., *Ruminococcaceae*, *Clostridiales* and members of the Faecalibacterium genus) and inversely with zonulin. Direct correlations between some polyphenol

metabolites in serum and changes of GM were observed [5]. Another marker, associated to specific alterations of the GM and induced by the PR-diet, was identified as indole 3-propionic acid, a compound that has been previously described as a marker of "healthy microbiota". However, this effect was observed in subjects with normal renal function, but not in subjects with impaired renal function [6]. Finally, the effects of PR-diet on metabolome, GM and IP revealed to be related to the urometabotypes. Urometabotype B (UMB) showed a 2-fold higher improvement of zonulin levels ($p<0.05$), and it was characterized for alterations in specific metabolic pathways (e.g., kynurenine pathway of tryptophan catabolism, and microbial metabolization of phenolic acids) that were correlated with the reduction of serum zonulin levels and modifications of gut bacteria (increased *Clostridiales*, including, *Rumnococcus lactaris*, and *Gemmiger formicilis*) [7].

Conclusions

Overall, results from the MaPLE trial indicate that a PR-diet can reduce IP in older subjects, by regulating the composition of the GM and triggering the production of specific metabolites. These findings may be important when defining appropriate dietary interventions to promote health in older adults, and point out that the different responses to polyphenols consumption (metabotypes) may be carefully taken in account to tailor personalized nutrition interventions. On this regard, nutrimetabolomics reveals to be a suitable tool.

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OR2

Discovering natural and healthy pigments: analysis of Anthocyanins on wheat by mass spectrometry

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Summary: *Anthocyanins are known as natural pigments in food and as great antioxidants. Due to their structural differences, as they may be bounded to various sugar moieties, their identification results difficult. This study is focused on the analysis through LC-ESI/MS/MS of anthocyanidins, obtained from hydrolysis of anthocyanins on pigmented wheat.*

Keywords: *HPLC-ESI-MS/MS; anthocyanins; color-grained wheat.*

Introduction

Anthocyanins naturally occur in plants and food and are the main responsible for some pigments. In fact, several studies have focused about their use as natural dyes as alternative to synthetic ones in the food market. These molecules are known to be great antioxidants and they may result of great interest because of the health benefits they produce as anti-inflammatory agents [1]. Their basic structure, consisting of two phenyl rings and a heterocyclic one, is usually bounded to sugar moieties at different hydroxylated positions. The anthocyanidins are anthocyanins not bounded to sugar residues and may be all conducted to six structures, which differ in the number of hydroxyl or methoxy groups: cyanidin, delphinidin, malvidin, peonidin, pelargonidin and petunidin. Thanks to this characteristic, in nature, a wide variety of anthocyanins could be found but finding a method that can identify each one of them turns out to be a difficult process, since the standards available are limited. In literature, UV-visible spectrophotometric identification is widely used because of the speed of the analysis; nevertheless, the single wavelength measurement considers all compounds with reddish colour, which causes an overestimation of anthocyanins. Furthermore, anthocyanins are unstable molecules and their stability depends on pH, solvent, temperature and light [2], which provides other difficulties to their characterization. For this purpose, the use of LC-Chromatography associated to Mass Spectrometry ESI(+)-MS/MS turns out to be the best choice for their analysis as it can discriminate eventual interfering. The aim of this study is the analysis of the content of anthocyanins in terms of anthocyanidins, which could be obtained after hydrolysis and have correspondent available standards.

This work was carried out within the financial support from the Project “PIGRANI” (MISE Decreto 235629 del 16/09/2020).

Experimental

Standards of the six anthocyanidins, cyanidin chloride, delphinidin chloride, malvidin chloride, peonidin chloride, pelargonidin chloride, petunidin chloride, and of the anthocyanins cyanidin 3-glucoside chloride, delphinidin 3-glucoside

chloride, malvidin 3-glucoside chloride, peonidin 3-glucoside chloride, were purchased from Sigma-Aldrich. The standards of anthocyanidins were dissolved in a solution of methanol containing HCl 2 M, while the standards of anthocyanins were dissolved in water:methanol 1:1 solution containing 2% HCl [3] and were all stored at 4°C. A study about the time, the pH and the temperature of hydrolysis was evaluated. In detail, solutions of cyanidin 3-glucoside at first, and other available anthocyanins afterwards, were analysed on LC-ESI(+)-MS/MS at specific times of hydrolyses, at time 0, after 10, 20, 40, 60 and 80 minutes, at 100°C. In Fig. 1 it is possible to observe an increase in the cyanidin peak at 3.8 min and a decrease in the cyanidin 3-glucoside peak.

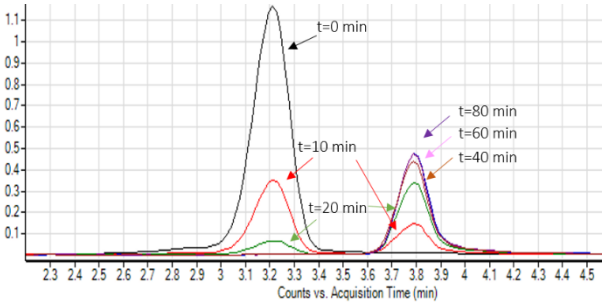

***Figure 1**. Chromatograms of cyanidin 3-glucoside hydrolysis into cyanidin analysed at 0 min, 10 min, 20 min, 40 min, 60 min and 80 min at a temperature of 100°C*

In the extraction and hydrolysis steps, pigmented wheat samples were dispersed in 10 ml of methanol containing HCl 2 M and sonicated for 20 minutes. After a centrifugation of 4000 *g* for 4 minutes, these passages were repeated adding other 10 ml. Once collected, the extracts were transferred into Digitubes centrifuge tubes and placed in a preheated bath; subsequently it was hydrolysed at 100 ± 2 °C for 80 minutes. After hydrolysis, the extracts were cooled and appropriately diluted for the analysis. To have an idea about the efficiency of the extraction and hydrolysis reaction, a solution of triphenyl phosphate was used as Internal Standard. Chromatographic analyses were performed on Infinity 1260 from Agilent Technologies. As column, a 2,1 x 100 mm, 1,8 µm, Zorbax Eclipse Plus C_{18} from Agilent was used for separation. Electrospray mass spectrometry was performed with Agilent 6410 Triple Quadrupole. Elution was performed using as mobile phase A a solution of ammonium formate 5 mM and acid formic 0,1% (*v/v*) and as mobile phase B acetonitrile:methanol 1:1, with a flow rate of 0,3 ml/min and the following gradient was used to separate the analytes: 0-2 min, from 95% A to 50% A; 2-8 min, from 50% A to 40% A; 8-12 min, from 40% A to 10% A; 12-14 min, 10% A; 14-14,5 min from 10% A to 95% A; 14,5-21 min 95% A.

Results

Identification and peak assignment of anthocyanidins were based on comparison of their retention time and mass spectrometric data with that of standards [4]. To obtain the best transitions from precursor ions to product ions, an optimization of MRM fragmentations was done. The selected transitions of anthocyanidins and

acquisition conditions are summarized in Table 1 (the quantitative transition for each analyte is reported in bold type).

Table 1. Mass Spectrometry acquisition conditions: MRM transitions of Anthocyanidins and relative Fragmentor and Collision Energy.

Anthocyanidin	MRM transitions (*m/z*)	Fragmentor (*eV*)	Collision Energy (*eV*)
Cyanidin	287→**137**/213/241	120	**35**/25/25
Delphinidin	303→**229**/149/257	120	**25**/40/25
Malvidin	331→**270**/149/299	120	**25**/35/25
Pelargonidin	271→**197**/173/215	120	**25**/25/25
Peonidin	301→**286**/230/258	120	**25**/25/25
Petunidin	317→**302**/274/285	120	**25**/25/40
Internal Standard	327→**215**/152	120	**25**/25

The developed method was applied to samples of pigmented wheat provided by CREA–CI–Foggia. The analyses showed a high content of anthocyanidins on coloured-grain wheat, as 34 mg/kg, 22.27 mg/kg and 21.96 mg/kg. These values are comparable to blue and purple wheat of a recent study, whose content was respectively 9.26 and 13.23 mg/kg [5]. The most abundant anthocyanidin found was cyanidin and on some samples were found also delphinidin and peonidin. To determine the linearity, seven different concentrations of standards of anthocyanidins were used. The standards were prepared diluting the solutions prepared before, with the matrix extract, to reduce the matrix effect. The regression analyses revealed correlation coefficients (*r*) higher than 0.999. The limit of quantification (LOQ) was estimated by a function of the software Agilent MassHunter. The LOQs were 4 mg/kg for delphinidin and 2 mg/kg for all the other anthocyanidins. The recovery values were 87.92-102.50%.

Conclusions

In this study, a new method for the analysis of the total content of anthocyanidins on pigmented wheat was developed through the use of HPLC-ESI(+)-MS/MS. The identification of each anthocyanidin was possible after the analysis of hydrolysis applied on the samples, thanks to the selected transitions for the Multiple Reaction Monitoring (MRM). The present method was validated in terms of its repeatability, accuracy and linearity, and furthermore it was applied to the analysis of pigmented wheat obtained from CREA-CI-Foggia. The highest total content of anthocyanidins found on samples was 34 mg/kg, which may be considered a good amount of anthocyanins for this kind of matrix, considering the usual content found on color-grained wheat [5].

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OR3

Determination of phyllobilins in the peels of apples cv. 'Gala' (*Malus* x *domestica* Borkh.) at five different ripening stages using high-resolution quadrupole-time-of-flight-mass spectrometry

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Summary: *Phyllobilins are the products of chlorophyll degradation. These compounds are formed in fruits during ripening via pheophorbide a oxygenase/phyllobilin (PaO/PB) pathway. Chlorophyll catabolites could be used as ripeness indicator for apples.*

Keywords: *Phyllobilins, chlorophyll degradation, fruit ripening*

Introduction

Chlorophyll breakdown is a natural phenomenon visible at naked-eye in leaves and fruits [1]. This process takes place in different life phases of plants, including ripening. During fruit ripening chlorophyll is degraded via pheophorbide a oxygenase/phyllobilin (PaO/PB) pathway obtaining chlorophyll catabolites, called phyllobilins (PBs) [1]. PBs are linear tetrapyrroles having different structures [1]. In this work we qualitatively analysed chlorophyll catabolites in peels of apples cv. 'Gala' (*Malus* x *domestica* Borkh.) at different ripening stages.

Experimental

50 Apples (*Malus* × *domestica* Borkh.) of cv. 'Gala' (clone 'Schniga') were harvested on September 3rd, 2018 from an experimental field in Laces (670 m a.s.l.), Val Venosta, South Tyrol, Italy. Fruits were selected from trees randomly allocated in the orchard and collected at their optimal harvest date with a starch index of 4.2, based on the CTIFL scale using the Starch Iodine Test. Apples were harvested from the centre of the canopy to get representative fruits. They were stored at room temperature (20-25 °C), ambient daylight and 60-70 % relative humidity to mimic ripening. Three apples per ripening stage (0, 7, 14, 21, 30 days after harvesting) were examined. The peel of each apple was removed using a kitchen peeler and stored at −80 °C until analysis. The extraction of PBs was performed with 10 mL of methanol into two steps (7 + 3 mL). The combined extracts were centrifugated and the supernatant was evaporated to dryness under nitrogen gas flow and reconstructed in 50 µL LC-MS eluent [methanol (solvent B): 4 mM aqueous ammonium acetate (solvent A), 1:1 (*v/v)*]. The combined methanolic extracts were centrifuged (12,000 rpm, at 4 °C, for 10 min), filtrated (PTFE filter, 0.2 µm), and the supernatant was stored at −80 °C until analysis. An UHPLC–HRMS-Q–TOF instrument was used for PBs

measurement. The solvent gradient was: 0−5 min, 20% B; 55 min, 70% B; 60 min, 95% B; 70 min, 95% B; 75 min, 20% B; 85 min, 20% B. ESI source was set with positive ion polarity, 500 V for end plate offset, 4,500 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*, 2.0 Hz spectra rate with Fullscan (FS) and Data Dependent (DDA) mode based on an "Precursor Ions List" containing all known and hypothesized pseudo-molecular ions of PBs (in total 51) using the three most abundant MS signals for fragmentation. All data were analysed with the Bruker Compass DataAnalysis software 4.2. PBs were identified using retention times (< 0.2 min differences to known PBs), molecular ions (< 5 ppm mass accuracy) and mass fragments (minimum three fragments per PB with mass accuracy < 5 ppm).

Results

We tentatively identified nine PBs in the peel of apples cv. 'Gala', all of them were previously reported in literature from different plant species: five nonfluorescent chlorophyll catabolites (NCCs), one yellow chlorophyll catabolite (YCC) and four nonfluorescent dioxobilane-type chlorophyll catabolites (DNCCs). Furthermore, we putatively detected a novel catabolite among DNCCs present in apple peels: DNCC-990 (Fig. 1). Some of PBs detected were previously found in leaves of apple cv. 'Golden Delicious', except for DNCCs [2]. We also observed the appearance of each PB at different ripening stages. Most catabolites occur in later periods (after 14 days of maturation).

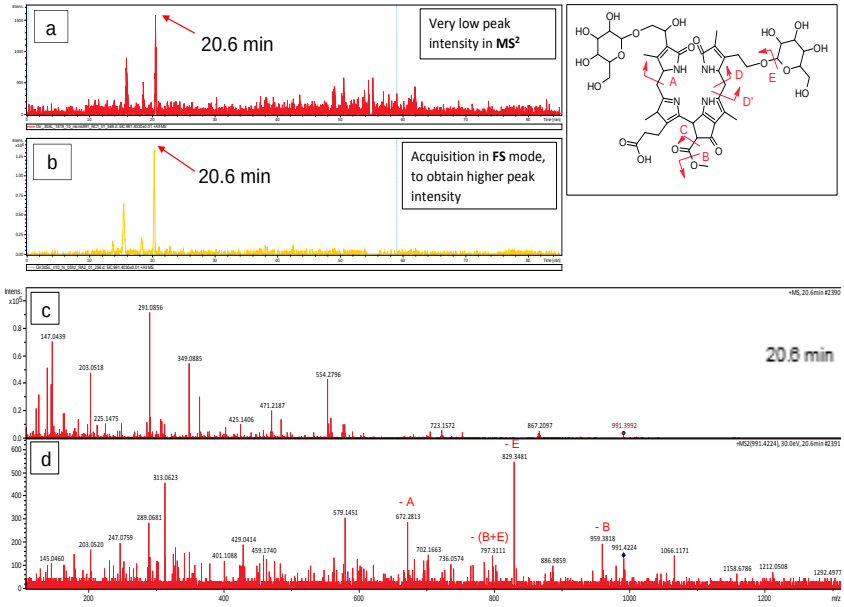

***Figure 1**. Extracted Ion Chromatogram's (EIC's) of DNCC-990 acquired with high-resolution mass spectrometry (a: obtained in data dependent method, b: obtained in full scan (FS) mode, c: FS mass spectra and d: MS^2 spectra are shown). The (tentative) constitutional formula and the fragmentation sites are shown (top right)*

Conclusions

In this study we performed a qualitative analysis to identify PBs in apples cv. 'Gala'. A total of ten catabolites belonging to different classes (NCCs, DNCCs and YCCs) were detected. Based on the high number of PBs, apple peel is a rich source of these catabolites. Considering their appearance in fruits, PBs could be used in future as indicators to determine the optimal harvest period of crops. Further investigations are necessary regarding their emergence during climacteric fruits ripening.

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OR4

Phytochemical investigation of seven unripe tomato cultivars (*Solanum Lycopersicum*)

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Summary: Unripe tomatoes are the main waste produced during tomato processing. They represent a matrix with high content of glycoalkaloids and polyphenols, potentially very useful in the development of new nutraceutical. The present study fully characterized seven unripe tomato cultivars and identified “Datterini” tomatoes as cultivars with the best metabolomic profile.

Keywords: *characterization unripe tomatoes, composition analyses, glycoalkaloids.*

Introduction

Tomato (*Solanum Lycopersicum*) is one of the most widely harvested fruit crops, with a world annual production over 180 million tons in 2019.
During industrial processing, about 15 million tons of waste are produced [1]. The evaluation of the tomato industrial by-products has proved to be an appealing research field to recover the natural matrix for pharmaceutical and nutraceutical applications. Unripe tomatoes represent the main waste produced during tomato harvest and a unique source of active ingredients as glycoalkaloids and polyphenols. These compounds make this waste food matrix of great interest for the development of new nutraceutical products. The aims of the present work on unripe tomatoes are:

1. Develop an efficient extraction method of the active ingredients from the unripe tomato waste
2. Compare the chemical compositions of seven unripe tomato cultivars grown in Sicily and Campania regions.
3. Analyse glycoalkaloid and polyphenolic profiles by HPLC-HESI-MS/MS and HPLC-DAD-FLD and compare the cultivars.

Results

The chemical analysis of unripe tomato cultivars was carried out with the extraction with hydroalcoholic solvents. Five extraction methods were developed using alcoholic, hydroalcoholic and aqueous solvents. They differed for the polarity and the acidity of the extraction solvent.
Hydroalcoholic mixtures were selected as the solvents for the quantitative analyses. The extracts were characterized by HPLC-DAD-HESI-MS/MS analysis. The workflow of the phytochemical investigation is reported in Fig. 1.
Glycoalkaloid profile was established in positive acquisition mode, while polyphenols, organic acids, phytohormones and oxylipins were detected in negative acquisition mode. Full scan and data dependent acquisition (DDA) were

used for the qualitative analysis. Comparisons with analytical standards allowed the identification of 17 components, in which α-tomatine and chlorogenic acid were the main glycoalkaloid and polyphenol of the unripe tomatoes. Quantitative analysis of 24 compounds was performed to compare the phytochemical profile of the seven unripe tomato cultivars. Quantification of 7 glycoalkaloids was performed by HPLC-HESI-MS/MS analysis with a multiple reaction monitoring (MRM) scan mode. One MRM transition was monitored for each compound, using as fragment ion the aglycon due to the cleavage of the sugar moiety.

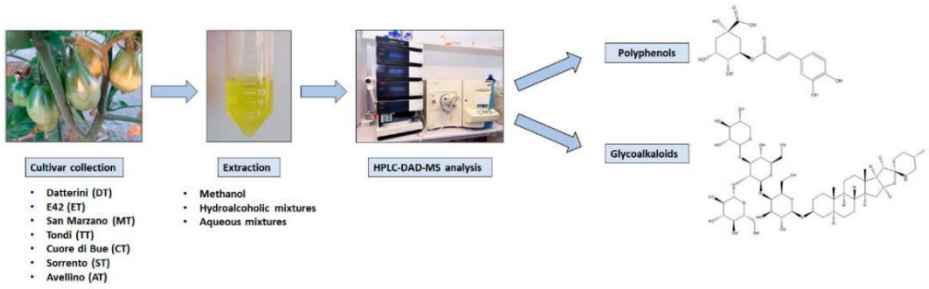

***Figure 1**. Workflow of the analysis of glycoalkaloids and polyphenols*

The quantitative analyses of the seven unripe tomato cultivars clearly indicated "Datterini" (DT) and E42 tomatoes as the most attractive, with a tomatine content of 34.699 ± 1.101 and 34.354 ± 1.093 (mg/g DW), respectively.
However, the DT cultivar represented the most interesting variety due to the high content of secondary glycoalkaloids, which could contribute to the pharmacological properties of the matrix.
Quantification of 17 polyphenols was performed by HPLC-DAD-FLD analysis. As reported in glycoalkaloid quantification, DT appeared as the most attractive cultivar, with the highest content of chlorogenic acid, rutin and quercetin *O*pentosylrutinoside equal to 1.412 ± 0.010, 0.996 ± 0.003, and 0.148 ± 0.001, respectively (mg/g DW). The results of the quantitative analysis are in agreement with literature data [2].
Two HPLC methods for the analysis of the content of glycoalkaloid and polyphenolic compounds have been validated by the assessment of their linearity, limit of detection (LOD), and limit of quantification (LOQ), accuracy, and precision. The validations were performed according to the ICH validation guideline (ICH.Q2[R1], 1995) [3]. In both methods, the calibration curves and correlation coefficients were calculated using a linear regression model. Good linear regression ($R2 > 0.99$) was calculated for all quantified compounds. In the validation of the HPLC-MS/MS analysis for tomatine quantification, LOD and LOQ were equal to 0.111 and 0.336 ppm, respectively. For intraday and interday precision and accuracy, the RSD value ranged from 0.461 to 2.790 % ppm and from -2.579 to -1.543 %. These results assessed that the developed method was satisfactory with acceptable precision, accuracy, and reproducibility. Furthermore, a biplot of the principal component analysis (PCA) was performed on the quantitative data set to explore the relationship between the quantitative polyphenolic and glycoalkaloids content, as shown in Fig. 2. The analysis showed great differences in metabolites concentration between the seven

cultivars. Positive value at PC1 indicated samples with high glycoalkaloids, glycosylated phenolic acids and flavonols content. Instead, negative values at PC1 were characterized by high flavanols and phenolic acid aglycones content. The second and third PCs had a minor ability to describe the system variability. As shown in the quantitative analysis, DT was the cultivar with the greater metabolomic profile, due to the high concentration of α-tomatine, rutin and quercetin *O*pentosylrutinoside.

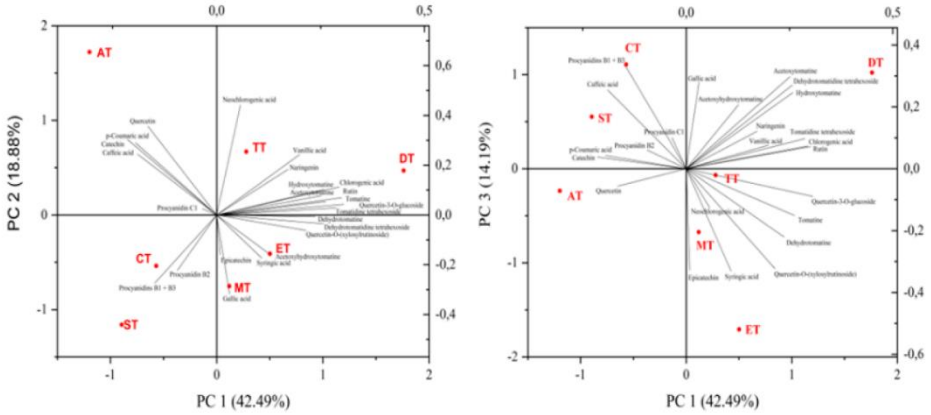

***Figure 2**. Biplot of the principal component analysis (PCA) of 7 unripe tomato cultivars and 24 quantified compounds*

Conclusions

During the processing of the tomato industry, a large amount of unripe tomatoes is the main waste produced. The present work suggests the possibility to recover this fruit for the development of new nutraceuticals for its distinctive phytochemical profile. The HPLCDAD-HESI-MS/MS analyses were used for the identification of 76 compounds, mainly glycoalkaloids and polyphenols. Moreover, using the multivariate statistical analysis, we identified the “Datterini” tomato as the cultivar with the best metabolomic profile.

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OR5

QuEChERS method combined to Liquid Chromatography and High-Resolution Mass Spectrometry for the accurate and sensitive simultaneous determination of Pyrrolizidine and Tropane Alkaloids in cereals, spices & herbs

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Summary: This work aims to elaborate and validate an analytical method based on QuEChERS sample preparation approach, exploiting the UHPLC coupled to HRMS to simultaneously identify and quantify 21 PAs and 2 TAs in different food matrices. The goal is to address the industrial needs to comply with forthcoming European legislation.

Keywords: *Tropane Alkaloids (TAs); Pyrrolizidine Alkaloids (PAs); High Resolution Mass Spectrometry (HRMS)*

Introduction

Alkaloids, which means alkali-like substances, are basic heterocyclic nitrogenous compounds of plant origin that are physiologically active. Tropane and Pyrrolizidine Alkaloids can be found in food or animal feed, originating either from edible plants (e.g. vegetables, herbal teas) or when these non-edible plants and/or its seeds are co-harvested with the crop [1;2]. For this reason, the great and increased consumption of plant-based product as an alternative to meat and/or for nutritional and health reasons, caused food research institutions and authorities to raise concerns regarding human exposure to these natural toxins in food and feed [3;4]. Awareness has also been raised by the forthcoming European legislation on the maximum levels of these alkaloids [5;6]. For the analytical determination, most of the methods described in the literature use liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and face separately the two different TAs and PAs issues. This study proposes the exploitation of the Ultra High Performance Liquid Chromatography (UHPLC) coupled to the High Resolution Mass Spectrometry (HRMS) to evaluate in a simultaneous way the most relevant 2 Tropane Alkaloids and 21 Pyrrolizidine Alkaloids in different food matrices, such as wheat, maize, buckwheat, oregano and rosemary.

Experimental

For a better optimization of the extraction, ready-to-use QuEChERS allows to clean up and minimize the matrix effect [7;8]. Solid analytical standards were purchased from Phytolab (Germany) and have a chemical purity of 99%. The methanol extract of a "blank" buckwheat sample for Tropane Alkaloids and the

methanol extract of a “blank” oregano sample for Pyrrolizidine Alkaloids was prepared. At the end, to reach the desired concentration, the 7 solutions calculated for PAs and for TAs, were mixed 50:50 directly in the vials for HPLC. A seven-points matrix matched calibration curve of both TAs and PAs was obtained to quantify the analytes.
The separation of TAs and PAs was achieved by using a Luna Omega C18 column (150 x 2.1 mm; 1,6 µm; Phenomenex, USA), heated at 50°C. Mobile phase was 2 mM ammonium formate and 0,2% formic acid in water (eluent A) and in methanol (eluent B), respectively; flow rate was 0,250 ml/min.
Despite the Ultra High-Resolution Chromatography and slow gradient to maximise separation, reverse phase chromatography allows the separation of 17 of the 21 PAs due to the co-elution of Intermedine and Lycopsamine (m/z 300.1805, RT 5.25 min) and their respective N-Oxides, Intermedine-N-Oxides and Lycopsamine-N-Oxide (m/z 316.1754, RT 6.34) because structural similarity (beside same mass/charge ratio and same product ions). For this reason, with these isomers, we have created an overall calibration curve, considering the sum of Intermedine + Lycopsamine and their respective N-Oxides (Fig. 1).

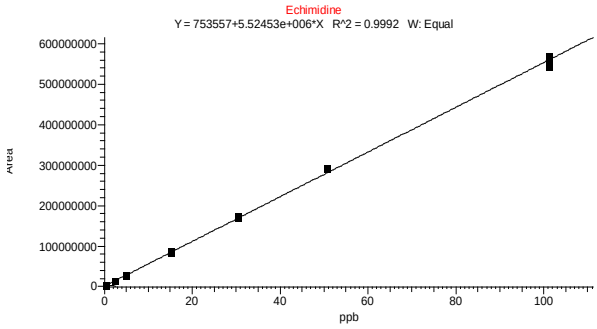

Figure 1. *Overall calibration curve for Echimidine (PA)*

Results

Results underline that the presence of TAs in wheat, buckwheat and maize samples remain below the limit of quantification (LOQ). Interestingly, for PAs, oregano samples are below the LOQ, while for rosemary, Retrorsine N-Oxide, Senecionine N-Oxide and Seneciphylline N-Oxide were detected with concentration of 8.5 µg/Kg, 1.8 µg/Kg and 17.3 µg/Kg, respectively.
Performance of the method was further investigated analysing a certified reference material (FAPAS), different commercial samples purchased at the supermarket, maize, buckwheat, oregano, rosemary and herbal tea, in relation to a comparison with analytical outcomes coming from a highly qualified external laboratory (Table 1) set with separated methods for each category of alkaloids and for specific matrixes.
Atropine and scopolamine were not detected in any of the cereals analysed in this study, except in the FAPAS certified sample, where the results of our method are in line with those of this quality control material. On the contrary, for PAs, the highest concentrations were detected in one sample of oregano for Europine and its relative N-oxide with an amount of 542.1 µg/Kg and 26.9 µg/Kg, respectively. The PAs pattern in one sample of Herbal tea is dominated by Retrorsine and the

co-eluting compounds Intermedine NO + Lycopsamine NO with a concentration of 19,5 µg/Kg and 46.7 µg/Kg, respectively. It is interesting to note that during the repeated analyses on the same sample batch for PAs, there were different results probably due to a not homogeneous distribution of the toxin within the sample matrix.

Table 1. Comparison of internal results with those of the external laboratory for rosemary supplier's sample

Positive Analyte	**Internal Method results**	**LOD**	**LOQ**	**External Laboratory results**	**LOD**	**LOQ**
Retrorsine NO	***8.5 µg/Kg***	0.2	0.5	***7.7 µg/Kg***	2	5
Senecionine NO	***1.8 µg/Kg***	0.2	0.5	< LOQ	2	5
Seneciphylline NO	***17.3 µg/Kg***	0.2	0.5	***15 µg/Kg***	2	5

Conclusions

The results of the comparison between the internal and the external laboratory show that our method was able to detect TAs and PAs in a simultaneous way, and also to obtain lower values of LOD and LOQ. This is probably due to the higher potentialities (in terms of selectivity and accuracy) related to HRMS instrument exploited in our method with respect to a triple-quadrupole instrument adopted by the external laboratory. Even if the presence of matrix effect and the attested uneven distribution of these Alkaloids will be furtherly investigated, validation data successfully demonstrated the overall robustness of the method addressing the industrial needs to comply with the imminent European legislation (being significantly lower than the indicated LOQ), also including the applicability on a set of different relevant matrixes, cereals, spices & herbs.

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.

OR6

Liquid chromatography/electrospray ionization with multistage mass spectrometry for L-Dopa determination in food matrices

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Summary: *Fabaceae vegetables* are natural *sources of L-dopa. In this work a LC/MSn study on some samples of Vicia faba, Mucuna pruriens and Fagioli di Sarconi beans (Phaseolus Vulgaris) PGI (i.e. Protected Geographical Indication) was carried out to identify L-Dopa and its potential derivates.*

Keywords: *L-Dopa, mass spectrometry, separation*

Introduction

L-Dopa or Levodopa (LD) is a catecholamine used as gold standard for the pharmacological treatment of Parkinson's disease (PD). PD arises when neuronal dopaminergic cell of the *Substantia Nigra* pars compacta die, unable to produce dopamine (DP). Therefore, LD drug crosses the blood–brain barrier where is decarboxylated into DP, by improving its bioavailability [1]. Complementarily, it's strongly suggested to use some natural adjuvants compounds avoiding oxidative stress, which plays a fundamental role in the PD pathogenesis [2].

LD occurs in a variety of Fabaceae vegetables at high, medium and low abundances. Recently, it was highlighted the occurrence of a high amount of LD in *Mucuna pruriens* seeds *Vicia faba* L. beans that could be involved like innovative food supplements adjuvants in the diet of patients with Parkinson [3,4]. Here, a combined MS approach for the structural characterization of LD in these and other Fabaceae family vegetables was exploited, based on reversed-phase (RP) LC/ESI in positive ion mode coupled to mass spectrometry. In addition, identification and characterization of LD potential derivatives were ascertained.

Experimental

Standard solutions of LD (3,4-dihydroxyphenyl-L-alanine ≥98%, purchased by Sigma-Aldrich) were prepared by solubilizing the analyte in 0.1 M HCl solutions (Merck KGaA) in Milli-Q water (produced by using Millipore, Billerica, MA, USA). *Mucuna pruriens* Bio powder supplement was purchased online, Fagioli di Sarconi IGP beans (*Phaseolus Vulgaris* L.) were provided by local farmer and *Vicia faba* L. beans were purchased at a local market. LD was extracted after optimising extraction conditions from the method proposed by Polanowska et al. [5]. In brief, the best extract conditions involve the use of dry weight/volume extracting solution (HCl 0.1M) in a ratio of 1:10, sonication and centrifugation. Chromatographic separation was performed via a Supelco Discovery C18 reverse-phase analytical column, 250 mm x 4.6 mm, 5 µm packing material

particle size. The extracts were eluted in isocratic flow with a mobile phase made up of 3% methanol (B) and 97% acetic acid 0.2% v/v (A) at 1 mL/min flow rate and characterized by positive electrospray ionization (ESI-MS) coupled with a quadrupole linear ion trap (LIT, Thermo Fisher Scientific, Bremen, Germany) or Orbitrap (Q-Exactive, Thermo Scientific, Waltham, MA, USA) mass spectrometers.

Results

Assisted by the flexible MS/MS capability of mass spectrometer, the characterization of main chromatographic peaks of all extracts analyzed and an unambiguous identification of the LD potential derivatives were ascertained. In Fig. 1 is shown a representative LD tandem mass spectrum of *Vicia faba* L. extract. The spectrum was acquired in the positive (+)-ESI mode, since the analyte has an easily protonable amino group. The molecular ion $[M+H]^+$ at nominal *m/z* 198 was selected and fragmented, by giving two abundant fragment ions: $[(M+H)-NH_3]^+$ at nominal *m/z* 181 and $[(M+H)-H_2O-CO]^+$ at nominal *m/z* 152. The fragmentation pathways interpretation was helpful to confirm the presence of LD derivatives already known. (e.g. dopaquinone and dopamine), but also to identify new potential LD derivates. Specially in the Fagioli di Sarconi beans (*Phaseolus Vulgaris* L.), an isobaric LD compound characterized by fragments signals at *m/z* 180 and *m/z* 154 values, was shown.

The developed and optimized method proposed has proved useful for the LD determination in vegetables, such as *Phaseolus Vulgaris* L. beans, with a low content of the compound under investigation.

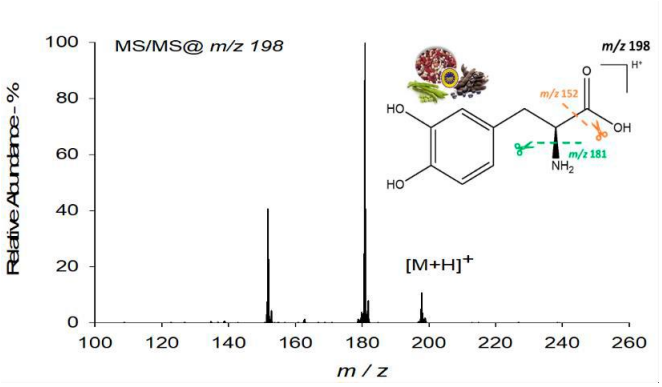

***Figure 1**. L-dopa CID-MS/MS spectra of Vicia faba L. sample, relative collision energy 19% was applied*

Conclusions

Our results show that by using an optimized extraction method and multistage mass analyses it was possible to carry out a useful study of fragmentation pathways and characterization of all compounds investigated. The good results obtained suggest that the methodology may be applied with success to other similar matrices in order to assist the researchers getting specific information related to the potential use of these vegetables in Parkinson's diet.

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OR7

An analytical platform for the screening an identification of pyrrolizidine alkaloids in food matrices with high risk of contamination

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Summary: *An analytical platform for the screening and identification of pyrrolizidine alkaloids in food matrices with high risk of contamination is proposed. It offers the possibility to detect both target and untarget pyrrolizidine alkaloids and resulted able to identify the target analytes with limits of identification of 4 µg L^{-1}.*

Keywords: *pyrrolizidine alkaloids, high-resolution mass spectrometry, targeted screening analysis*

Introduction

Pyrrolizidine alkaloids (PAs) are a large group of naturally occurring phytotoxins recently regarded as undesirable substances in plant-derived food products due to their genotoxic and carcinogenic activities [1-2]. An analytical platform for the screening and identification of pyrrolizidine alkaloids in food matrices with high risk of contamination (honey, pollen, black and green teas, herbal infusions, and plant-based dietary supplements) is proposed in this study. The sample preparation procedure included an initial pre-treatment of the samples followed by a salting-out assisted liquid-liquid extraction (SALLE). Then, the samples were analyzed through ultra-high performance liquid chromatography coupled with high resolution tandem mass spectrometry (UHPLC-HRMS/MS) [3]. A wide database of PAs (n = 779) was created and used to collect a set of precursor ions ($[M+H]^+$), which was used to fill the inclusion list associated to the Full MS/dd-MS^2 acquisition method. Then a wide-scope suspect screening method was developed and applied to the characterization of 10 PA-producing plants, which resulted in the collection of 84 spectra of PAs. These spectra, in addition to those of the available reference standards, allowed to build a HRMS/MS spectral library, which was used as identification tool for a high-throughput screening and identification method of 118 target PAs. The method was validated according to the European guidelines for qualitative screening methods [4-5] and applied to the analysis of a huge number of commercial samples.

Experimental

Sample preparation: An appropriate amount of each sample was pre- treated with an acidic water solution (H_2SO_4, 0.05 M). Aqueous extracts of each matrix were subjected to the SALLE procedure, according to our previous study [3].

UHPLC-HRMS/MS analysis: The UHPLC system was equipped with a Luna

Omega Polar C18 column. The chromatographic separation was achieved using a binary gradient of water (A) and acetonitrile (B), both containing 0.1% of formic acid.
MS data were acquired in Full MS/dd-MS^2 mode. The resolution of Full MS scans (scan range 250-500 m/z) was set at 70k (FWHM) while the resolution of the dd-MS^2 scan at 15k. The inclusion list associated to the method was filled with 112 masses of precursor ions ($[M+H]^+$).

Results

Wide-scope suspect screening method: A systematic flowchart (Fig. 1) was designed by observing the HRMS/MS spectra of the reference standards, online spectral libraries, and previous studies. The flowchart was divided into two subsets since the literature studies immediately allowed to delineate the different behavior of PAs and PANOs regarding their fragmentation patterns. The PA and PANO's subsets of the flowchart are shown in the Fig. 1 and 2, respectively.

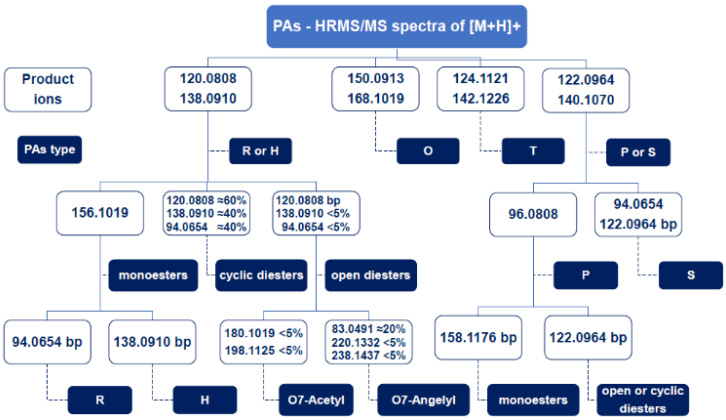

***Figure 1**. PAs' subset of the strategy*

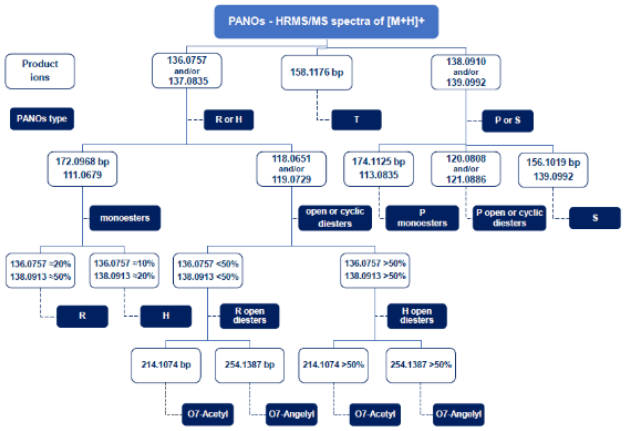

***Figure 2**. PANOs' subset of the strategy*

Then, a wide-scope suspect screening method was developed using TraceFinder software (ThermoFisher Scientific) by associating each precursor ion of the inclusion list to a set of characteristic diagnostic product ions selected from the flowchart. This allowed the software to process the raw data, flagging as putative PAs/PANOs the only peaks with a molecular formula corresponding to that of the compounds of the database (± 5 ppm) and at least three product ions (± 5 ppm). The identity of the detected compound was then confirmed by comparison with the reference standards (MSI, L1), or putatively assigned based on literature and online databases information (MSI, L2). When no spectrum or literature information was available, the detected compound was tentatively assigned to the structure of the database which corresponded to the proposed identification strategy, if present (MSI, L3).

High-throughput screening and identification method: An HRMS/MS spectral library was built by collecting spectra from the characterization of 10 PA-producing plants and reference standards. The spectral library was associated with a high- throughput target screening method for the rapid screening and identification of 118 PAs/PANOs. The method was developed using Trace-Finder software. The identification criteria were a retention time variation of ± 0.2 min, a response threshold of 10e4, a mass tolerance of 5 ppm for both precursor and product ions, a minimum of three product ions required for the identification, and a library match score higher than 70%.

Validation of the method: The proposed screening and identification method was validated in terms of specificity, accuracy (expressed as extraction efficiency, EE), limit of identification (LOI), and precision (expressed as false negative rates), according to the performance criteria of qualitative screening methods established by the European analytical guidelines. Under optimal conditions, the proposed procedure provided satisfactory EEs (69-113%). The LOIs of the target analytes ranged from 0.6 to 16 μg L^{-1}. The overall false negative rate of the proposed method was of 4.7% at 4 ng mL^{-1}.

Analysis of commercial samples: The proposed procedure was applied to the screening of a huge number of commercial samples (n = 282). The qualitative analysis of the samples revealed the presence of 58 compounds in 59% of the analyzed samples; among these, 21 compounds belonged to the list of PAs to be monitored under the 2040/2020/EC Regulation [4], 9 compounds were their co-eluent isomers, and 28 compounds were PAs included in the HRMS/MS spectral library but not mentioned in the Regulation. Among the studied matrices, honey was found to be the most contaminated one as 89% of the samples tested positive to the presence of PAs. In decreasing order of contamination follow plant-based dietary supplements (58%), pollen (50%), herbal infusions (46%), and teas (39%).

Conclusions

A general platform for the simultaneous screening and identification of 118 hepatotoxic pyrrolizidine alkaloids in matrices with high risk of contamination was developed for monitoring purpose in the present study. Target-PAs could be detected at sub to low ppb levels within a fast analysis of 30 min, including the easy and cheap salting-out assisted liquid-liquid extraction and the

chromatographic separation. High resolution mass spectra were acquired in Full MS/dd-MS^2 acquisition mode, which allowed to verify the identity of the target PAs through the matching with the spectra of the HRMS/MS spectral library or to further inspect the samples to detect untarget PAs. Furthermore, the method can be easily and continuously expanded to accommodate additional target compounds and it can be even re-interrogated without having to re- analyse the samples to search for PAs untarget at the time of the analysis but discovered in a future time.

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OR8

Incidence of perfluoroalkyl substances in marine and lake fish toward "One Health" perspective as key approach to consumer protection

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Summary: *Perfluoroalkyl substances are emerging contaminants and endocrine disruptors. The main source of exposure is the diet, especially fish. The aim of this study was to investigate the occurrence of PFASs in the most consumed sea fishes and lake fish.*

Keywords: *Perfluoroalkyl substances, UPLC-HRMS, fish*

Introduction

Perfluoroalkyl substances (PFASs) are highly fluorinated aliphatic substances with high chemical, thermal stability and high surface activity, used since decades in a wide range of industrial applications as for paper, photo paper, packaging materials, textiles, carpets, furniture, shoes, cleaning agents, floor polishing agents, paint and varnish, wax, fire-extinguishing liquids and insecticides [1]. PFASs are considered among the contaminants of emerging concern (CECs) and classified as endocrine disruptors (EDs), able to bioaccumulate and to bio-magnify in the different trophic levels of food chain. The main source of PFASs exposure is the diet, and principally fish [2]. PFAS occurrence in fish depends on fish species, geographical areas, age, diet, position in the trophic level, etc. Moreover, with the new omics techniques, their toxicological potential is in continuous exploration, encouraging the discovery of new molecules, working in accordance with a One Health approach [3]. The aim of this study was to investigate the occurrence of PFASs in the most consumed sea fishes (sea basses, sea breams, salmons, mussels and clams) from different FAO areas and in lake fish (eels, agones, whitefishes and perches) from the major lakes of the Northern Italy. At the end was assessed a risk characterization for the different fishes to verify compliance with the Tolerable Weekly Intake (TWI) according to the recent EFSA note [4].

Experimental

The collected sea fish samples were: 34 seabasses (*Dicentrarchus labrax*) and 34 seabreams (*Sparus aurata*) from Italy, Croatia, Greece, and Turkey; 66 wild and farmed salmon from 5 geographical areas and 3 different FAO zones (Norway (FAO 27), Scotland- North East Atlantic (FAO 27), Canada (FAO 67), USA- Pacific Ocean (FAO 77)); 50 mussel and 39 clam samples from different FAO zones (Mediterranean Sea (FAO 37.1.2.3), Atlantic Ocean (Spain, France, FAO 27), Pacific Ocean (Thailand, Chile, FAO 71; FAO 87), Black Sea (FAO 37.4) and New Zeland (FAO 81)).

As regard lake fish we collected: 90 farmed eels from Lake Garda, 34 European whitefishes (*Coregonus lavaretus*), 36 perches (*Perca fluviatilis*) and 38 Agones (*Alosa agone*) collected from the representative lakes of Northern Italy (Lake

Garda, Lake Como and Lake Iseo). Five grams of homogenized sample were spiked with internal standards to have a concentration of 5 ng g^{-1} in matrix and added of 10 mL of acetonitrile for PFASs extraction and protein precipitation. The extract was purified by STRATA PFAS cartridges and finally analysed by UPLC-HRMS system consisting of a Vanquish (Thermo Fisher Scientific, Waltham, United States) coupled to a Thermo Orbitrap™ Exploris 120 (Thermo Fisher Scientific, Waltham, United States), equipped with a heated electrospray ionization (HESI) source.

Results

In general, mussels and clams, as filter feeders were the most contaminated with up to 11 compounds (both acid and sulfonate forms) detected in almost all clam samples, showing an evident higher contamination in terms of frequency and concentration than in mussels. The most contaminated clam pool was fished in the FAO area 37.2. The most abundant compound in clams was PFOA, with 97% of positivity and the highest concentration of 31.03 ppb. Of the tested compounds, PFBA was present at the highest concentration (both for mussels and clams).

If we compare sea and lake fish, generally the second ones showed the higher concentrations and frequencies. In particular, of all 17 searched PFASs, only PFBA and PFOS were found both in sea and lake fish, with higher concentration in the second ones. Moreover, PFBS was found only in lake fish. In particular Agones from Lake Garda and Lake Como were the more contaminated species. In particular, the higher average concentrations of PFBA (8.07±7.92 ppb) and PFBS (1.10±2.22 ppb) were detected in Agones from Lake Garda, instead the higher average concentration of PFOS (9.90±6.46 ppb) in those from Lake Como. Regarding farmed sea fish, PFBA average concentration were comparable between sea bass (4.96±2.46 ppb) and sea bream (4.75±1.50 ppb), while PFOS was dectected at higher frequency and concentrations (0.15±0.20 ppb) in sea bass, especially those from Turkey.

About salmons, PFBA was found in both wild and farmed ones, with similar incidence (29%) and slightly higher concentration in wild-caught. The highest concentration (34.51 ppb) was in a sample from Canada. PFOA was frequently detected in farmed samples (48%) at concentrations slightly higher than in the wild ones, in the order of few ppb. Only one wild salmon sample from North Atlantic-Scotland showed PFOS at 1.77 ppb.

Regarding eels, the results showed the presence of several PFASs, up to 11 in the same eel. The distribution of the various contaminants, in the order of ppb, was mostly similar in each sample, representing the low contamination level of the lake, without any relation to the weight, length or the percentage of animal fat.

Conclusions

Based on our results, PFASs were detected in higher concentrations and frequencies in molluscs than in other fish species. In particular, they better accumulated in clams due to their high affinity to the mineral parts that characterize the deep-sea habitat. If we instead consider a comparison between sea fish and lake fish, PFASs bioaccumulated more in the second ones because

they were collected from closed basins with anthropogenic activities nearby. Since there are no MRLs for these substances, the risk characterization performed for the different species showed no situations of concern.

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OR9

Perfluoroalkyl substances (PFASs) analysis in chicken eggs from different poultry farms by a sensitive LC-MS/MS method in food

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Summary: *PFASs are persistent compounds with negative impacts on the environment and human health. Diet is an important source of exposure and eggs are notable contributors. A fully validated and sensitive LC-MS/MS method was applied to quantify the most relevant PFASs in eggs obtained with different types of poultry farming.*

Keywords: *PFAS; egg; LC-MS/MS.*

Introduction

Per- and polyfluoroalkyl substances (PFASs) are a family of synthetic organic compounds that have been intensively produced since the late 1940s. Despite their interesting technological features, PFASs are highly persistent substances, characterized by bioaccumulative potential in both the environment and biota, yielding harmful effects for human health. Apart from specific cases of occupational exposure or polluted areas, food is the main source of exposure for humans [1]. In 2020, the European Food Safety Authority (EFSA) established a new safety threshold for the four most dangerous PFASs (PFOA, PFOS, PFNA, PFHxS) setting the tolerable weekly intake (TWI) at 4.4 ng kg^{-1} body weight per week. Maximum limits in food have not yet been set because there is still a lack of data regarding food contamination, especially in Italy. In order to answer the authorities' request for data, a sensitive analytical method was developed and fully validated. The procedure allows the determination of 19 PFASs in food at ppt levels using ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS).
Eggs are a very important food commodity and their consumption is relevant for human diet. The aim of this study was to assess the PFASs contamination level in chicken eggs obtained from different poultry farms, using the developed method. This would give an idea of the PFASs intake from eggs and how it relates to the breeding system.

Experimental

Twenty-nine egg samples were collected in different laying hens rearing plants: small-scale rural (n=10), barn (n=7), battery cage (n=6) and organic farming (n=6). Each sample was a pool of eggs of at least 100 g. Small-scale rural eggs

were taken from private courtyards located in the Marche region, while the other categories included commercial eggs from all over Italy purchased in local markets or supermarkets.
Samples were investigated for 11 perfluorinated carboxylic acids (PFCAs: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA) and 8 perfluorinated sulfonic acids (PFSAs: PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS and PFDoDS) using a method developed and validated for PFASs in food of animal origin. The method originated from the protocol for feed developed by Stramenga et al. and it was applied to food with slight modifications [2]. Briefly, 2 grams of the homogenised eggs, spiked with isotopically labelled internal standards, were doubly extracted with acetonitrile. After overnight freezing, the extract was purified by weak anion exchange SPE followed by a dispersive solid phase extraction (d-SPE) with ENVI-Carb and finally subjected to instrumental analysis. The analytes were detected with a triple quadrupole Xevo TQ-S micro IVD System (Waters), equipped with an ESI source operated in negative mode. The acquisition was accomplished in MRM mode. Chromatography was performed on a Waters ACQUITY I-Class UPLC system and the separation optimised on a Luna Omega PS C18 (100 × 2.1 mm, 1.6 µm, Phenomenex) accompanied with a C18 security guard column 2.1 mm, thermostated at 40 °C. ACN and CH_3COONH_4 2 mM were the mobile phases.

Results and discussion
Method development and validation for PFASs determination in food of animal origin were performed on three different dietary test matrices (chicken muscle, chicken egg and cow milk), considered representative for the entire application field. Linearity, trueness, precision, limits of detection (LOD), limit of quantification (LOQ) and method robustness were assessed, obtaining results consistent with the validation criteria.
In all commercial eggs investigated in the survey (free-range, caged and organic farming), the level of each PFAS was below the LOQ (0.010 ng g^{-1} for all the PFASs, 0.20 ng g^{-1} for PFBA). Only in three samples, two free-range eggs and one cage farmed egg, traces of PFOA were estimated over the LOD (0.003 ng g^{-1}). Despite the different rearing systems, the eggs contamination was limited and no comparison was possible. In contrast, 8 PFASs (PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFOS) out of 19 were detected above the LOQ in the 10 home-produced egg samples. In all of them at least two substances were quantified and in 3 samples all the 8 above mentioned PFASs were found. ∑8PFASs concentrations were in the range of 0.074 to 0.633 ng g^{-1}. PFOS was the predominant analyte with a mean concentration of 0.121 ng g^{-1} followed by PFTeDA (0.063 ng g^{-1}) and PFTrDA (0.041 ng g^{-1}). PFOS contributed 44.3% to the ∑8PFASs mean, followed by PFDA (13.3%) and PFNA (10.0%) while PFOA contribution was only for 3.0% (Fig. 1).
Rural flock eggs as an emerging source of PFOS was recently reported by the work of Gazzotti et al., in which only PFOS, PFOA, PFNA and PFHxS, listed as priority in the last EFSA opinion, were monitored in backyard hens egg yolks sampled from different sites throughout Italy [3]. Zafeiraki et al. analysed 11 PFASs in eggs collected in the Netherlands and Greece and also in that study the levels of perfluoroalkyl substances were predominantly below the LOQ in

supermarket eggs, while long-chain PFASs (C ≥ 8) were detected in home-produced eggs [4].

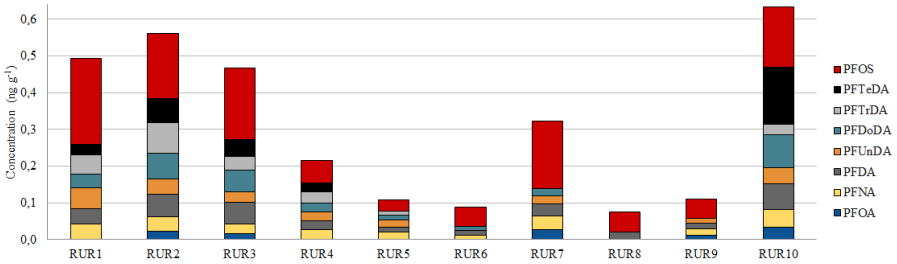

***Figure 1**. Concentrations (ng g^{-1}) and contributions of the detected PFASs in small-scale rural egg samples*

Conclusions

A fully validated sensitive method for the analysis of 19 PFASs in food of animal origin was developed and validated, obtaining completely satisfactory results. It was applied in a preliminary survey on chicken eggs (Italy) and homegrown eggs from Marche region. The analysis highlighted PFASs in samples from laying hens reared in small-scale rural farming, while the eggs collected in retail showed negligible concentrations. Contamination levels and pattern were relatively uniform in all the contaminated samples with PFOS as major contributor, regardless the geographic location. Most likely the contamination could be attributable to soil ingestion. Therefore, the consumption of homegrown eggs could contribute to the PFAS dietary intake and further studies are surely needed to investigate the issue.

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PL2

Going -omics to reveal the food-gut microbiota-host triangle

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Dietary patterns, or the food we eat, are the sum of many small molecules foreign to the body. After being ingested and digested, nutrients are altered by the trillions of microorganisms that inhabit our gastrointestinal (GI) tract, shaping the chemical structures of such compounds and thus modifying the lifespan, bioavailability, and biological effects. In this context, dietary patterns modulate the gut microbiome and alter its functions by modulating the production of gut microbial metabolites (GMMs). Lastly, these GMMs can regulate gut homeostasis and the risk of GI disease. We studied how GMMs may prevent GI disease, such as colorectal cancer, and promote gut health by modeling diet-microbiome-host interactions.

To tackle this enormous challenge, we use different OMICS approaches. First, we investigated food-gut microbiota interactions by combining different in vitro models and omics approaches. This strategy provides us with a clear picture of gut microbiota functionality. Secondly, we mirror the gut microenvironment using 3D intestinal models closely recapitulating homeostasis and carcinogenesis. At this point, we can investigate human colon organoid and tumoroid responses to specific GMMs by different OMICS approaches. Understanding the human genetic diversity at microbial and intestinal levels will open new horizons in personalized prevention and nutrition.

OR10

Characterizing the food protein digestome by mass spectrometry: *in vitro* and *in vivo* perspectives

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Summary: *Peptides resulting from the digestion of dietary proteins can exert health-promoting properties or induce adverse responses. High resolution tandem mass spectrometry-based peptidomics is the "gold standard" for the comprehensive characterization of food-derived peptides produced by either in vitro digestion models or in vivo as well as to assess their bioavailability.*

Keywords: *food-derived peptides; high-resolution mass spectrometry; peptidomics*

Food digestion is a complex, multi-compartmental, multi-scale physiological process. The characterization of the products of food digestions, which are collectively definable as the "digestome", is essential to establish relationships between food and effects on human health. "Digestomics", that is - in analogy to the "omics" sciences - the comprehensive characterization of the products of food digestion, combines information deriving from food sciences, analytical chemistry, physiology, bioinformatics among other disciplines.

Dietary proteins are digested into very heterogeneous mixtures of free amino acids and peptides of various sizes. Because of inherent structural features as well as of other concomitant factors (co-administration of nutrients, age, gender, physio-pathological status), some food-derived oligo-/poly-peptides (5-50 residues long) can survive digestion and interact with intestinal cell receptors or with immunocompetent cells residing in the gut lymphoid tissue. Thus, besides supplying nutritionally relevant catabolites (*i.e.*, amino acids), dietary proteins can be source of health-promoting peptides or epitopes that induce foodborne adverse responses.

Several static or dynamic *in vitro* models of digestion have been devised to assess the fate of food nutrients. Recently, a static *in vitro* model of the oral-gastro-intestinal digestion has been harmonized and standardized based on physiologically relevant parameters [1]. Obviously, the kinetics of food degradation *in vivo* is much more complex than *in vitro* because it involves a series of dynamically changing factors.

High resolution liquid chromatography - mass spectrometry is an irreplaceable tool for characterizing the protein digestome (Fig. 1). The application of mass spectrometry-based proteomics and peptidomics enables a virtually comprehensive characterization of a protein digestome, although some technical shortcomings can limit the description of the "deep digestome". Several examples of food protein digestion *in vitro* will be surveyed, including characterization, achievements, shortcomings, and knowledge gaps with relevant physiological and immunological implications.

Mounting evidence appears to support the concept that *in vivo* small amounts of

food-derived oligopeptides that escape hydrolysis may enter the blood circulation and exert physiological and immunological effects even at a systemic level. Indeed, it is difficult to establish whether an extremely low level of food-derived peptides can be able to exert any systemic hormone-like bioactivity, also because peptides are subjected to further degradation by plasma peptidases and exhibit half-life time within the seconds-few minutes range. On the other hand, food allergies are a fact and demonstrate that at least at a very low extent or probably under specific circumstances (*e.g.*, infanthood, altered intestinal permeability, dysbiosis), immunological active food peptides can enter the body. Monitoring the course of digestion *in vivo*, for instance through the detection of food-derived peptides in biological fluids, is extremely challenging due to experimental drawbacks, unpredictable specificity of protein cleavage, limited dynamic range of the analysis related to the scant amounts of dietary peptides and presence of endogenous interfering compounds.
The very recent detection of dietary peptides in human biological fluids (*e.g.*, breast milk, urine, plasma) using high resolution mass spectrometry opens up new perspectives for the assessment of the relationship between nutrition and human health [2-5].

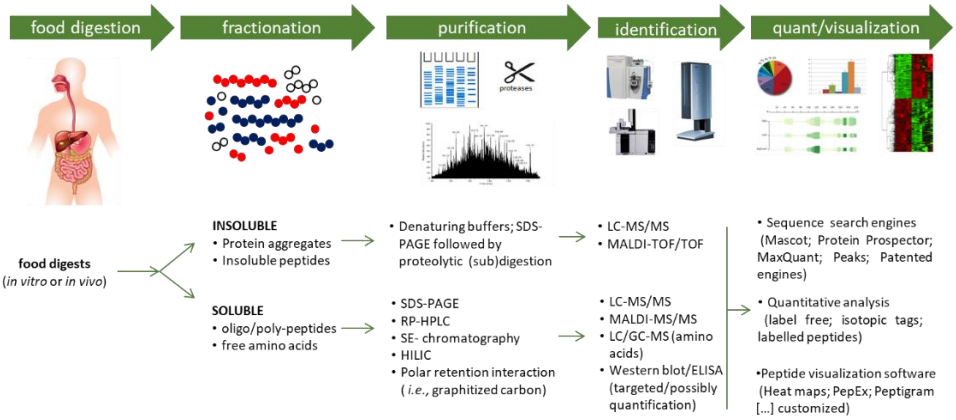

Figure 1. *General analytical workflow of proteomics and peptidomics in digestomics. Different options can be selected at each step, depending on the nature of the food matrix and on the experimental endpoint*

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OR11

Oleuropein-rich leaf extract affects intestinal microbiota and free fatty acids in Apc-mutant and wt rats

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Summary: *An oleuropein-rich leaf extract (ORLE) was administered for one week to wt rats and toApcmutated PIRC rats, spontaneously developing intestinal tumors. Fecal microbiota composition and free fatty acids were analysed to understand possible relation with the beneficial effect in intestinal carcinogenesis observed in ORLE-treated rats.*

Keywords*: Oleuropein; intestinal microbiota, FFAs*

Introduction

Epidemiological and experimental studies point out to several risk factors for colorectal cancer (CRC) such as dietary habits, a pro-inflammatory status and dysbiosis in the intestine [1]. Much attention has been paid to explore the beneficial properties of natural products in the treatment/prevention of CRC. It has been reported that Oleuropein, a secoiridoide polyphenol, present mainly in the leaves of olive tree (Olea Europeae L.), but also, in moderate quantities, in extra virgin olive oil, shows anti-cancer activity in different cell lines [2]. We previously reported that an oleuropein-rich leaf extract (ORLE) has beneficial effect on Apc-mutated PIRC rats, an experimental model of CRC [3]. Since it has been documented that Oleuropein is able to modify the composition of the intestinal microbiota in experimental models of metabolic diseases [4], we were also interested in investigate whether the beneficial effect of ORLE observed in PIRC rats may be linked to variation in the microbiome and in metabolites such as free fatty acids FFAs.

While many studies are uniquely devoted to the determination of short-chain fatty acids (SCFAs) arising from gut microbiota metabolism, the determination of additional FFAs is also interesting. Accordingly, our recent data suggest that the level of medium-chain fatty acid (MCFAs) such as octanoic and decanoic acids may be associated to gastrointestinal diseases, including CRC [5]. Therefore, it was developed an isotopic dilution gas-chromatography coupled mass spectrometry (ID/GC-MS) method for the targeted analysis of both linear and branched FFAs (SCFAs, MCFAs, and LCFAs) in fecal water samples as specific markers for both microbiota and host metabolic variations.

Experimental

We studied the effect of treatment with ORLE in PIRC rats (F344/NTac-Apcam1137) mutated in the onco-suppressor gene Apc (Adenomatous polyposis coli) and developing spontaneous tumours in the colon, as well in F344 wt rats. PIRC and wt rats were randomly assigned to control diet (AIN-76) or to the same diet containing ORLE (2,7 g/kg of diet) for one week.

Fecal samples were collected at the end of the treatment and analysed for microbiota composition [6]; FFAs in fecal waters were analysed by ID/GC-MS method by using an Agilent GC–MS system composed with single quadrupole mass spectrometer, gaschromatograph and autosampler as described by Vitali and colleagues [6].

Results

We previously showed that ORLE was able to inhibit tumour and macrophage iNOS in the PIRC rats [3]. Here we document that ORLE promotes apoptosis and decreases proliferation in colon tumours and normal mucosa of Apc-Mutant Rats.

Regarding microbiota composition, PCoA (principal coordinate analysis) based on Bray–Curtis distances showed a significant effect of the treatment with ORLE in both PIRC and wt rats. In addition, Lefse analysis able to determine the taxonomic units that most likely explain differences between the groups, showed a significant increase in the abundance of the genera Sporobacter, Anaerotruncus and Oscillibacter in ORLE group compared to the CTR group.

Regarding FFAs, while SCFA were similar among groups, we observed that MCFA were higher in PIRC rats compared with wt rats, with no effect of dietary treatment.

Conclusions

Our previous data indicate that ORLE decreases inflammation, promotes apoptosis and decrease proliferation in PIRC rat tumours [3]. Our present data, although preliminary, indicate that in PIRC rats but also in wt animals, ORLE, is able to modulate intestinal microbiota, a result that could be linked to the beneficial effects observed in carcinogenesis. Regarding FFAs, while we did not observe variation due to ORLE treatment, the fact that MCFA are higher in PIRC rats than in wt animals, may be linked to the presence of intestinal tumours, as also observed in CRC patients [5].

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KN1

Mass spectrometry: the Terminator of Mycotoxin occurrence in Foods

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Summary: *Mycotoxins are an undervalued risk by consumers while their toxic effects have a severe impact on human health. Mycotoxins risk evaluation and management require large database based on mass spectrometry analysis. This is the fittest techniques for complex matrixes such as raw, finished foods and their byproducts.*

Keywords: *Multi-mycotoxin, High Resolution Mass Spectrometry, food safety*

The growing interest in the consumers' Health Risks associated with mycotoxins has led to the development of several research projects during the past decade in several countries. All these efforts have generated a great deal of information on the natural occurrence of these unwanted contaminants in raw foods, feeds, vegetables, and transformed foods and, consequently, to evaluate human exposure to these natural toxins in human samples like biological fluids. Mycotoxins are substances that pose a serious risk to animal and human health and may be occurring at various stages of its field production, food processing, transport, or food storage and may result from environmental contamination or from the low quality of foods or the management of part or entire food chains. The main emphasis of the presentation is to show effective, rapid, and reproducible methods easily applied to the identification of mycotoxins in routine food analysis. The most frequent analytical methods adopted by research laboratories and surveillance government agencies are based on liquid chromatography coupled with MS. The use of ultra-high-performance liquid chromatography (UHPLC) provides higher sensitivity, a reduction in mobile phase consumption with consequent reduction of the environmental impact of analyses, and an increase in resolving power and peak shape. High-resolution mass spectrometry (HRMS) provides sensitive and specific measurements for the quantification of targeted compounds, with the additional features of making retrospective data analysis and the identification of untargeted compounds based on exact mass measurements. This presentation provides an overview of the occurrence of mycotoxin and other contaminants in vegetables, foods, food supplements, and biological fluids that can serve as a basis for risk management-based regulatory decisions in charge of public institutions to shield consumers' health.

Conclusions

Results of many surveys show that the mycotoxin contamination of foods aren't limited to few commodities or finished foods. The multiple mycotoxin contamination is the norm and often five or more mycotoxins are cooccurrence per sample. Mainly mycotoxins detected are produced in field, but the presence of storage-type mycotoxins was not ancillary. Fusarium mycotoxins were the

most frequent with FA, DON, FBs and 15ADON being the contaminants most common of cereals and their derived but aflatoxins, ochratoxins aren't to be forget. The synergic effects due to two or more mycotoxins lead a strong increase of quality and safety of foods and feeds and products like milk for adults or baby or cheese. The real point to evaluate is the continue exposition to mycotoxins, their storage in the body and their cumulative effects on human health. The database research using mycotoxins, foods and mass spectrometry generates at moment 2.293 citations and the 50% have been published in the last ten years. Appear to be clear that high-resolution mass spectrometry are the best tool to evaluate the mycotoxins occurrence, their metabolism and their occurrence in biological samples like urine or hair. This large data is the base to produce the best answer at mycotoxins risks.

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OR12

Veterinary Drug Analysis for Meat Supply Chain Safety

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Summary: *The analysis of antibiotics and coccidiostats in meat supply chain requires low limit of quantification in very complex matrixes. The use of HPLC-MS technique allowed reaching high sensitivity keeping under control matrix effect. The results obtained by proficiency test confirms excellent method performance for the analysis of real samples.*

Keywords: *Veterinary drugs, Meat supply chain, HPLC-MS*

The presence of veterinary drug residues in meat supply chain is an important current topic because of the animal administration of these substances. Among veterinary drugs, the permitted substances, such as antibiotics and coccidiostats, have a maximum residue limit (MRL); therefore, the presence of these molecules in meat supply chain is regulated. Furthermore, the growing consumption of products belonging to antibiotic free supply chains requires lower and lower limits of quantification (LOQ) of this analytes in meat.

Antibiotics include many compound classes (e.g. beta-lactams, quinolones, sulfonamides, macrolides, tetracyclines) characterized by very different molecular weights and functional groups resulting in diverse chemical properties such as polarity and solubility. Similarly, coccidiostats include both ionophores and non-ionophores molecules characterized by different polarity and consequent chromatographic behaviour.

Moreover, the veterinary drug residue determination in meat supply chain corresponds to the analysis of very different matrices (such as feeds, water, meats, offal and animal urine) characterized by different matrix interferences (e.g. proteins, phospholipids and fats).

High performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) analysis allows the required selectivity and sensitivity for discriminating analytes and matrix interferences. For these reasons, two methods for the analysis of antibiotics and coccidiostats in meat were developed using HPLC-MS analysis, without purification steps. The correct analyte quantification is allowed by matrix-matched calibration. The method validation was performed by the replicated analysis of spiked meat samples at two different concentration levels (limit of quantification and a higher level) in order to evaluate method recovery (percentage recovery included in the range 70-120%) and repeatability (relative standard deviation less than 15%). Moreover, the metrological approach was used for the calculation of methods uncertainty. Furthermore, the participation to proficiency tests with the achievement of satisfactory z-scores for all tested analytes confirmed the excellent methods performances.

KN2

Mineral oils in vegetable oils: background, analysis and the role of MS

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Summary: *Mineral oils are widespread food contaminants of health concern. Because of their lipophilic nature and the presence of multiple sources of contamination along the processing chain, their presence in vegetable oils has received much attention, encountering particular analytical difficulties that will be discussed, along with the role of MS analysis.*

Keywords: *Mineral oils, vegetable oil, contamination*

Introduction

Mineral oil hydrocarbons (MOH), which are complex mixture of thousands of saturated (MOSH) and aromatic hydrocarbons (MOAH) of petrogenic origin, are widespread environmental and processing contaminants, that can reach food by different routes (environmental contamination, agricultural practices, harvesting operations, food processing, migration from food packaging). While MOSH accumulate in human tissues/organs based on their structure and molecular weight, MOAH with 3 or more aromatic rings include suspected genotoxic and carcinogenic compounds. Due to the lipophilic nature of these contaminants and the presence of multiple sources of contamination along the processing chain, vegetable oils are among the most contaminated foods [1]. Although no legal limits have entered into force so far, recently (at a meeting of the Standing Committee on Plants, Animals, Food and Feed), the EU Member States agreed in recommending a common limit for MOAH (2 mg/kg for fats and oils).
In addition to providing a basic knowledge of these emerging contaminants and their complex analytical determination according to the on-line high-performance liquid chromatography (HPLC)- gas chromatography (GC)- flame ionization detector (FID) reference method [2], the purpose of this contribution is to discuss, through examples, the importance of GC-mass spectrometry (MS) as a confirmatory analysis, as well as the role of GC×GC-FID/MS for in-depth characterization of contamination and as valuable aid in identifying sources of contamination.

Analytics and the role of GC-MS

Due to the presence of huge amounts of triglycerides and other endogenous interferents (olefins, *n*-alkanes), the determination of MOH in oils and fats is very complicated, especially when low detection limits are required [2]. For this reason, analytical determination must be preceded by adequate sample enrichment (saponification) and purification [2, 3]. Because of the calibration problem encountered with mass spectrometry, FID is required for MOH quantification. Nevertheless, the poor selectivity of FID and the possible presence of residual interferences (even after optimal sample preparation) make MS a useful tool for confirming mineral oil contamination in cases of doubt

(checking for the presence of markers such as steranes and hopanes [4]). Very recently, comprehensive two-dimensional GC (GC×GC) with parallel MS/FID detection was used for MOH characterization of oils physically extracted from olives before and after harvesting and lubricants/greases used during harvesting operations [5]. The use of this platform, alongside the reference method, made it possible to unequivocally identify the source of contamination and, thanks to MS information, added useful information on MOH composition

Conclusions

MOH analysis in vegetable oils is very complex. It requires sample enrichment and purification before on-line HPLC-GC-FID. Comprehensive two-dimensional gas chromatography (GC×GC) with parallel MS/FID detection represents a valuable tool for achieving reliable quantification and further MOH characterization.

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OR13

Artificial Intelligence strategies based on GC×GC-MS/FID patterns capture extra-virgin olive oil aroma blueprint and unique identity

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Summary: *The contribution illustrates the potentials of GC×GC-MS/FID platforms in the context of Artificial Intelligence Smelling and computer vision tools for extra-virgin olive oils characterization and identitation. By accurate quantification of key-aromas and odorants strongly correlated to sensory defects, samples' aroma blueprint is captured and used to discriminate oils based on peculiar hedonic features.*

Keywords: *extra-virgin olive oil volatiles; comprehensive two-dimensional gas chromatography; Artificial Intelligence algorithms*

Introduction

Since its introduction, comprehensive two-dimensional gas chromatography (GC×GC), has unrevealed its potentials in many fields helping scientists to better understand the Nature's complexity, facilitating highly-informative screenings, supporting markers discovery in *omics* applications, and offering many opportunities to implement system biology-like strategies for investigation, the *integrationist* approach [1].

In *food-omics* the analytical platform design and configuration plays a key role to achieve the suitable information capacity, resolution and sensitivity to answer the many questions posed by application needs. The contribution deals with the challenging task of designing a multidimensional platform for high-quality extra-virgin olive oil (EVOO) volatiles (quantitative)-screenings. By combining effective separation by GC×GC with low-resolution, fast-scanning quadrupole mass spectrometry and parallel FID detection, a single measure can answer many questions about product qualities (e.g., sensory quality, freshness, authenticity, presence of sensory defects etc.).

Within this context, the key-role of Artificial Intelligence (AI) algorithms for computer vision (i.e., "…*a field of AI that enables computers and systems to derive meaningful information from digital images*…" [2]) and smelling (e.g., AI smelling machine [3]) is discussed and proof-of-evidence on the feasibility and effectiveness of such "comprehensive" approaches presented through the

authors research experience on high-quality extra-virgin olive oil.

Experimental

Brazilian olive oils (n=28) from the 2020 harvest year were selected by Embrapa research team (Rio de Janeiro, Brazil) from those available on local markets or directly supplied by the producers. They were obtained from olives of different cultivars and proved compliant with the analytical parameters necessary to classify them as EVOO, except for sensorial analysis.

EVOOs from Italy (n=111) were supplied within the VIOLIN project (Progetto Ager, 2016). They were obtained by olives of different cultivars harvested over several Italian regions. Italian EVOOs were all certified as compliant by accredited laboratories (ISO 17025:2018) and by the official sensory panel test.

GC×GC was run with a polar × semi-polar column combination followed by qMS/FID parallel detection. HS-SPME automated sampling, performed in HS linearity conditions, was by a divinylbenzene/carboxen/polydimethyl siloxane (DVB/CAR/PDMS) fiber (df 50/30 µm; 2 cm length - Supelco, Bellefonte, PA, USA) at 40°C for 50 min on a 0.500 g of EVOO in a 20 mL HS vial. Untargeted/targeted fingerprinting (*U*T fingerprinting) work-flow was carried out combining template matching strategies on the 2D-patterns of volatiles collected by qMS and FID detection. By Multiple Headspace SPME, quantification was possible for an extended list of target volatiles (n=42) including potent odorants and geographical tracers. Quantification was by external standard calibration and FID predicted relative response factors (RRF) based on combustion enthalpies [4].

Results

The olive oil volatilome has a high chemical dimensionality reflecting many different biological phenomena influencing the global metabolome (*e.g.*, olive trees genetic traits, harvest region pedo-climatic conditions and soil composition, olives ripening stage, processing technologies applied to obtain oils, and shelf-life conditions). Diagnostic patterns might include known analytes (targets) and unknowns (untargeted features); their tracking across samples is confidently approached by GC×GC-qMS/FID by retention times ($^{1}t_R$, $^{2}t_R$) and EI-MS spectral similarity match.

Brazilian olive oils were here studied for their peculiar yet unique detectable volatilome, characterized by 262 UT features, and compared to a large selection of Italian oils. The quali-/quantitative distribution of all 262 UT peaks enabled effective modeling providing the best classification performances in terms of accuracy, sensitivity, and specificity. By limiting the fingerprinting breadth to target features (i.e., 105 known analytes), the model loses its sensitivity to 60% while keeping excellent scores for accuracy and specificity. Insights on characteristic/diagnostic components gave access to high-level information. An example is that of unsaturated alkenes [(5E)-3-ethyl-1,5-octadiene; (5Z)-3-ethyl-1,5-octadiene; and (E,E)-3,7-decadiene] whose relative abundance was lower in Brazilian oils, suggesting a different strategy to assess optimal harvest for olives destined for oil production. Fig. 1 shows the pattern of unsaturated alkenes detected in an Italian EVO sample.

Their pair-wise comparison evidenced compositional differences with direct tracking to UT features identities over the 2D chromatographic plane. Computer vision results, cross-validated by high-resolution fingerprinting *(i.e.*, UT fingerprinting), and modeling evidenced how 2D patterns of chemicals can be treated as *identitation* fingerprints opening many other investigation possibilities across the sample set.

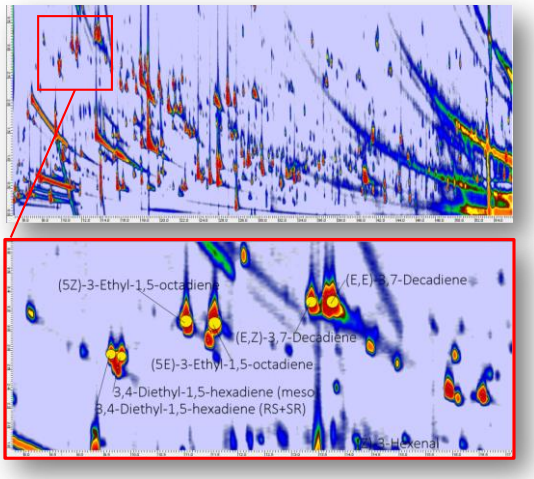

***Figure 1**. pattern of unsaturated alkenes detected in an Italian EVO sample*

The strategy adopted offered many other options. By a computer-vision approach, composite-class images were realized for Brazil and Italy origins. Last but not least, thanks to the implementation of the accurate quantification procedure with MHS-SPME, an extended list of volatiles was monitored for their actual amounts in all samples. These data feed the first database of volatiles quantitative signatures in Brazilian oils while adding further information to the existing knowledge on Italian EVOOs. In particular, when observed through the OAV concept, results suggest which sensory features might discriminate between Brazil and Italy EVOOs.

Conclusions

The potentials of this “omics” strategy can be explored even more. A larger and more representative sample set might answer questions related to cultivar phenotyping when trees are transplanted out of their native geographical area and the impact of local-pedoclimatic conditions within Brazil regions.

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OR14

Analysis of 3,5-Stigmastadiene in Extra virgin Olive Oil by GC-MS

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Keywords: *Stigmastadien, Extra Virgin, UE2568/91*

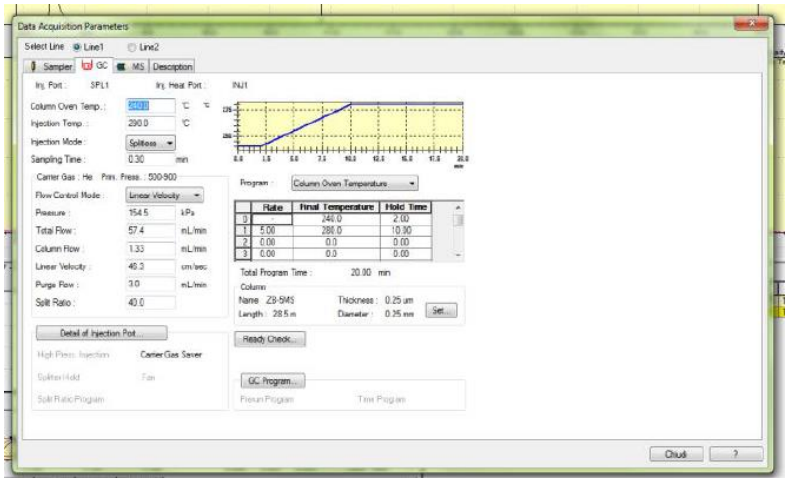
Introduction

For the determination of 3,5-Stigmastadiene there are 3 methods: UNI-EN.ISO 17788-1: 2001, EU Reg. 2568/91 and subsequent amendments. and the method COI / T.20 / Doc. No 11 / Rev. 4.
All these methods involve determining in GC / FID.
These methods, around the limit, have poor reproducibility and for lower values (0.02 mg / kg) exceed the expected value.
In addition, for the determination in Extra Virgin Olive Oils (EVOO) a saponification on 20 g of oil and a subsequent purification on a silica gel column is foreseen.
We have tried an analytical system that provides the direct purification of EVOO with a single ion determination in GC / MS.

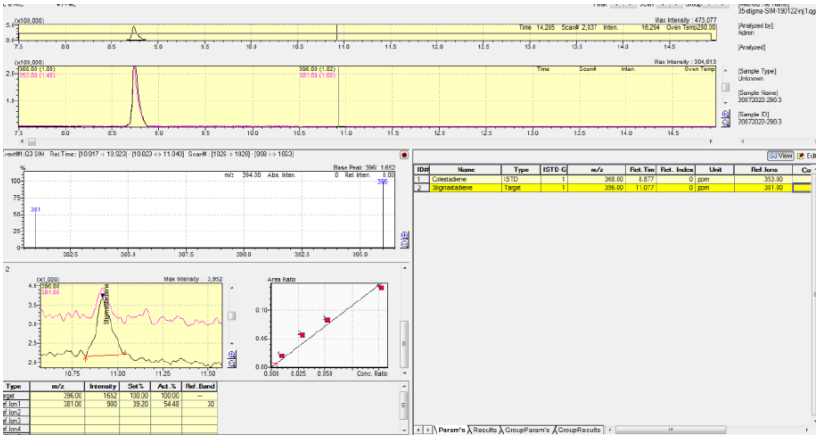
Experimental

Not having available the pure reference standard of 3,5-Stigamstadien, we used a reference material (RM) of an olive oil from a proficiency test with a 3.5-Stigmastadien content of 31.65 mg / kg.
For dilution of this RM in EVOO with a content of 3.5-Stigmastadien below the detection limit, we have built a calibration curve of five points (from 0.01 to 0.10), using 3,5-Cholestadien how Internal Standard.
Every oil of the calibration curve has been purified for direct elution on a column of silica with n-hexane.

GC-Condition



MS-Condition



Results and conclusion

The repeatability of the analytical system was verified at three levels, as reported in the following table.

3,5-Stigmastadien

Assigned Value (mg/kg)	P1	P2	P3	P4	P5	P6	P7	P8	Mean	Sr	CVr%	r	U (±)
0,02	0,013	0,015	0,018	0,022	0,023	0,017	0,016	0,024	0,019	0,004	21,6	0,01	0,014
0,05	0,045	0,057	0,05	0,046	0,042	0,06	0,048	0,053	0,049	0,006	12,6	0,02	0,025
0,10	0,101	0,094	0,102	0,096	0,093	0,108	0,112	0,094	0,101	0,007	7,0	0,02	0,037

U = r+(RSD%)*(Mean)/100

From the results obtained, verified around the limit of EU Reg. 2568/91, the CVr% is 12.6% and the repeatability is 0.02 mg / kg, the LOQ evaluated is 0.01 mg/kg.

These results have agreed with the statistical data of the UNI-EN.ISO 17788-1: 2001 method.

Our analytical system has been applied in this year on 85 EVOO samples: the 85 % are below LOQ, 8 % between 0.01 and 0.03 mg/kg, 3 % between 0.03 and 0.05 mg/kg and 2 samples are over the limit.

This method is faster than the official methods in GC / FID and moreover the consumption of reagents is just over at 60 ml of n- hexane, much lower than that of the official methods that also carry out an oil saponification before the purification of the stigmastadien fraction.

At the moment we are evaluating an upgrade of this system, reducing the quantity of silica gel to 3 gr and using a detection with GC-MS/MS.

OR15

Response Surface Methodology optimization of HS-SPME-GC-MS method for the analysis of pentene dimers and terpenes in extra virgin olive oil

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Summary: *A HS-SPME-GC-MS method for the simultaneous analysis of pentene dimers and the main mono- and sesquiterpenes in virgin olive oil was proposed. A Doehlert Design was employed and Response Surface Methodology was applied for optimizing the pre-concentration step. A quantitative approach using internal standards was then set up and validated.*

Keywords: *Volatile hydrocarbons; method operable design region; experimental design*

Introduction

The volatile fraction of virgin olive oil (VOO) includes hundreds VOCs [1], present in concentrations ranging from ng/kg to mg/kg [1,2], affects the sensory characteristics of VOOs and is affected by several factors. It is recently widely used for the quality control of VOO [1,3,4].

Several volatile hydrocarbon such as pentene dimers (linear and branched C10 hydrocarbons from the LOX-pathway) and mono- and sesquiterpenes have been detected in the volatile fraction of VOO but are less investigated to date [1,4,5], and are thought to contribute to the pleasant notes of EVOOs. Terpenes might be varietal and geographical differentiators [1,4,5]. Some analytical methods for analysis of hydrocarbons were very time consuming; therefore, a suitable method for the simultaneous analysis of pentene dimers and terpenes in EVOO is required. HS-SPME-GC-MS is the most common approach for analysis of VOO volatile profile [1], being cost-effective, solvent-free, easy to adopt, fast and versatile, and not requiring sample preparation. The Design of Experiments (DoE) with Response surface methodology (RSM) can be suitable for optimization of the HS-SPME pre-concentration step, in particular using the method operable design region (MODR) [6], which is the zone where the requirements are fulfilled with a certain probability.

The aim was developing a HS-SPME-GC-MS method for the simultaneous analysis of pentene dimers and terpene hydrocarbons in EVOOs. RSM has been applied for the optimization of the critical process parameters (CMPs) of VHCs pre-concentration by HS-SPME, thus obtaining the MODR and leading to the selection of a working point to be used for routine analysis. A quantitation method was set up using a number of external and internal standards, it was then

validated and applied to a group of monovarietal EVOOs.

Experimental

Samples: Stock solutions of external (ESTD) and internal standards (ISTD) of terpenes and pentene dimers were prepared in a refined olive oil. Eight levels of calibration scales were prepared by mixing the same amount of ISTD and increasing amounts of ESTD. Four monovarietal EVOO were used for preliminary trials. A pooled sample of Coratina and Altomira cvs was used for the Doehlert Design experiments. Eight EVOOs were analyzed using the validated method: 4 of the Moraiolo and 4 of the Tonda Iblea cultivar.

Sample amount, extraction time, extraction temperature and desorption time of HS-SPME step were optimized using a Doehlert Design experiment. A 50/30 µm DVB/CAR/PDMS 1-cm SPME fiber was employed for extraction of VHCs from the HS of 20-ml screw vials at the selected conditions. The VHCs were desorbed at 260 °C in a 6890N GC system with a 5975-MS detector (all from Agilent, Palo Alto, CA, USA), and separated in a HP-Innowax capillary column (50m×0.2mm id, 0.4 µm ft). Oven: 2 min at 40°C; to 156°C at 4 °C/min; to 260°C at 10°C/min. MSD worked in scan mode at m/z 29-350 Th, IE energy 70 eV. Peaks were identified using commercial standards when available; in the other cases the retention index evaluated analyzing C9–C30 linear alkanes and the NIST08/Wiley98 library were used.

Eight-levels calibration lines were built, and the response factors were calculated after normalizing the peak area using the ISTDs. Validation by considering repeatability, LOQ, LOD, linear range of calibration, accuracy, sensitivity and selectivity was performed.

Results and Conclusions

65 VHCs were identified, including pentene dimers and terpenes. HS-SPME pre-concentration step was optimized by RSM, making previsions all throughout the experimental domain. The domain of the CMPs were: sample amount (SaAm), 2.1500-8.6000 g; extraction time (ExTi), 20-80 min; extraction temperature (ExTe), 30-90 °C and desorption time (DeTi), 1-5 min. The responses included both cumulative areas of groups of VHCs and areas of individual compounds of interest. The responses related to sesquiterpenes content were selected for building the MODR. Quadratic polynomial models relating the factors to the responses were hypothesized and the coefficients of the model were calculated by means of a Doehlert Design, a matrix with high efficiency (i.e., low n° of experiments). Each factor was studied at a different number of levels uniformly distributed for an experimental plan with a total of 23 experiments. Three replicates at the center were performed, enabling the estimation of the experimental variance. Logarithmic transformations of responses and model refining were done excluding the factors that were found to be not significant, obtaining very good results in terms of quality of the models. All the models were significant, while validity was verified for the majority of the responses. All the models were considered acceptable due to the small residuals and to the high values of Q^2, which indicated a good prediction quality [7]. Graphic analysis of effects made it possible the direct evaluation of the significant effects of the CMPs on the responses. The trend of the predicted values of the responses

could be easily visualised by drawing the four-dimensional contour plots (Fig. 1 a-c).
The conditions which made it possible to optimize both these responses corresponded to the red zone, located at high ExTi, medium ExTe, low SaAm and low DeTi values.
Taking into account Doehlert Design results, target values for the three critical method attributes CMAs related to sesquiterpenes were defined and the sweet spot plots with the zone where all the CMAs are fulfilled were drawn.

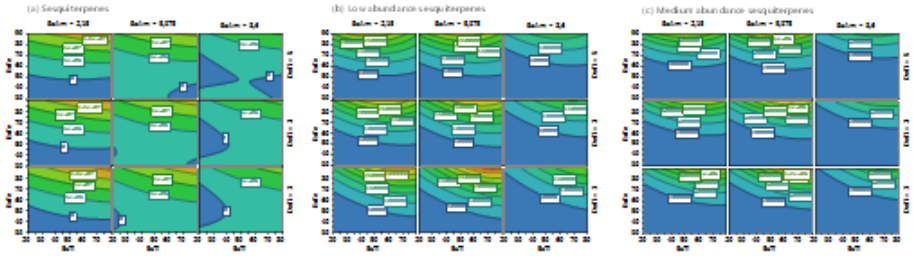

***Figure 1**. Contour plots for the responses of (a) Sesquiterpenes; (b) LA sesquiterpenes; (c) MAsesquiterpenes*

In the next step, the MODR was defined, which includes any combination of the variables that provide assurance of quality of the data produced by the method [6]. The MODR around the set-point is in green in Fig. 2. Inside the MODR, the working point was chosen as the same set-point originally selected. It was at: SaAm, 3.27 g; ExTi, 65 min; ExTe, 90 °C and DeTi 1.70 min. Using these optimized conditions, a quantitative method was developed and validated and applied to samples of the Moraiolo and Tonda Iblea cultivars, which showed different VHCs profiles.

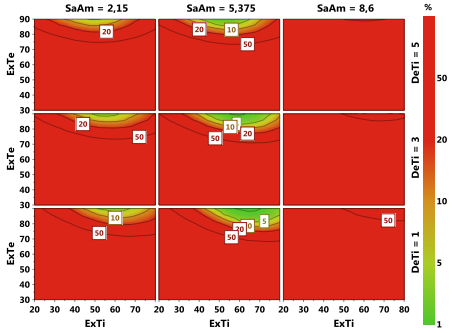

***Figure 2**. Probability maps obtained by plotting ExTe, vs. ExTi, at 3 values of SaAm and DeTi. 1, 3, 5 min. The MODR is the green zone included in the 10% isoprobability line*

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OR16

Potential of Trapped Ion Mobility combined with LC-HRMS in food authenticity studies

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Summary: *An innovative LC-TIMS-QTOF method was developed for food authenticity studies.*

Keywords: *TIMS, LC-HRMS, CCS*

Food analysis is incessantly requiring the development of more efficient, cost-effective, and robust method to investigate the quality and traceability of food commodities with respect to legislation and consumer demands. Within this context, this study aims to elucidate the characterization and identification of stereochemical isomers associated with the health claim in Greek olive oil and evaluate the discrimination of olive oil samples from different agricultural backgrounds (variety, geographical origin).
An innovative trapped ion mobility spectrometry (TIMS) coupled to ultra-high-performance liquid chromatography–electrospray ionization quadrupole time of flight tandem mass spectrometry (UHPLC-QTOF) analytical method was developed for the analyses of 48 samples of Koroneiki variety from 3 different geographical origins in Greece (Peloponnese, Lesvos, Crete) and 33 samples of 5 different Greek varieties: Koroneiki, Kolovi, Adramytiani (Lesvos), Chiotiki (Chios), Thrumba (Samos). The discrimination studies, based on an untargeted approach, and Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) statistical models, showed the capability of TIMS-TOF technology to discriminate samples from different varieties and geographical origins, also combining the positive and negative polarity acquisitions. In particular, thanks to the TIMS activation, was possible to obtain the isomers separation (i.e. 5 different mobility peaks and MS/MS spectra were detected from 1 chromatographic peak) and isomer identification (applying a structure elucidation-aimed workflow). Therefore, an efficient geographical discrimination of the samples and a list of isomers as potential authenticity markers were obtained.

PL3

Applications of high-resolution MS metabolomics in the traceability of the agri-food products

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In a framework of the whole food chain, the set of conditions a food undergoes in his production cycle is able to affect its chemical profile. Starting from the field, a specific cultivar interacts with the environment (the so-called "genotype *x* environment" effect), and climatic and edaphic conditions, as well as agronomic and post-harvest management (including tradition and local common practices), significantly shape the metabolome of a food. Thereafter, processing and storage conditions also contribute to determining unique chemical fingerprints.
Starting from this concept, the hypothesis-free untargeted nature of metabolomics, due to its broad characterization of chemical profile in foods, may harvest information on the authenticity and geographical origin of foods. The downstream multivariate interpretations (unsupervised as cluster analysis and PCA, rather than supervised like OPLS-DA) and Artificial Neural Networks allow supporting food traceability and mining markers of origin.
The speech will present some case studies based on the use of UHPLC-ESI/QTOF-MS untargeted metabolomics to support the authenticity of agri-food products. The aspects related to MS and MS/MS annotations in non-model systems, as well as the relevance of multivariate statistics (both multivariate statistics and Artificial Neural Networks) will be introduced. Some examples will deal with profiling approaches (i.e., profiling phenolic compounds and sterols), while some others are comprehensive metabolomic approaches.
Among case studies, the effect of environment (geographical origin and altitude) and the blending with different but genetically related cultivars) on Taggiasca Ligure olive oil metabolomics profile will be described. The metabolomics dataset originated from about 300 samples over three consecutive seasons will be used to specifically point out the effect ascribable to the factors under study, and to identify markers of each condition.
Thereafter, the possibility to trace hazelnuts origin will be presented, by highlighting as supervised OPLS-DA allows identifying the markers of provenience, even when the cultivar effect is hierarchically prevalent.
To conclude, few other examples (cocoa, vanilla and saffron) will be given to highlight the potential of metabolomics even in completely different matrices.

OR17

Integrating TD-(+/-)DART-HRMS, data fusion and LASSO method for rapid authentication of grounded black pepper

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Summary: *This contribution reports the application of TD-(+/-)DART-HRMS, combined with data fusion strategies and LASSO method, for the authentication of grounded black pepper samples. High predictive performances of this rapid authenticity assessment suggested that the technology may be useful to screen the samples in a quality control frame.*

Keywords: *adulteration, data fusion, ambient mass spectrometry*

Introduction

Economically motivated adulteration of spices and herbs encompasses the fraudulent deliberate substitution of valuable commodity with cheaper materials or plant sub-products (1). A recent survey carried out by the Joint Research Centre of the European Community in 2021 revealed that 17% of black pepper in the market is suspicious of adulteration (1). This contribution describes the development of a non-targeted method for the authentication of black pepper by thermal desorption direct analysis in real time high resolution mass spectrometry (TD-DART-HRMS) coupled to data fusion and least absolute shrinkage and selection operator (LASSO). In the present study, the positive and negative TD-DART-HRMS was applied to investigate the volatile profiles of black pepper and explore its potential in the detection of adulterations. Once combined the two datasets, a LASSO statistical method was applied to develop a classification model that is predictive of authentic and adulterated samples.

Experimental

In our study, a total of 39 samples (25 adulterated samples and 14 genuine samples) were analysed by TD DART-HRMS in positive and negative ion mode. The authentic samples were originated from a variety of countries (Indonesia,

Vietnam, Brazil, Cambodia, Madagascar, Costa Rica, Ecuador and Sri Lanka). The adulterated samples included 12 different types of adulterants (spent, pinhead, papaya seeds, red beans, garlic, olive kernel, olive pomace, black mustard, green lentils, plaster, coriander, chili, aniseed green) in a concentration range between 15%-35%. A labmade heating device generated a temperature gradient between 25°C and 150°C in a few seconds. Once located ~ 40 mg of sample on the heater, the generated plume of volatile molecules was ionized by the DART SVP 100 ion source (IonSense, Saugus, MA, USA) and analysed by an Exactive Plus orbitrap from Thermo. The data were statistically analyzed using MetaboAnalyst 5.0 web portal (www.metaboanalyst.ca) and Rstudio 3.6.1 software with the *caret* package. The two datasets were concatenated by low-level data fusion. Once obtained a unique fingerprint, the data were submitted to partial least-squares discriminant analysis (PLS-DA), to verify a possible discrimination of the two groups of samples. Then the data was split into training (28 samples) and test sets (11 samples). A LASSO classifier was created on the training set, and its performances established by 5 times repeated 5 fold cross-validation. The performances of the classifier were then evaluated on the withheld test set. Accuracy, sensitivity and specificity rates of the classifier were calculated and then a receiver operating characteristics (ROC) curve was generated.

Results

Representative TD-DART-HRMS spectra of genuine and adulterated black pepper are reported in Fig. 1. The PLS-DA score plot showed a tendency to discrimination of the two groups of samples (Fig. 2A).

Our LASSO classifier, built with a high number of different adulterated samples analysed by TD-(+/-) DART-HRMS data, was created with the aim pf precisely assessing adulterated samples. The model achieved great performances on training set, achieving good results in terms of overall accuracy, sensitivity, and specificity. The ROC curve shows an area under the curve (AUC) of 0.96 on test set, demonstrating a good response of the model (Fig. 2B). The model achieved an overall accuracy of 90.9%, specificity 75% and sensitivity 100%.

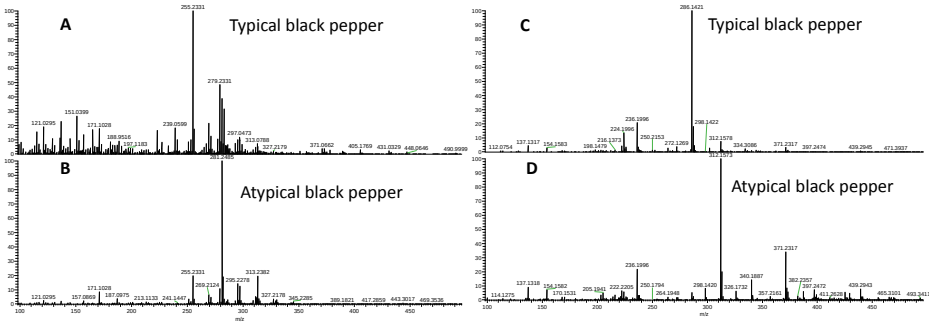

Figure 1*. Representative (+/-) TD-DART-HRMS spectra of authentic (A and C) and adulterated (B and D) black pepper spiked with 30% of papaya seeds*

Note that on test set, only one Brazilian authentic sample was not correctly

classified. While the specificity rate (75%) is a measure of the typical black pepper correctly predicted by the model, the sensitivity (100%) indicates how well the model classifies atypical samples. Therefore, high sensitivity is desirable in a quality control of raw material in an industrial frame.

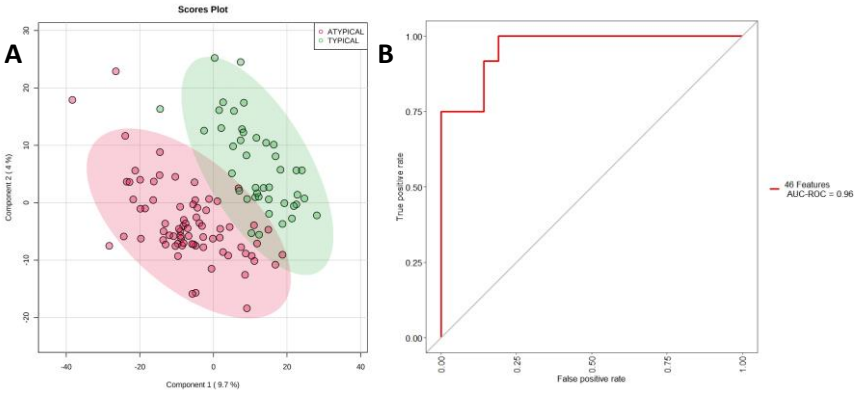

***Figure 2**. A) PLS-DA score plot. A tendency to discrimination of authentic (typical) and adulterated (atypical) samples can be observed. B) ROC curve obtained from the validation of LASSO classifier on the test set*

Conclusions

We demonstrated that the volatile profile acquired by TD-DART-HRMS can be useful in fraud assessment of spices. While TD-DART-MS was already applied in forensic and environmental sciences, this is its first successful application to spices authenticity. We are working on the enlargement of the training set and the validation of the method with an independent set of samples and different users.

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OR18

Assessing chicken meat authenticity within divergent farming systems (organic *versus* antibiotic-free) using SWATH-MS-based proteomic analysis and chemometrics multivariate tools

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Summary: *Faming systems influence physiological and metabolic functions of the animals and impact meat quality. Organic meat production aims to provide high quality, safe, and sustainable products. From an analytical standpoint, few methodologies have been used to authenticate organic meat. In this work, proteomics and chemometrics were used to discriminate chicken meat from different strains and farming systems.*

Keywords: *Proteomics, meat authenticity, organic meat*

Introduction

Poultry meat is among the most consumed meats in the world. The physiological and metabolic functions of the animals are influenced by many factors such as the production systems and pre-slaughter stress, with consequent impact on the quality of the final product [1]. Organic meat production systems aim to provide more sustainable products with high levels of quality and safety and are based on high animal welfare standards [2]. To date there has been a paucity of published literature on the application of high-throughput omics methods such as proteomics to further our understanding and characterisation of these production systems and potential impacts on quality. This study aims to understand the impact of an organic farming system, compared to an antibiotic free system (that can be considered as conventional system), at the level of the proteome of *post-mortem Pectoralis major* muscle in the Ross 308 strain.

Experimental

Twenty Ross 308 and 20 Ranger Classic chickens were used in this study for a comprehensive proteomics investigation. Early *post-mortem* muscle biopsies samples were provided by Fileni® industry (Cingoli, Italy). From each group, 10 chickens were reared antibiotic-free inside ground farming (ARO and ARA) and 10 according to the Council Regulation (EC) No 834/2007 on organic production and labelling of organic products (ORO and ORA). All animals were slaughtered under standardised systems, within the one batch and within one hour. *Pectoralis major* muscle (breast) biopsies were taken within 3 h under standardized conditions and with randomisation between the left and right sides, and stored at -80°C until analysis. For shotgun proteomics, the protocol of Zhu *et al.* [3] was used for total protein extraction and preparation of the protein bands. Quantitative proteomics was performed using a TripleTOF 6600plus (Sciex,

Redwood City, CA, USA) using a Data-Independent Acquisition proteomics approach using SWATH-MS (sequential window acquisition of all theoretical fragment ion spectra mass spectrometry) as in Chantada-Vázquez *et al.* [4]. The data after normalization and imputation were analysed by multivariate partial least squares discriminant analysis (PLS-DA) to discriminate the groups of interest using the most significant proteins. Therefore, the variable importance in projection ≥ 1 and permutation diagnostics (1000 random permutations) were calculated to consider the most influential protein markers. Subsequently, pathway enrichment analyses (Gene Ontology (GO), KEGG, Reactome terms) was performed on the discriminatory proteins identified by the PLS-DA using Metascape® following the guidelines of Gagaoua *et al.* [3].

Results

The SWATH-MS proteomics applied in this study on individual samples allowed the identification of 660 quantifiable proteins in chicken *Pectoralis major* muscle. The PLS-DA score plot allowed visualizing a clear separation between the 4 groups based on their proteome. A slight overlap could be noticed between the organic groups (ORA and ORO) (Fig. 1A).

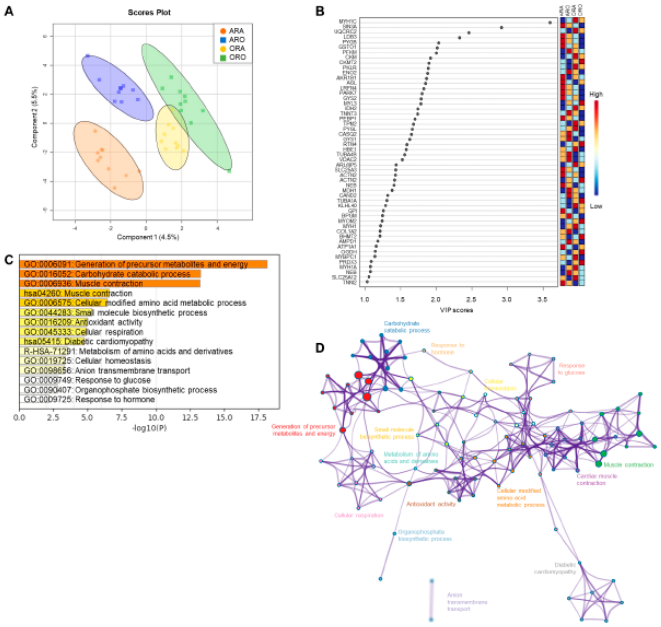

Figure 1. *A) Partial least squares-discriminant analysis (PLS-DA) score plot of meat proteome distribution according to chicken strain and production system. B) Variable Importance in Projection (VIP) plot obtained from meat samples classified as ARA, ARO, ORO and ORA. VIP cut-off of 1.0 has been used. C-D) Bioinformatic enrichment analyses (Gene Ontology, KEGG, Reactome) on the 48 proteins with VIP > 1. C) Top significantly enriched terms. D) Network layout based on the enriched pathways using the list of 48 proteins. Each term is represented by a circle node, where its size is proportional to the number of input genes under that term, and its color represent its cluster identity. Terms with a similarity score > 0.3 are linked by an edge (the thickness of the edge represents the similarity score)*

Fig. 1B shows the Variable Importance in Projection (VIP) plot values set at a cut-off of 1.0. The pathway enrichment analysis of the 48 VIP (Fig. 1C) identified 15 significantly enriched terms.
The “generation of precursor metabolites and energy (GO: 0006091)” was the top GO term explaining the differences within the groups followed by others related to the carbohydrate catabolic process, muscle contraction, metabolic and biosynthetic processes, antioxidant activity, cellular respiration and response to glucose and hormones. These enriched cluster terms allowed to construct a process network of the pathways (Fig. 1D).

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OR19

Ultra-high sensitivity quantification of veterinary drug residues in animal by-products

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Ultra-high sensitivity quantification of veterinary drug residues in animal by-products The use of pharmacologically active substances in veterinary settings has been scrutinized for several years due to their sometimes inappropriate or intensive application. Therefore, these substances must be limited to mitigate negative consequences. One way to implement controls is to perform analytical testing of animal by-products. Several compounds found in these by-products have a maximum residue limit (MRL) to minimize their use, and some compounds are prohibited due to their inherent toxicity. To limit these compounds within the food industry, it is important to achieve LOQ values that are as low as is reasonably possible.

Here, we present a method for analyzing over 180 compounds used in the veterinary industry that canachieve LOQ values as low as 0.005 ng/mL.

PL4

High resolution mass spectrometry as an efficient tool in cannabis research

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OR20

Rheological and nutritional profile of spaghetti and bread fortified with hemp flours

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Summary: *The objective of this work was to study rheological and chemical qualities (fatty acids, total phenolic and amino acids content, antiradical capacity) in samples of spaghetti and bread made from "Ciclope" durum wheat semolina fortified with different percentages of hemp flours cv Futura 75. Hemp flours were sieved at 0.530 mm (Hemp -1) and 0.236 mm (Hemp -2).*

Keywords: food fortification, hemp flour, durum wheat cultivar, amino acids, fatty acids

Introduction

In recent years, the demands of food consumers have changed considerably. Food today is not only intended to satisfy hunger and provide the necessary nutrients, but also to prevent food-related diseases and improve the physical and mental well-being of consumers.
Functional foods are formulated to contain healthy components, which when consumed daily as part of the diet, can have beneficial health effects.
Cereal-based products, especially pasta and bread, are well suited for adding nutrients. Both bread and pasta, traditionally produced using durum wheat flour, can be prepared also using "non-wheat flours" in variable percentages or by incorporating food by-products, capable of increasing their nutritional value [1].
Unfortunately, fortification often affects the quality of cereal-based products, in terms of texture, color, cooking quality and sensory properties. Therefore, one of the main challenges of the food industry is to increase the healthiness of foods without sacrificing sensory attributes.
In this study the rheological and chemical qualities of spaghetti and bread samples were evaluated, obtained by using a durum wheat cultivar, called "Ciclope", fortified with different percentages of hemp flour, called "Futura 75". The hemp flours, obtained after grinding the seeds and the subsequent separation of the oil, were sieved at 0.530 mm (Hemp -1) and 0.236 mm (Hemp -2).

Experimental

In order to determine the total phenolic content (TPC) in samples, analysis was

performed using Folin-Ciocalteau reagent. The results were expressed in mg gallic acid equivalent/g.
DPPH method was used to test antiradical capacity. The results are expressed in Trolox equivalent antioxidant capacity (TEAC) as mmol/g and in IC50. Identification and quantification of fatty acids were carried out by GC-MS.
The amino acid analysis was performed after hydrolysis of the proteins with 6M HCl. Pre-column derivatization with FMOC-Cl (9-fluorenylmethylchloroformate) was required prior to amino acid analysis with HPLC-FLD.
The durum wheat Ciclope was chosen among the durum wheat varieties made up by CREA, Research Centre for Cereal and Industrial Crops of Acireale (Catania - Sicily). The dough mixing properties of the control and different mix were examined with the Brabender Farinograph, according to the constant flour weight procedure (AACC n° 54-21). According to the standard procedure, the following farinograph indices were determined: (1) water absorption of blend (WA), (2) development time of dough (DT), (3) stability of dough (S), and (4) the degree of softening of dough (DS). The alveographic test was used to analyze the effect of additions on the dough rheological behavior performed by Chopin alveograph (Chopin,Villeneuve La Garenne, France) according to the standard alveographic (UNI n° 10453 method). Each sample was analyzed in five repetitions and deformation energy W (strength) and P/L (tenacity/extensibility ratio) were calculated [2].

Results

Preliminarily, Ciclope durum wheat semolina, Hemp-1 and Hemp-2 flours were analyzed. As it was possible to predict, the total phenolic content was high for hemp flours, (but without substantial differences between the two flours, about 6.3 mg GAE/g); Ciclope semolina had a TPC of 2.4 mg GAE/g. These data were inevitably in accordance with the IC50 and TEAC results. From the point of view of fatty acids, Ciclope semolina has linoleic acid as the main one (59.9) followed by oleic and palmitic acid (about 17%) and by a small percentage of linolenic acid (3.65%). Regarding fatty acid composition, the two hemp flours did not show substantial differences. Linoleic acid is the main one (53.6%) followed by alpha linolenic acid (15.5%) and oleic acid (14.5%). Amino acids showed a particularly interesting profile. Ciclope semolina showed the presence of high concentrations of leucine, proline, isoleucine, tyrosine and glutamic acid. Hemp flours had significant differences. Higher was the amino acid content in Hemp-2 with isoleucine, proline, tyrosine and glutamic acid. As mentioned, using Ciclope semolina, bread samples were prepared with more degrees of fortification with the two hemp flours (0, 5, 7.5 and 10% replacement). Higher values of TPC and antiradical activity were obtained with 10% substitution with Hemp-1. The best fatty acid profile was observed at the 10% substitution, but there were no differences between the two hemp flours. Bread samples that had the highest concentration of amino acids were those obtained with 10% fortification with Hemp-2, reporting high values of proline, leucine and isoleucine.
Spaghetti fortified with hemp flour show higher values of TPC and antiradical activity when compared with samples with 0% fortification (CTRL). In order to determine the real nutrient content, the spaghetti were cooked. TPC and DPPH were determined on the cooked and lyophilized spaghetti. Also, in this case the

best results were observed in the samples fortified with Hemp-1 at 10% substitution. Of course, the comparison between raw and cooked spaghetti indicates a loss in cooking of some nutrients that varies from 10 to 50%. Higher fatty acid content was observed in spaghetti with 10% substitution, but there were no differences between the two hemp flours.
Comparison between CTRL spaghetti prepared with only Ciclope semolina and fortified spaghetti, showed a net increase in the amino acid content. In particular, spaghetti fortified with 10% Hemp-2, highlights an increased content of glutamic acid, tyrosine, proline, and essential amino acids such as leucine and isoleucine.
The results of the rheological characteristics show significant differences in the properties of the dough; the different percentages of substitution of wheat semolina with hemp flours induced significant differences both on alveographic and farinographic parameters. The W value, comparing the CTRL sample and the formulation containing 10 % of hemp flour has decreased by 10 % with Hemp-1, while about 15%, with Hemp-2. Regarding the P/L value, in all the replacement percentages, a slight increase was observed which allows to obtain a good balance between the toughness and extensibility of the mixtures.
Regarding farinographic parameters, the water absorption did not show significant differences, while the development time underwent a decrease, in particular at the 10% replacement with both types of hemp flour.

Conclusions

The enrichment of bread and spaghetti with hemp flours offers an effective method for the improvement of their biological value. The addition of hemp caused a significant increase in the content of phenols, PUFA, amino acids and their antioxidant activity, compared with the control products.
In particular, a higher concentration of amino acids in Hemp-2 emerges from this preliminary study, on the contrary TPC and anti-radical activity were similar. Naturally, their contribution in fortified foods (bread and spaghetti) was observed at the maximum percentage of substitution (10%). At this fortification value high TPC values and antiradical activity were shown both in bread and spaghetti fortified with Hemp-1.

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OR21

The challenging identification of isomers by HR-MS/MS: a case study from pre-cannabinoids

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Summary: *ESI-QqToF fragmentation patterns of pre-cannabinoids in negative ion mode, together with energy-resolved CID mass spectra, proved to give valuable information to achieving the discrimination among constitutional isomers. Workflow guidelines for a proper identification, aimed at getting new insight into industrial hemp-based products and by-products.*

Keywords: *industrial hemp, HR-MS/MS tools, pre-cannabinoids, isomer discrimination*

Introduction

In the last years, the food sector has been enriched with industrial hemp-based products and by-products (e.g. hemp seeds, oil, flour, and processed products therefrom), claimed as regard to their functionality. In this context, the rapid and unequivocal identification of cannabinoids therein, until now addressed mainly by GC-EI/MS or LC-ESI/MS in positive ion mode, is an issue to tackle [1,2]. Since pre-cannabinoids (acidic cannabinoids) constitute the large part of the cannabinoid profile of hemp-based food products, exploiting the acidity of their carboxylic groups, better suited to deprotonation in the ESI source, can lead systematic investigations, thus providing general rules for straightforward discrimination of constitutional isomers.

Experimental

Industrial hemp pollen was extracted by ultrasound assisted maceration (UAM) in hexane/$CHCl_3$ solution and chemically characterized by employing UHPLC-ESI-QqTOF techniques in negative ionization mode. Energy-resolved CID curves were also obtained, varying the collision energy in HRMS/MS experiments in the range 5−50 V (E_{Lab}).

Results

The extract obtained from industrial hemp pollen underwent UHPLC-HRMS/MS analysis and twenty pre-cannabinoids were tentatively identified (Fig. 1).
The deep study of HR tandem mass spectra, based on the occurrence and/or relative intensity of pivotal fragment ions, resulted in a complete rationalization of fragmentation patterns of the main olivetoid compounds. Moreover, in order to avoid misunderstanding, due to changes in experimental parameters affecting ion intensity, the integrated energy framework of their dissociation pathway was studied through energy-resolved CID experiments. It was found that precursor ion fragmentation up to 70-75% of the initial abundance provided the most useful information for discrimination purposes. In fact, key neutral losses (44, 86, 112, and 166 Da) were in accordance with THC-, CBL-, CBD- and CBC-type

skeletons, whose confirmation derived from the intensity ratio between dehydrated and decarboxylated ions. Thus, a general workflow was built up, and it was extended also to viridinoids (three carbon alkyl chain) and orcinoids (one carbon alkyl chain), and also to superior homologues. Finally, the applied systematic and rational approach led to the preliminary differentiation of other phytocannabinoids belonging to sub-classes different from the previously mentioned ones [3].

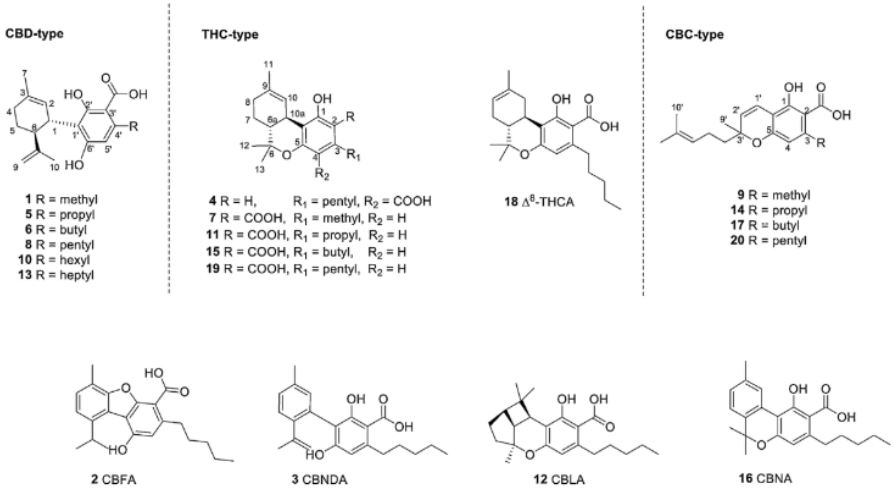

***Figure 1**. Structures of the pre-cannabinoids tentatively identified in the hemp pollen extract under study*

Conclusions

CBD-, THC- and CBC-type pre-cannabinoids were successfully discriminated in mixture by UHPLC-HRMS/MS tools. Guidelines for rapid identification were provided as part of the systematic investigation, exploitable also for quantitative purposes in MRM experiments. Moreover, the energy-resolved CID technique demonstrated that mass fragmentation involved mainly the molecular skeleton, independently from the alkyl chain, allowing us to apply key rules to all the detected homologues.

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OR22

Advancing MOSH/MOAH analysis towards speciation and contaminants identification

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Summary: *Two-dimensional comprehensive gas chromatography (GC×GC) with parallel FID/HRMS detection is investigated for advancing MOSH/MOAH analysis. The methodology is exploited to achieve more insightful and detailed characterization of fractions preliminarily separated by HPLC. This solution increases the capacity to classify the hydrocarbon profiles and achieve more confident identification of contamination sources.*

Keywords: *MOSH/MOAH analysis, speciation,* GC×GC

Food contamination attributed to mineral oil (MO), revealed by the presence of saturated (MOSH) and aromatic (MOAH) hydrocarbons in various food products, has been for years at the center of attention for its potential impact on consumers' health. Especially the MOAH compounds raise concern for increased risks due to their known toxicity and suspect carcinogenicity and genotoxicity, in particular for species with 3 or more aromatic rings and low alkylation degree.

Current methodologies, based on the LC-GC-FID hyphenation, allow individual quantification of the aliphatic and aromatic contaminant fractions. Nevertheless, the task is often challenging due to high matrix complexity and interferences. Moreover, FID detection does not permit to obtain qualitative information about the type of MOSH or MOAH present, the occurrence of synthetic hydrocarbons such as polyolefins (POH) and polyalphaolefins (PAO) or hydrocarbons of endogenous origin (terpenes, olefins residues, etc.).

Laboratories tasked with MOSH/MOAH analysis need access to advanced investigation tools for improved characterization of both fractions for samples positive to contamination.

This contribution presents the development and optimization of a platform for MOSH/MOAH analysis based on a preliminary HPLC separation followed by two-dimensional comprehensive gas chromatography (GC×GC) in combination with FID detection and high-resolution mass spectrometry. This solution significantly increases characterization capability and thus delivers a more detailed and insightful classification of the hydrocarbon profiles. A real-life case study highlights the value added for a confident identification of contamination origin in extra-virgin olive oil.

OR23

Autenticity and Fraud: regulatory and analytical point of view by IRMS and HRMS

Giancarlo Quaglia

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Honey is one of the most popular and marketable products in the world. Authenticity, healthiness and compliance of the product with the regulation must therefore be the priority of the entire production chain from producers to distributors.
Thanks to a long-standing activity on the national and international territory with the highest producers of honey and beehive products, Lifeanalytics organizes a highly qualified speech to deepen the subject both from a regulatory and an analytical point of view.

PL5

Chemistry and analysis of chlorogenic acids from coffee

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Chlorogenic acids (GGAs) are ubiquitous phenolic plant secondary metabolites, abundant in coffee. By definition CGAs are hydroxycinnamoyl esters of quinic acid thus existing in nature as sets of regioisomeric compounds.
Most plants including coffee produce sets of multiple isomers eg all six isomers if dicaffeoyl quinic acid.[1] Using a variety of isomer sensitive mass spectrometry methods including tandem mass spectrometry, ion mobility mass spectrometry or energy resolved mass spectrometry we could introduce methods that not only distinguish CGA isomers, but also provide methods for unambiguous structure elucidation.[2] In coffee roasting the 45 CGA derivatives in a green arabica coffee bean are converted to an estimated 200 new CGA derivatives. Again, these can be identified using tandem mass spectrometry approaches.
Finally, I complement my presentation by sharing some interesting findings on general CGA profiles from the world of coffee, [3] including some latest findings on CGA biological activity, including promise to reduce Covid-19 infections.[4]

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OR24

Identification and quantification of sinapoylquinic acid isomers in green coffee (*Coffea arabica* L. and *C. canephora* Pierre ex Froehner) extracts

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Summary: *The focus of this paper is to quantify sinapoylquinic acid isomers (SiQA) in green coffee extracts (Coffea arabica and Coffea canephora) using an extract of Gardenia Fructus as a surrogate standard. The quantification of 3-SiQA and 4-SiQA in addition to 5-SiQA in Arabica coffee is reported for the first time.*

Keywords: *sinapoylquinic acids, coffee, UHPLC-ESI-MS/MS*

Introduction

Chlorogenic acids (CGAs) are a large class of esters formed between quinic acid and hydroxycinnamic acids. They are present in coffee as a complex mixture of positional and geometric isomers, where caffeoylquinic acids (CQA) are the most abundant, followed by dicaffeoylquinic acids (diCQA) and feruloylquinic acids (FQA) [1]. Sinapoylquinic acids (SiQA) are the most widely distributed of the less common chlorogenic acids, being reported in Gentianales [2] (the order with the greatest range), Aquifoliales, Asterales, Caprifoliales, and Solanales. The use of a Gardenia fructus extract analyzed by LC-MS/MS allows the fingerprinting of this specific class of compounds and this extract can be used as qualitative standard. After optimization of a LC-MS/MS method for identification and quantification of SiQA, different matrices were analyzed, and the presence of these compounds was confirmed in Robusta coffee. In Arabica coffee, in addition to 5-SiQA isomer, previously identified and quantified [3], the 3- and 4- isomers are quantified for the first time.

Experimental

Gardenia Jasminoides fruits (zhi zi) extract was purchased from Qiu Tian Srl (Acquaviva, San Marino Rep.), Robusta lyophilized coffee extracts were prepared in our lab from an aqueous ethanolic extract, Arabica commercial extract was purchased from Natural Origin® (Lozanne, France). Ethanol, methanol, acetonitrile, sinapic acid and formic acid were purchased from Merck. Chlorogenic acid (5-caffeoylquinic acid) was purchased from Phytolab (Germany). Sample were extracted with water/ethanol (30/70 v/v) for 30 min at 60°C in an ultrasound cleaning bath Sonorex RK100 (Bandelin), 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 (Allegra 64R Centrifuge, Beckman Coulter, Indianapolis, IN, USA) and filtered with regenerated cellulose (RC) membrane 0.20 µm (Reliaprep Ahlstrom-Munksjö Oyj, Helsinki, Finland) and diluted with water if needed. LC-MS analysis were performed on a Agilent 1290 system coupled to a Sciex triple quad 4500. Chromatographic separation

was achieved with an Acquity BEH C18 column (Waters) using an elution gradient of aqueous formic acid 0.1% v/v (A) and acetonitrile (B) at a flow rate of 400 ml/min with the following elution program: 0 min 95% A, 5% B; 8 min 85% A, 15% B; 13 min 60% A, 40% B; 14,90 min 60%A, 100%B; 15 min 0% A, 100% B; 17 min 0% A, 100% B; 17,5 min 95% A, 5% B. The column was set at 40°C and injection volume was 4 µl.
MS was operating in negative mode, ESI source set at 350°C, best operating conditions and MRM transitions were optimized with infusion of 5-caffeoylquinic acid solution as previously reported [4].

Results

All data for chlorogenic acids presented in this manuscript use the recommended IUPAC numbering system. Generally, peak assignments have been made on the basis of the structure-diagnostic hierarchical keys published in literature [5], supported by examination of the UV spectrum and retention time relative to 5-caffeoylquinic acid. The identification method is a Multiple Reaction Monitoring mode (MRM) with specific transitions of SiQAs: with negative ionization the parent ion is m/z 397, and the base peak of the three isomers are m/z 223 for 3-SiQA, m/z 191 for 5-SiQA and m/z 173 for 4-SiQA. Quantitative analysis was performed and results expressed as sinapic acid equivalent. The method was validated in terms of specificity, linearity, concentration range, limit of detection (LOD), limit of quantification (LOQ) accuracy and repeatability according to the criteria specified in EU Commission Decision 2002/675/EC.
The validated method allowed to quantitate SiQAs in different samples: food supplement containing Zhi zi and coffee (both Arabica and Robusta). As far as we know this is the first time that 3- and 4-sinapoyl quinic acid isomers are quantified in green *Coffea arabica*. To our knowledge, as far as sinapoyl derivatives are concerned, only 5-sinapoylquinic acid was quantified and one caffeoyl-sinapoylquinic acid isomer was detected in Arabica green coffee extract [3,6]. Further studies are needed to elucidate the role played by sinapoyl derivatives as possible phytochemical markers for differentiation between Arabica and Robusta green coffee.

Conclusions

In conclusion we proved that the use of a matrix particularly rich in secondary metabolites of interest as a standard for both identification and LC-MS/MS method optimization is an effective and cheap strategy to fulfil characterization of those secondary metabolites in other different matrices.
Once the identification of the synapoylquinic acid isomers in the studied samples was performed, we unexpectedly observed that these isomers are all present in amount >LOQ in both Arabica and Robusta green coffee. A quantification method for these compounds was validated.
We strongly believe that the presently adopted approach to identify new compounds in a coffee matrix is particularly useful and effective and it can help research team to improve and to widen the characterization of minor compounds, especially when already described in literature, without the need of time-consuming organic synthesis or expensive commercial standards purchase (if possible).

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OR25

Phenotyping Green and Roasted Beans of Nicaraguan Coffea Arabica Varieties Processed with Different Post-Harvest Practices

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Summary: *We propose the 1H-NMR-metabolomic characterization of seven different Arabica varieties of green and roasted coffee beans to determine the farm of origin looking at the same restricted geographical area. We also evaluate on the same batches, the effect of post-harvest procedures on the coffee metabolomic profile, identifying some aroma precursors.*

Keywords: *metabolomics; nuclear magnetic resonance spectroscopy; coffee*

Introduction

Green coffee beans are one of the most traded commodities, and coffee is the most consumed beverage after water [1]. Its popularity is due to the attractive organoleptic and energetic characteristics of coffee [2]. The quality of coffee principally derives from the grade of green coffee beans that are influenced by several factors, including genetics, geographic localization, altitude of the plantation, climate, agricultural and postharvest processing factors [3,4]. Moreover, the different processing techniques of coffee beans can impact the final product influencing the organoleptic properties and the quality of the final product [5], which can be described also by the presence and the concentration of certain metabolites (small molecules < 1500 Da) in coffee beans [6]. These differences in metabolites can serve as indicators of coffee quality and metabolites can be potentially used to direct the agronomic and post-harvest procedures to a high-quality grade final product [7]. Metabolomic techniques have already been used to characterize two of the most common coffee species, *C. arabica* and *C. canephora*, but no studies have focused on the characterization of green and roasted coffee varieties of a certain species.

Experimental

Here, 1H-NMR-based metabolomics is applied to characterize seven different coffee (green and roasted) varieties of the same species (*C. arabica*) and the same cultivation type (Bourbon-Typica) localized within the same geographic

area of Nicaragua. For each variety, two points of fermentation time (12 h vs. 24 h) and two types of drying procedures (under shade and direct sun) have been considered to evaluate how they differently react to the same post-harvest procedure. We also evaluated how they react to the same roasting time and temperature. The experimental design (Fig. 1) also allowed us to evaluate the differences between the same varieties grown by different farms located within the same territory.

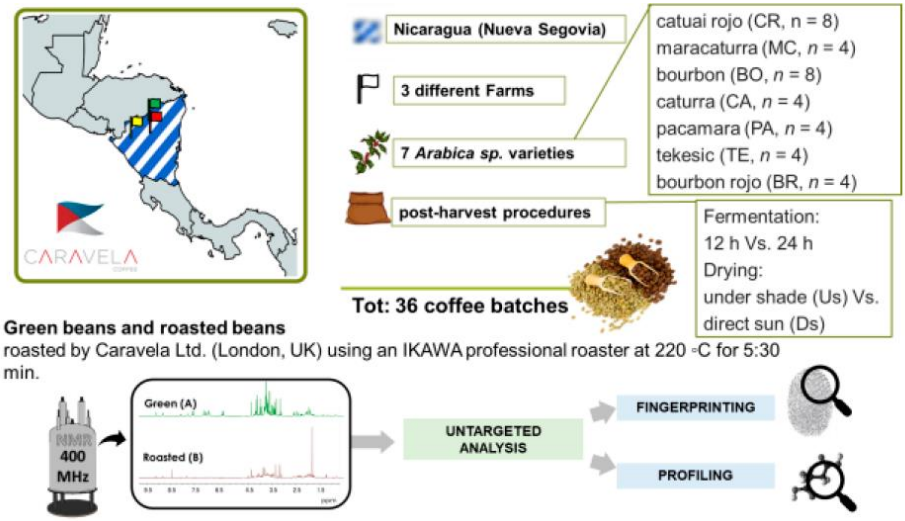

Figure 1. *Experimental scheme*

Results

The results demonstrated that NMR spectra of both green and roasted coffee beans can be used as fingerprint to recognise coffee varieties with high accuracies (87.2% and 86% using, respectively green and roasted NMR spectra to build the model). Moreover, it was also possible to characterize, the metabolomic profile of the distinct coffee farms cultivating the same varieties within the same restricted geographical area of Nicaragua. This demonstrate that, even when coffee batches are processed following the same post-harvest procedure, the characteristic fingerprint of each farm can be derived with high predictive accuracies (>90%). Moreover, we detected changes in the metabolomic profile of coffees undergoing different post-harvest procedures, such as the different fermentation times, which are responsible for different flavours in the cup. This demonstrate that post-harvest treatment procedures can differently affect the amount of aroma precursors within distinct coffee varieties and that the kind of processing should be optimized specifically for each variety.

Conclusions

This study provides proof of concept for the ability of NMR to phenotype coffee, helping to authenticate and optimise the best way of processing coffee.

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OR26

Quantification of glyphosate in milled and brown rice in LC-ICP-MS/MS

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Summary: *We developed a new method for the quantification of Gliphosate in rice by coupling an Agilent bio-inert 1260 HPLC system with an Agilent 8900 QQQ ICP MS/MS System. The result is a very sensitive and selective method with a minimum sample preparation; LOQ are lowed than the EU community limits and this methos can be extended to other food matrix*

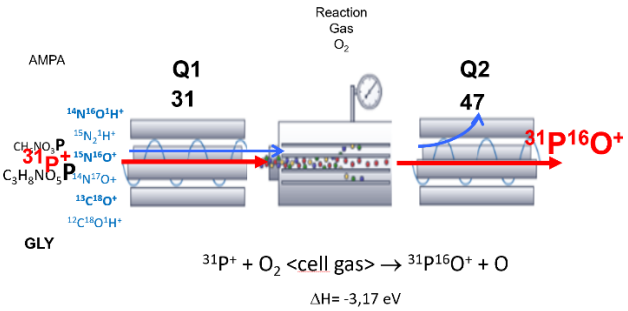
Keywords: *LC-ICP-MS/MS – Gliphosate - Rice*

Introduction

Gliphosate (GLY) is a potential carcinogenic pesticide regulated by UE regulation 293/213 in food and in particular in rice with a limit of 0.1 mg/kg considered the actual instrumental LOQ. A new method developed coupling an Agilent bio-inert 1260 HPLC system with an Agilent 8900 QQQ ICP MS/MS System is able to improve of classical LC-MS method LOQ with a simplified sample preparation.

Experimental

ICP-MS/MS is a very selective and sensitive detector for phosphorus, a controlled reaction with oxygen can remove all the potential on mass polyatomic interferents.



Rice samples prepared by extraction and after filtration is introduced in a coupled LC-ICP-MS/MS system, different instrumental set-up has been tested on milled and brown rice

Results

Method shows a very good linearity and reproducibility with excellent LOQ both in milled and brown rice with a good recovery even on very low spike amount.

Product	Spiked level (gly mg/kg)	Recovery (%)	Standard deviation (SD)	RSD (%)
Milled Rice	0,01	76	8	11
	0,03	90	6	6

LOD
2,7 µg kg-1
LOQ
16 µg kg-1

Product	Spiked level (gly mg/kg)	Recovery (%)	Standard deviation (SD)	RSD (%)
Brown Rice	0,05	94	8,3	8,8
	0,14	99	1,3	1,4
	0,27	97	2,6	2,7

LOD
5,5 µg kg-1
LOQ
45 µg kg-1

Conclusion

This work isa proof that actual triple quadrupole ICP-MS-MS system can be extended to organic method when coupled to HPLC system, with the advantage of a simplified sample preparation and an improvement of LOQ.
This method can be implemented to other food matrix and to other organic pollutant containing elements like phosphorus and/or sulphur.

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OR27

On-line mass spectrometry-based high-throughput analysis of volatile aging markers in long-life milk

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Summary: *High-throughput analysis of ultra-high temperature (UHT), or long-life, milk was performed with on-line mass spectrometry in an accelerated shelf-life (ASL) study to explore potential volatile markers related to milk aging during storage. The ketone 2-heptanone was found to be a prospective aging marker in semi-skimmed and whole milk.*

Keywords: *PTR-TOF-MS, Headspace analysis, Autosampler*

Introduction

Use-by dates for ultra-high temperature (UHT) treated milk, also termed long-life milk, are assigned based on evidential data of representative samples, thus represent general estimates that are not necessarily applicable to any individual product. In the case of long-life milk, these estimates are generally quite conservative, since the stability of these milk products typically extends well beyond this designated shelf-life. This situation leads to unnecessary food waste, as consumers have a tendency to rely on use-by dates printed on packaging and dispose of foods that have exceeded this date, rather than use their sensory faculties to assess the product for edibility and the need to discard it. To overcome this problem, there is a pressing need to better understand aging and spoilage mechanisms and to introduce measures for more accurate shelf-life assessments and use-by date assignments.

In the present study, the stability of long-life milk during storage was investigated via an accelerated shelf-life (ASL) test regime. On-line mass spectrometry in the form of proton transfer reaction-time-of-flight-mass spectrometry (PTR-TOF-MS) was employed to analyse and seek volatile aging markers in the headspace of milk samples of different fat content. An automated sampling system (autosampler) was used to enable high-throughput analyses of aliquot milk samples on different days of storage.

Experimental

UHT milk samples with different fat content (0.1 %, 1.5 % and 3.5 %) were subjected to storage at 20 °C, 30 °C and 40 °C for adjusted periods according to the theory on accelerated shelf-life regimes. Specifically, it is expected that the aging rate of the milk doubles for a 10 °C increase in storage temperature or, in other words, the corresponding shelf-life is halved. Using this assumption, as well as a generally assigned 150- day shelf-life for room temperature storage, milk samples were stored in a staggered process such that all sample groups were expected to reach their end-of-shelf-life on the same day. Samples were taken out of storage on specific days towards and beyond the end-of-shelf-life

for analysis.
The detection of constituent volatile organic compounds (VOCs) in the milk samples was made via headspace analyses of milk aliquots, with triplicate analysis per sample. Chemical analysis was performed using a PTR-TOF-MS instrument (PTR-TOF 8000, IONICON Analytik GmbH, Innsbruck, Austria) coupled to an autosampler. Complementary VOC analyses were made by gas chromatography-ion mobility spectrometry (GC-IMS) using a FlavourSpec system (G.A.S. Dortmund mbH, Dortmund, Germany). Further, a trained sensory panel evaluated the milks samples at different times during ASL storage according to appearance and odour using the check-all-that-apply (CATA) approach.

Results
The PTR-TOF-MS datasets identified a series of ketones to relate quantitatively to the duration and temperature of storage. Specifically, 2-heptanone in the headspace of the milk samples was observed to increase linearly with storage period and this increase occurred at a higher rate in samples stored at higher temperature. While these observations were made in the 1.5 % and 3.5 % fat milk samples, the concentration of 2-heptanone in the 0.1 % milk samples was low across all samples, irrespective of storage period or temperature, indicating that the presence of fat plays a role in its production. Using 2-heptanone as a marker, aging acceleration factors for the milk samples according to ASL conditions were calculated, as listed in Table 1. According to these values, a storage temperature elevation of 10 °C led to an accelerated aging of the milk by a factor of 5 in both the 1.5 % and 3.5 % fat content samples. The GC-IMS data yielded similar observations and acceleration factors based on this marker compound. Sensory evaluations indicated that sensory defects in milk samples held at 40 °C were predominantly associated with temperature-induced chemical changes, e.g., Maillard reaction products, suggesting that this ASL storage temperature is less suitable for aging studies.

Table 1. *Aging acceleration factors calculated from the relative changes of 2-heptanone as a representative volatile spoilage marker of long-life milk; note that no changes were observed in the skimmed milk (0.1 % fat content).*

Fat content	Aging factor at 20 °C	Aging factor at 30 °C	Aging factor at 40 °C
0.1 %	1.0	n/a	n/a
1.5 %	1.0	5.2	25.1
3.5 %	1.0	5.0	23.8

Conclusions
PTR-MS is a well-established technology for the analysis of VOCs, or aroma compounds, in food science [1, 2], including markers relating to sensory defects or spoilage of milk [3, 4], with its viability for high-throughput analysis in related applications being previously demonstrated [5]. This work presents a novel

experimental design for accelerated shelf-life tests and aging assessments of long-life milk via high-throughput, on-line mass spectrometry analysis. A key finding of this study was the identification of 2-heptanone to be linked to shelf life and storage temperature, indicating its potential as a suitable marker to quantify aging effects in long-life milk. Based on the quantitative changes in 2-heptanone across the samples, an acceleration factor of 5 was estimated for a 10 °C increase in storage temperature. A secondary finding of this study was that ASL storage at 40 °C is deemed less suitable for studying aging effects, as the (sensory) changes observed were indicative of thermally-induced effects. This work offers the basis for further studies on accelerated shelf life tests and use-by date predictions and highlights the utility of on-line mass spectrometry for high-throughput analyses.

Acknowledgements
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OR28

Understanding the generation of volatile organic compounds by yeast during beer fermentation

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Summary: *The goal of this research is to gain a more comprehensive understanding of the biotransformation reactions responsible for hop flavour development in beer and hence be able to better predict how hop additions will impact on the aroma of finished beer.*

Keywords: *Hop terpenes, biotransformation, beer flavour*

Introduction

Beer flavour is impacted, in part, by hop-derived volatiles and their transformation products [1]. To meet consumer demand for hop-flavour driven beers, there is increasing interest controlling, optimising and predicting hop flavour generation during fermentation. Somewhat surprisingly, many of the hop aroma compounds, such as terpenes, noticeable in beer are not detected in raw hops owing to the transformations they undergo during fermentation [2]. There is, however, little understanding of the nature of hop biotransformation due to yeast metabolism during fermentation. The volatile organic compounds (VOCs) produced due to yeast biotransformation during fermentation were initially assessed off-line using SPME-GC-MS. This was followed by an experiment involving on-line PTR-ToF-MS dynamic headspace measurements to gain insights into the complex interactions that occur in the VOC profile over time.

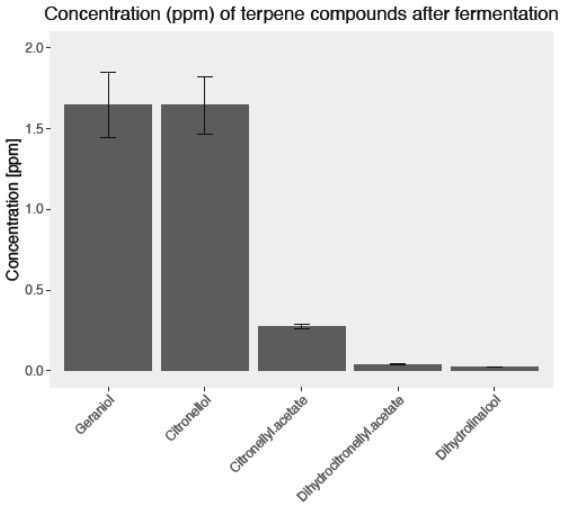
Method and materials

Geraniol (10 ppm) was added into a model wort SafAle US-05 (*S. cerevisiae*; Fermentis yeast), the VOCs present after fermentation were analysed using SPME-GC-MS. The VOCs produced by two *S. cerevisiae* strains (US-05 and WB-06; Fermentis) and two *S. pastorianus* strains (W-34/70 and S-23; Fermentis) in a model wort throughout fermentation (5 days at 20oC) were assessed using PTR-ToF-MS.

Results

Terpenes were not detected in wort with only yeast (blank). Only the terpene that was added to each sample was detected in the uninoculated wort (absence of yeast) after a 5-day incubation, thereby confirming that yeast do not produce measurable terpene compounds endogenously during fermentation and that the terpenes are unable to spontaneously transform. In contrast, in the presence of yeast, a range of terpenes were found in the wort at the end of fermentation, despite only a single terpene being added initially, thereby demonstrating that biotransformation of the spiked terpene was occurring. Fig. 1 displays the concentration of compounds at the end of fermentation by SafAle US-05 (*S.*

cerevisiae) when only geraniol (10 ppm) had been added to the wort. There was a decrease in the concentration of geraniol (to 1.64 ppm) and dihydrolinalool, dihydrocitronellyl acetate, citronellyl acetate and citronellol were detected at concentration of 0.02, 0.04, 0.27 and 1.6 ppm respectively. A further trial which compared the dynamic biotransformation (s)of geraniol by 4 yeast strains during fermentation using PTR-ToF-MS was conducted. The scores plot (Fig. 2), which reflects the analysis of the whole database, shows that the difference between samples was primarily due to yeast strain, confirming that yeast metabolism plays a fundamental role in the volatile composition of beer.
The corresponding loadings plot (Fig. 1b) provides information about the volatile profile (Fig. 2).



Compound	Concentration (ppm)
Geraniol	1.64 ± 0.20
Citronellol	1.64 ± 0.18
Citronellyl acetate	0.62 ± 0.01
Dihydrocitronellyl	0.04 ± 0.00
Dihydrolinalool	0.02 ± 0.00

***Figure 1**. Biotransformation of (10 ppm) geraniol by SafAle US-05 (S. cerevisiae) yeast. Samples were measured after 5-days incubation at 20°C using SPME GC-MS. The mean concentration of the spiked compound and the terpenes produced are displayed. The error bars represent the standard deviation of three replicates*

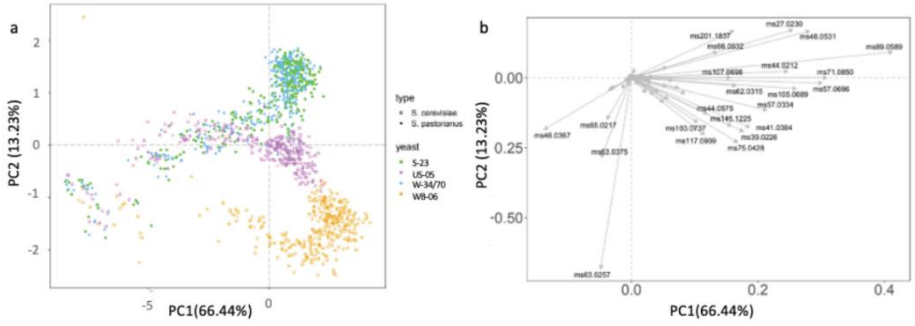


***Figure 2**. Score plot of the principal component analysis (PCA) of volatile organic compound (VOC) (a) and loading plot of PCA of the mass peaks (ms) (b) for yeast: S. cerevisiae (US-05 and WB-06) and S. pastorianus (W-34/70 and S-23). Samples were measured every 6 hours over a 5-day incubation at 20°C using PTR-ToF-MS*

Discussion

The biotransformation of citronellol from geraniol by both ale and lager yeast strains has previously been reported [3-5]. Novel results from the current study were the detection of citronellyl acetate, dihydrocitronellyl acetate and dihydrolinalool after fermentation, highlighting that multiple pathways from terpene biotransformation were occurring. Hops contain very low concentrations of citronellol, which is described as having a rose leaf and oily petal aroma [4] and beers which contains a higher concentration of oxygenated terpenes such as citronellol, linalool, α-terpineol are generally preferred [5]. The development of desirable hop flavour in beer can be maximised by selecting hops with a high concentration of the required terpenes and yeast which have a good ability to biotransform them. The study of many other peaks detected by PTR-ToFMS during fermentation is ongoing. This evidence that the development of hop VOCs in beer is strongly influenced by yeast species will help brewers to design the yeast / hop combinations required to achieve the flavours they wish to highlight in the beer they produce.

Conclusions

The fermentation of wort containing a single terpene resulted in the production of range of terpenes thereby demonstrating the role yeast play in their biotransformation. The terpenes produced will influence the aroma of beer and lead to the production of unique volatile profiles. SPME-GC/MS provided an accurate identification of volatile compounds, while PTR-ToF-MS successfully followed the changes in VOCs profiles in real-time during fermentation. The ability to monitor changes in VOCs during fermentation provides valuable information on the mechanism of production and biotransformation reactions by yeasts, thus supporting brewing science and technology. In addition, such research provides brewers with the knowledge required to control and optimise beer aroma to meet consumer preferences.

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OR29

Green analytical approach meets sustainable food processing: PTR-ToF-MS applications for VOCs monitoring during food fermentation

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Summary: *Fermentation represents a driver of sustainable innovation in food systems. VOCs are microbial metabolites of interest for bioprocess monitoring, also influencing food's sensory perception. PTR-TOF-MS is a versatile, efficient and green analytical tool for volatilome profiling. Here we report case studies showing the potential of PTR-TOF-MS in the study of food fermentation.*

Keywords: *Volatile Organic Compounds (VOCs); food fermentation; proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF-MS)*

Introduction

The term fermentation summarises a 'family' of microbial-based processes that are commonly considered sustainable drivers of innovation in food systems from different points of view: social (e.g. food safety, food security, wellness, cultural heritage), environmental (e.g. biodiversity preservation, energy and resources saving) and economic (e.g. improved added value, food sovereignty, decrease food waste) [1]. Fermented goods can be described as "foods made through desired microbial growth and enzymatic conversions of food components" [2]. Important variables deservedly to be explored are the different raw materials and the numerous and different genera/species/strains of bacteria, yeasts, and filamentous fungi that intervene in the different fermentation processes, leading to a wide range of diverse fermentation metabolites produced [3]. This contributes to explaining the fermentative process's potential in modulating the final product's global quality and the relevance of fermented foods in the human diet. Volatile organic compounds (VOCs) in fermented foods can also be associated with microbial metabolism and are also called mVOCs (microbial volatile organic compounds) [4]. Hence, by analysing the volatilome in fermented foods and beverages, we might have polyphasic information on the quality of the matrices (including data on VOCs of non-microbial origins) and obtain the monitoring of microbial-based bioprocesses (mVOCs profiling) [5]. Direct injection mass spectrometry (DIMS) techniques, direct injection coupled with high sensitivity, robustness and resolution of modern mass spectrometry approaches allowed interesting applications to study VOCs profiling online, allowing the evolution of phenomena in the time [6]. Within this context, proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF-MS) offers a good compromise, optimising ionisation parameters and operational simplicity [4,6]. In

reason of specific characteristics (e.g. non-invasive/destructive analysis, low amount of sample needed, no toxic reagents needed, no toxic analytical waste produced), PTR-MS proved compliance with the guidelines of Green Analytical Chemistry. In addition, we designed a procedure centred on coupling PTR-ToF-MS with an autosampler and tailored data analysis implements, to enhance the degree of automation [7]. Here, we report a list of case studies to focus on the potential of the technique, highlighting the strengths and limitations of the proposed approach. In particular, we point out the versatility of PTR-ToF-MS to boost research and development activities in the sector, underling how the low-impact attributes of the analytical approach are coherent with the sustainable character of the fermentative processes.

Experimental

VOCs released during fermentation in the different case studies were constantly measured by directly linking the sample's headspace to the PTR-MS instrument's drift tube, where ionisation occurs. Experiments were achieved with a PTR-TOF 8000 apparatus (Ionicon Analytik GmbH, Innsbruck, Austria). Measurements were automated by using multipurpose gas chromatography (GC) automatic sampler (Autosampler, Gerstel GmbH, Mulheim an der Ruhr, Germany). A gas calibration unit (GCU, Ionicon Analytik GmbH, Innsbruck, Austria) was used to generate zero air for flushing sample headspace. A multigas controller (MKS Instruments, Inc) was employed to monitor the argon flow rate (in case of high ethanol contents during the fermentation process, an argon dilution system was applied to minimise primary ion depletion and ethanol cluster formation. For all the instrumental parameters, please refer to the methodological work by *Capozzi et al*. [7] and the cited literature for the complete procedures followed for the experimental samples preparation.

Results

In this contribution, after an overall outline of the DIMS application in fermented foods and beverages, we propose an in-depth overview of the applications of PTR-MS studies on the online monitoring of fermentative bioprocesses of food interest (Table 1).

The results show how versatile this green technique is, online monitoring VOCs associated with the *i)* different categories of fermented foods and beverages (i.e. bread, dairy, plant-based dairy-like and alcoholic beverages) and *ii)* the two principal categories of 'virtuous' eukaryotic and prokaryotic microorganisms, yeast and lactic acid bacteria, respectively. Evidence shows that PTR-TOF-MS can be a powerful tool for discriminating and evaluating protechnological 'microbiodiversity' in food contexts, allowing the definition of markers for rapid selection and strategies for massive screening. In addition, the findings indicate how the instrumental approach can be interestingly applied in evaluating the connections of microorganisms with different raw materials and the interaction among diverse 'virtous' microbes during food fermentations.

***Table 1**. A non-exhaustive list of PTR-TOF-MS studies for detecting VOCs in fermented foods and beverages. Lactic fermentation (LF), alcoholic fermentation (AF), malolactic fermentation (MLF). Lactic acid bacteria (LAB), Yeasts/Y)*

Fermented product	**Type of fermentation/ microbes**	**Variable studied**	**Ref**
Bread	AF/Y	Different commercial starter cultures	[8]
Yoghurt	LF/LAB	Different commercial starter cultures	[9]
Bread	AF/Y	impact of flour, yeast and their interaction	[10]
Wine	MLF/LAB	Wine from different origins, different starter cultures	[11]
Beer	AF/Y	Effect of diverse hops during the brewing process	[12]
Wine	AF/Y	Inoculation of mixed starter culture	[13]
Kefir	AF-LF/Y-LAB	Backslopping/complex cultures and LAB strain to improve vitamin B2 content	[14]
Kefir-like cereal based	AF-LF/Y-LAB	Backslopping/complex cultures and LAB strain to improve vitamin B2 content	[14]

Conclusions

PTR-TOF-MS can represent a valuable tool for speeding up and optimising research and development in the field of microbial resource management to improve the quality of fermented products. Coupling green analytical techniques and low-impact microbial-based innovations in the field of fermented foods and beverages, we propose new strategies to pursue sustainable transition in food systems.

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OR30

RADIAN™ASAP: Ambient Mass Spectrometry for food authenticity and adulteration

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The emergence of ambient ionization techniques and their combination with smaller, cheaper mass spectrometers is beginning to make real the possibility of mass spectrometry measurements being made routinely outside of traditional laboratory settings.
Many of the methods currently employed within the food manufacturing industry for quality control purposes are based on spectroscopic techniques. By comparison, direct Mass Spectrometry (MS) is a relatively new technique which has proven to offer comparable speed and ease-of-use, alongside the advantages of higher selectivity, sensitivity, and diagnostic chemical information. Numerous MS based methods have been explored for food authenticity analysis based either on the target detection of adulteration markers or the development of multivariate classification models.
The aim of this study was to evaluate the performance of the RADIAN ASAP in combination with LiveID for chemometric modelling and subsequent real-time quality control testing. Different case studies were presented providing an overview of the chemical profile of each sample generated using the RADIAN ASAP. The species diagnostic region of the mass spectral profile was used to generate a multivariate model using the PCA/LDA algorithm in LiveID. The predictive accuracy of the binary model was 100% *via* independent validation.
Finally, the model was then used for the real-time classification of a set of challenge samples.

OR31

Characterization of phenolic and aromatic profiles of Radler beers by HPLC-ESI-MS/MS and GC-MS techniques

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Summary: *Targeted and untargeted metabolic profiles of commercial Radler beers were investigated by HPLC-ESI-MS/MS and GC-MS, for obtaining evidences of their differences and quality characteristics. The addition of lemon juice to the classical beer improves the content of polyphenolic compounds, that are always present in beer along with volatile aroma compounds.*

Keywords: *Polyphenols, HPLC-ESI-MS/MS, GC-MS*

Introduction

Beer, one of the most consumed alcoholic beverage in the world, is a complex mixture of various nutrients like carbohydrates, amino acids, minerals and vitamins. Beer flavor and aroma depends on volatile and non-volatile compounds coming from different chemical classes and deriving from barley malts, hops and yeasts used during the brewing process. Health benefits of the beer are ascribed to the presence of chemical compounds such as polyphenols, especially hydroxybenzoic acids. These compounds have health beneficial effects thanks to their good antioxidant activity, but they are important also from a technological point of view, because they are involved in foam maintenance, physical and chemical stability and shelf life and they are considered as quality indicators for beer processing [1-3].

The radler beers, low-alcohol content drinks, are made by mixing beer with lemon juice. This kind of beer is very popular thanks to the rich fruity flavor, to the refreshing properties and also to the low alcohol content. The simultaneous effect of the low alcohol content and the addition of juices rich in bioactive compounds like flavonoids makes the radler beer a valuable and very attractive beverage.

Experimental

Two commercial Italian radlers (R1, R2), both lager made with Italian malts and lemon juice, one Italian beer (B), and one Italian lemonade (L), a soft drink made with Italian lemon, were analyzed by high performance liquid chromatography (HPLC), coupled with tandem mass spectrometry (MS/MS), with an electrospray ionization source (ESI) acquiring in selected ion recording (SIR) mode, using a method previously developed, [1-2], for the detection and quantification of 14 compounds and herein slightly modified to include 12 other compounds among hydroxybenzoic acids, hydroxycinnamic acids, caffeoylquinic esters, flavonoids and prenylflavonoids. The volatile fraction was analysed by GC-MS. Compounds were identified by comparison of mass spectra with NIST libraries.

Results

HPLC-ESI-MS/MS analysis in SIR mode, by using the improved and validated

method [4], provided different phenolic profiles for R1 and R2. Among the searched 26 compounds, 20 were identified in at least one sample and quantitated in most cases. The total content of the phenolic compounds of B, R1, R2 and L were summarized for classes, and reported in Fig. 1. Radlers had a higher amount of antioxidants respect to beer, with high level of hesperidin. The GC-MS untargeted analysis of the volatile fraction profile of R1, R2, B and L, evidenced 23 peaks tentatively assigned by comparison of the fragmentation spectra with NIST libraries (Table 1). Most of the compounds were found in the volatile profile of R1 and R2 where D-limonene was the dominant peak.

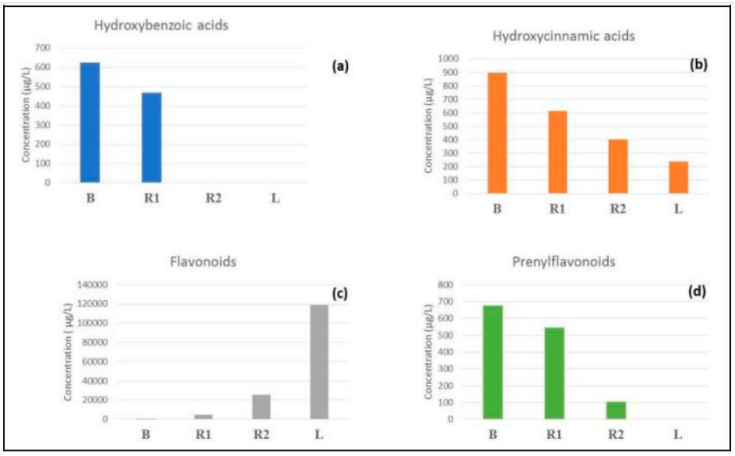

***Figure 1**. Phenolic content in the analyzed beverages (B, R1, R2 and L), assembled as total hydroxybenzoic acids (a), hydroxycinnamic acids (b), flavonoids (c) and prenylflavonoids (d)*

***Table 1** Compounds tentatively identified in the volatile fraction of B, R1, R2 and L, by GC-MS analysis and comparision of fragmentation spectra with NIST libraries.*

n.	Compound	tR (min)	B	R1	R2	L
1	heptane	9.48	x	x	x	x
2	isoamyl alchohol	10.71	x	x	x	-
3	diacetone alchohol	15.85	-	x	x	x
4	*m-xylene*	17.94	x	x	x	x
5	isoamyl acetate	18.22	x	x	x	-
6	Isoamyl n-eptanoate	18.34	x	x	x	-
7	ethyl caproate	23.60	x	x	x	-
8	α-pinene	23.63	-	-	-	x
9	isocineole	24.55	-	x	x	x
10	β-cimene	24.85	-	x	x	x
11	D-limonene	25.25	-	x	x	x
12	γ-terpinene	26.41	-	x	x	x
13	(+)-4-carene	27.67	-	x	x	x
14	N-hydroxymethyl-2-phenylacetamide	27.87	x	-	-	-
15	β-fenchol	28.53	-	x	x	x

Table 1. contd.

16	4-amino-1-pentanol	28.64	x	-	-	-
17	neodihydro carveol	29.66	-	x	x	x
18	cosmene	30.47	-	-	-	x
19	vinyl-o-xylene	31.05	-	-	x	x
20	terpinen-4-ol	31.15	-	x	x	x
21	α-terpineol	31.58	-	x	x	x
22	ethyl caprylate	31.78	x	x	x	-
23	phenylethyl acetate	33.78	x	x	x	-

Conclusions

The improved method was confirmed suitable for fast analysis of radlers, beer and the lemonade. R1 and R2 showed different phenolic profiles; R1 and B resulted not significantly different ($p<0.05$) for the most of the identified phenolic compounds. High levels of hesperidin, typical citrus fruits flavonoid and generally not present in beer, were found in R1 and R2, besides L. The analysis of the untargeted metabolic profile of the volatile fraction showed a strong effect of the lemon aromas on the radlers.

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OR32

HRMS profiling of grape glycosidic aroma precursors finalized to selection of Glera crossings resistant to the main vine diseases and suitable for Prosecco wine production

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Summary: *The environmental sustainability of Prosecco production may be achieved by creating a new resistant Glera variety while maintaining the aromatic characteristics as much as possible. The profile of glycosidic terpene precursors of Glera tonda and Glera tonda×resistent vitis sp. (Solaris, Bronner, and Kunleany) was performed by high-resolution mass spectrometry LC/QTOF.*

Keywords: *Glera tonda×resistent vitis sp., glycosidic terpene precursors, high-resolution mass spectrometry LC/QTOF.*

Introduction

Prosecco is one of most Italian sparkling wine consumed all over the world and the production is strictly regulated by several production specifications, such as Denominazione di Origine Controllata (DOC, the label guaranteeing the quality and origin of a wine) and Denominazione di Origine Controllata e Garantita (DOCG). Total production of Prosecco wine in 2021 exceeded 600 million bottles (Il Sole 24 Ore 7 June 2021). The main grape variety used is *V. vinifera* Glera tonda although minor *V. vinifera* varieties are admitted, such as Bianchetta trevigiana, Glera lunga, Verdiso, and Perera, with total maximum 15%. Prosecco DOCG is produced in northeast Italy, traditionally in the Treviso province area known as Conegliano-Valdobbiadene hills which has been recently recognized as UNESCO World Heritage Site in 2019 (UNESCO, 2019).

Unfortunately, in general the *V. vinifera* varieties need many treatments/year with phytochemicals against some severe vine diseases, such as powdery mildew and downy mildew, in particular during the seasons characterized by unfavorable climatic conditions (i.e., high temperature and rainfall). In the last century, many new vine varieties characterized by high resistance to these two diseases were produced by crossing *V. vinifera*×American vitis sp. and in recent years some of them were admitted for wine production by several Italian regions (e.g., Veneto, Trentino-Alto Adige, Friuli-Venezia Giulia).

Experimental

The profile of glycosidic terpene precursors of Glera tonda and Glera tonda×resistent vitis sp. (Solaris, Bronner, and Kunleany) was performed by high-resolution mass spectrometry LC/QTOF [1]. The skins of fifty grape berries were extracted with 35 mL methanol for 4 hours, then the solution was homogenized and centrifuged. The supernatant was concentrated to 10 mL at 40°C by rotary evaporator (Laborota 4000, Heidolph, Germany), and the residue was adjusted to 100 mL by deionized water. To remove polyphenols and tannins,

the solution was treated with 1 g Polyclar® AT (SERVA Electrophoresis, Heidelberg, Germany) under stirring for 20 minutes, and after centrifugation the clear supernatant was recovered. Ten milliliters of the extract were diluted 1:2 v/v with deionized water, and the solution was passed through a 1 g Sep-Pak® C18 cartridge (Waters, Milford, MA, USA). The glycoside derivatives fraction was recovered with 5 mL methanol. The organic phase was filtered with a Clarify-PTFE 0.22 µm filter (Phenomenex, Torrance, CA, USA) and collected in a vial for LC/MS analysis. Analyses were performed using an ultra-high-performance liquid chromatography (UHPLC) Agilent 1290 Infinity system coupled to an Agilent 1290 Infinity Autosampler (G4226A) and Agilent 6540 accurate-mass quadrupole time-of-flight (QTOF) mass spectrometer (nominal resolution 40.000) equipped with Dual Agilent Jet Stream Ionization source (Agilent Technologies, Santa Clara, CA, USA). The metabolites were identified using a homemade electronic database of glycosidic precursors, made with molecular information from the literature and from other electronic databases.

Results

A way to increase the environmental sustainability of Prosecco production may be the creation of a new resistant Glera tonda variety. At CREA-VE labs hundred accessions were produced by crossing Glera tonda×resistent vitis sp. such as Solaris, Bronner, and Kunleany. Some crossings resulted particularly interesting because have inherited the resistant character of American vitis sp. and currently their oenological aptitudes are under study. In particular, the research aims to verify that the profile of glycosidic terpene precursors of Glera tonda is transferred to the crossings because they are the most important of grape compounds which contribute to aroma of Prosecco sparkling wine [2]. LC/QTOF profiles of the selected crossings were studied by using the methods previously described [1] and a new database expressly constructed, and the findings are here reported.

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OR33

Mycotoxins comprehensive panel analysis

Emanuele Ceccon

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Summary: A more comprehensive toxins panel, is a real need.
Here a really interesting solution

Keywords: *QuPPe-Pesticides-Mycotoxins*

Introduction

Various food commodities are vulnerable to different types of fungal pathogens and could be contaminated with differential classes of mycotoxins as a result. It is Ideally to implement a generic method for simultaneous determination of multi-mycotoxins in different food matrices or agricultural products.
In this study, a simplified sample preparation procedure and a reliable LC-MS/MS analytical method was developed for comprehensive measurement of 38 regulated and emerging mycotoxins including 5 *Alternaria* toxins, 6 major ergot alkaloids and their corresponding epimers. Four different food matrices (baby wheat cereal, peanut, tomato puree, and blended flour) were chosen for method validation to demonstrate the applicability of this analytical method to a wide range of food types

Experimental

Sample extraction was performed using a formic acid-acidified 80:20 acetonitrile:water solution followed by extract dry-down and reconstitution in a 50:50 water:methanol solution for injection analysis on a Biphenyl LC column. Chromatographic analysis was performed using LC-MS friendly acidic mobile phases and completed with a short 11-minute cycling time for proper separation of ergot alkaloid epimers. Accurate quantification was achieved using matrix-matched calibration standards at the range of 0.4 to 400 µg/kg. The recoveries of all mycotoxins (except citrinin) in fortified samples were from 70% to 120%, and the relative standard deviation (RSD) was less than 20%. For the vast majority of analytes, the limit of quantification was at 0.4 µg/kg which was satisfactory to meet the regulatory levels

Results & Discussions

Linearity: It was shown that a consistent and most suitable linearity of all analytes could be obtained with a quadratic regression (1/x weighted). The lowest concentrated standards were varied due to the differential MS ionization of analytes and specific matrix effect of different food matrices. Nevertheless, most analytes were quantifiable at the full range of 0.4 – 400 µg/kg and all compounds showed proper linearity with r^2 >0.997 and deviations <30.
Accuracy & Precision: For each food sample, 3 batches of analyses were performed on different days with a total of 9 repetition of each fortified level. The average recovery and relative standard deviation (RSD) were shown in Table 1.

Table 1: Recovery & Precision

	Average Recovery (RSD, %)											
	Baby Wheat Cereal			Peanut			Tomato Puree			Blended Flour		
Concentration, µg/kg	5	50	200	5	50	200	5	50	200	5	50	200
Aflatoxin B1	105 (4.8)	100 (3.0)	79.8 (2.6)	98.2 (6.4)	97.0 (5.2)	89.0 (5.7)	92.7 (3.8)	97.6 (5.2)	103 (3.0)	101 (2.8)	95.5 (1.3)	89.0 (1.5)
Aflatoxin B2	110 (1.4)	109 (2.8)	106 (2.3)	102 (5.8)	99.3 (4.7)	91.3 (2.9)	91.7 (4.2)	93.3 (0.9)	94.7 (0.4)	100 (2.3)	101 (0.9)	88.7 (1.3)
Aflatoxin G1	105 (6.1)	107 (1.7)	102 (2.1)	98.2 (4.2)	97.3 (3.2)	91.2 (4.1)	91.3 (1.9)	92.2 (3.6)	93.3 (2.5)	99.3 (1.7)	100 (1.6)	93.6 (2.2)
Aflatoxin G2	108 (3.0)	109 (1.3)	104 (2.2)	104 (5.3)	102 (3.8)	93.5 (1.9)	86.8 (8.3)	96.4 (2.5)	98.5 (2.5)	98.7 (3.1)	102 (2.6)	94.5 (2.0)
Aflatoxin M1	109 (3.2)	109 (1.5)	101 (2.1)	91.5 (3.0)	96.0 (3.1)	93.1 (3.6)	92.5 (3.2)	92.8 (4.0)	93.3 (2.8)	99.2 (2.7)	101 (2.0)	95.3 (2.1)
Ochratoxin A	109 (1.8)	108 (2.1)	94.5 (1.5)	102 (1.9)	101 (1.1)	97.7 (0.9)	90.9 (3.5)	93.8 (3.3)	101 (5.9)	98.1 (1.6)	98.2 (1.3)	82.8 (1.7)
3- + 15-Acetyldeoxynivalenol	104 (6.3)	108 (1.8)	104 (3.3)	101 (6.5)	95.9 (5.8)	91.0 (4.4)	91.9 (4.3)	98.1 (2.7)	95.0 (1.8)	98.4 (5.2)	101 (2.9)	100 (0.9)
Deoxynivalenol	112 (4.0)	102 (2.6)	95.7 (1.3)	98.1 (3.5)	93.7 (4.8)	88.2 (3.4)	-	90.3 (6.4)	94.5 (2.6)	102 (3.5)	97.5 (2.6)	96.9 (0.8)
Diacetoxyscirpenol	105 (4.0)	107 (1.5)	103 (1.2)	93.2 (4.3)	95.4 (3.9)	93.8 (5.0)	90.9 (3.8)	94.5 (4.7)	94.0 (1.9)	98.1 (6.3)	101 (3.1)	98.7 (1.8)
Fumonisin B1	94.3 (4.6)	94.0 (2.8)	92.3 (2.6)	87.2 (3.1)	88.2 (4.5)	87.8 (6.6)	91.8 (3.6)	91.5 (1.9)	91.9 (0.7)	100 (3.2)	99.6 (1.7)	96.1 (1.2)
Fumonisin B2	93.3 (4.1)	95.1 (4.8)	90.3 (2.9)	95.4 (4.7)	92.5 (2.3)	88.8 (3.9)	89.9 (4.1)	92.9 (2.3)	92.4 (0.8)	104 (2.7)	99.6 (1.4)	94.4 (1.6)
Fumonisin B3	91.8 (4.9)	94.6 (4.9)	91.6 (3.1)	90.6 (2.7)	90.1 (3.8)	87.7 (4.7)	91.1 (3.6)	93.1 (1.8)	91.9 (0.9)	104 (2.2)	99.9 (1.4)	95.9 (1.2)
Fusarenon-X	99.0 (3.9)	100 (2.9)	103 (2.8)	86.9 (7.0)	90.3 (11.0)	88.3 (10.1)	-	92.0 (6.8)	94.3 (1.9)	101 (3.8)	100 (3.7)	98.3 (1.6)
HT-2	110 (2.4)	111 (1.4)	108 (1.1)	100 (2.7)	100 (2.0)	94.3 (3.0)	96.8 (3.1)	96.1 (2.1)	99.0 (1.4)	101 (1.6)	103 (2.2)	98.3 (1.3)
Nivalenol	-	-	-	-	98.3 (6.2)	89.0 (3.6)	-	92.5 (4.5)	93.7 (5.0)	-	95.5 (4.7)	92.9 (2.3)
T-2	111 (2.1)	110 (1.8)	108 (2.8)	99.1 (2.7)	101 (1.7)	95.9 (2.1)	92.0 (6.3)	94.7 (1.3)	98.6 (1.5)	102 (1.3)	103 (1.3)	96.9 (1.3)
α-Zearalenol	100 (4.9)	102 (5.2)	90.1 (5.8)	89.2 (8.1)	93.6 (5.5)	94.7 (3.4)	97.7 (3.2)	88.9 (4.2)	90.0 (3.4)	96.9 (3.7)	99.0 (3.6)	95.0 (3.3)
Zearalenone	110 (6.7)	110 (3.0)	105 (3.7)	98.3 (7.3)	97.4 (2.8)	91.3 (1.5)	95.0 (4.5)	93.6 (2.2)	95.7 (2.0)	101 (3.8)	102 (2.1)	92.3 (1.4)
Citrinin	26.1 (9.2)	26.6 (3.1)	30.1 (3.8)	24.1 (8.7)	25.1 (1.9)	25.8 (3.5)	71.9 (4.7)	76.4 (1.6)	77.1 (1.7)	32.3 (3.5)	32.2 (6.3)	35.8 (4.5)
Patulin	106 (4.6)	95.6 (5.6)	89.2 (5.1)	88.8 (12.0)	83.6 (9.0)	86.0 (7.2)	-	98.9 (3.6)	103 (4.5)	93.6 (4.4)	86.1 (3.1)	92.2 (2.9)
Alternariol	108 (4.1)	108 (1.6)	104 (1.0)	94.2 (3.4)	95.4 (2.4)	96.2 (2.7)	89.3 (4.6)	91.8 (2.5)	91.4 (1.3)	98.4 (2.3)	101 (2.5)	96.3 (3.2)
Alternariol monomethylether	108 (4.1)	109 (2.2)	99.3 (2.7)	93.5 (3.3)	93.5 (3.7)	89.8 (2.4)	91.3 (6.6)	88.7 (5.1)	93.9 (3.9)	104 (2.9)	101 (1.7)	93.7 (1.9)
Altenuene	110 (2.1)	109 (2.1)	105 (2.1)	99.6 (2.0)	99.5 (1.2)	95.4 (1.2)	98.4 (3.4)	92.4 (2.1)	92.8 (1.8)	101 (2.9)	101 (3.1)	98.2 (0.5)
Tentoxin	111 (3.6)	109 (2.5)	103 (1.4)	104 (2.9)	101 (1.1)	95.3 (1.4)	92.5 (6.2)	94.2 (2.2)	95.8 (1.4)	104 (4.2)	105 (2.1)	98.2 (1.9)
Tenuazonic acid	-	85.8 (1.7)	87.4 (6.3)	92.5 (4.7)	91.0 (2.1)	88.5 (2.4)	-	89.3 (4.1)	88.5 (2.0)	-	92.5 (8.8)	90.0 (9.5)
Ergocornine	109 (1.5)	109 (1.4)	102 (1.3)	93.8 (3.5)	93.2 (4.4)	91.2 (3.3)	91.5 (3.0)	93.1 (1.9)	92.9 (0.6)	102 (2.5)	101 (1.9)	97.6 (1.7)
Ergocorninine	109 (3.0)	109 (2.0)	101 (1.9)	105 (3.0)	104 (2.4)	99.5 (3.1)	89.9 (3.8)	92.3 (2.2)	92.5 (3.1)	101 (2.5)	102 (2.6)	95.7 (2.4)
Ergocristine	108 (3.1)	108 (2.9)	101 (4.4)	92.1 (3.8)	91.7 (5.1)	92.0 (2.2)	91.3 (2.9)	94.2 (2.0)	94.3 (0.8)	101 (1.7)	99.8 (2.0)	96.7 (1.8)
Ergocristinine	106 (3.5)	105 (1.4)	101 (0.8)	102 (4.8)	104 (4.3)	102 (4.6)	91.6 (5.9)	94.4 (1.8)	95.6 (2.7)	102 (2.9)	102 (3.0)	99.3 (4.5)
Ergocryptine	107 (2.0)	109 (1.9)	104 (3.4)	95.0 (3.0)	94.7 (4.1)	92.1 (1.7)	90.1 (3.0)	93.5 (2.2)	93.2 (0.7)	99.5 (2.7)	99.9 (1.2)	97.4 (1.4)
Ergocryptinine	106 (1.7)	108 (2.0)	101 (1.1)	103 (5.3)	105 (4.0)	101 (4.2)	91.1 (4.3)	95.1 (1.5)	98.1 (1.6)	101 (2.0)	101 (1.8)	95.4 (1.9)
Ergometrine	92.8 (7.3)	90.0 (4.2)	88.3 (3.6)	101 (2.3)	96.2 (2.6)	86.7 (1.9)	90.7 (3.6)	88.9 (6.1)	87.6 (3.5)	101 (1.8)	99.7 (3.2)	95.3 (1.3)
Ergometrinine	101 (4.2)	99.1 (1.9)	94.3 (0.7)	93.2 (4.3)	95.5 (1.7)	89.1 (2.2)	90.6 (3.9)	90.1 (4.4)	89.7 (1.9)	100 (3.5)	98.5 (1.9)	91.1 (1.9)
Ergosine	108 (2.6)	106 (5.6)	101 (3.2)	90.8 (2.0)	91.8 (2.2)	89.2 (2.6)	91.7 (2.2)	90.4 (3.1)	90.3 (1.5)	99.9 (2.7)	99.1 (3.0)	98.2 (1.1)
Ergosinine	111 (1.8)	109 (0.9)	103 (1.1)	100 (1.1)	102 (2.0)	97.7 (2.2)	92.7 (1.4)	93.6 (2.5)	93.8 (0.9)	99.2 (2.8)	98.4 (2.8)	97.5 (1.0)
Ergotamine	109 (1.9)	108 (1.7)	102 (2.8)	91.0 (2.8)	92.6 (2.8)	89.8 (3.6)	91.1 (2.2)	90.6 (3.7)	90.7 (1.3)	101 (2.9)	100 (3.1)	96.4 (2.2)
Ergotaminine	109 (1.0)	109 (0.7)	101 (0.6)	98.2 (2.0)	101 (1.5)	96.6 (1.3)	93.6 (3.5)	94.7 (1.7)	94.5 (0.6)	101 (2.3)	99.7 (1.3)	97.1 (1.5)

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