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

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*

Organizing Committee

Giacomo Bruni	*Carapelli, Firenze*
Diletta Balli	*Università di Firenze*
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Lorenzo Cecchi	*Università di Firenze*
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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),* ***Donatella Caruso*** *(Univ. of Milan)*

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),* ***Paola Pittia*** *(Univ. of Teramo)*

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

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 – 18:05 **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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Serena Rizzo, Rita Celano, Luca Campone, Luca Rastrelli, Anna Lisa Piccinelli

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Maria Mazzucotelli, Iuliia Khomenko, Emanuela Betta, Irene Cetto, Brian Farneti, Elena Gabetti, Andrea Cavallero, Eugenio Aprea, Franco Biasioli

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Karina Gonzalez-Estanol, Iuliia Khomenko, Monica Fontova-Cerda, Markus Stieger, Franco Biasioli

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BOOK of ABSTRACTS (full)

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ABSTRACTS

Plenary, Keynotes, Orals

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.

References

1. Del Bo', C.; Bernardi, S.; Cherubini, A.; Porrini, M.; Gargari, G.; Hidalgo-Liberona, N.; González-Domínguez, R.; Zamora-Ros, R.; Peron, G.; Marino, M.; et al. *Clin. Nutr.* **2021**, doi:10.1016/j.clnu.2020.12.014.
2. Nicoletti, C. *Expert Rev. Gastroenterol. Hepatol.* **2015**, *9*, 1467–1469, doi:10.1586/17474124.2015.1092872.
3. Sturgeon, C.; Fasano, A. *Tissue Barriers* **2016**, *4*, e1251384, doi:10.1080/21688370.2016.1251384.
4. González-Domínguez, R.; Jáuregui, O.; Mena, P.; Hanhineva, K.; Tinahones, F.J.; Angelino, D.; Andrés-Lacueva, C. *Int. J. Obes.* **2020**, doi:10.1038/s41366-020-0628-1.
5. Peron, G.; Gargari, G.; Meroño, T.; Miñarro, A.; Lozano, E.V.; Escuder, P.C.; González-Domínguez, R.; Hidalgo-Liberona, N.; Del Bo', C.; Bernardi, S.; et al. *Clin. Nutr.* **2021**, *40*, 5288–5297, doi:10.1016/j.clnu.2021.08.027.
6. Peron, G.; Meroño, T.; Gargari, G.; Hidalgo-Liberona, N.; Miñarro, A.; Lozano, E.V.; Castellano-Escuder, P.; González-Domínguez, R.; Del Bo', C.; Bernardi, S.; et al. *Mol. Nutr. Food Res.* **2022**, *n/a*, 2100349, doi:https://doi.org/10.1002/mnfr.202100349.
7. Meroño, T.; Peron, G.; Gargari, G.; González-Domínguez, R.; Miñarro, A.; Vegas-Lozano, E.; Hidalgo-Liberona, N.; Del Bo', C.; Bernardi, S.; Kroon, P.A.; et al. *Food Res. Int.* **2022**, *159*, 111632, doi:https://doi.org/10.1016/j.foodres.2022.111632.

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.

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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.

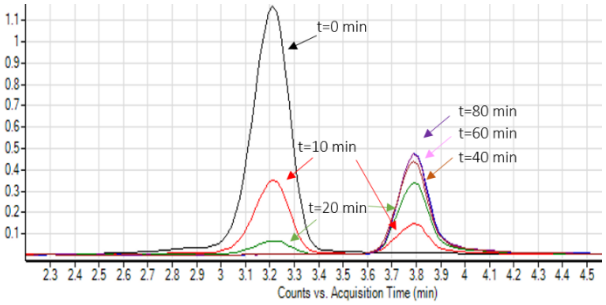

***Figure 1**. Chromatograms of cyanidin 3-glucoside hydrolysis into cyanidin analysed at 0 min, 10 min, 20 min, 40 min, 60 min and 80 min at a temperature of 100°C*

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].

References

1. N. Tena, J. Martin, A. G. Asuero; *Antioxidants*, 9 (2020).
2. D. Strack, V. Wray, *The Flavonoids: Advances in Research Since 1986*, Chapman & Hall, (1993), p. 1.
3. Y.-S. Shim, W.-J. Yoon, D.-M. Kim, M. Watanabe, H.-J. Park, H. W. Jang, J.

Lee, J. Ha; *Journal of Chromatographic Science*, 53 (2015), 1646-1653.
4. X. Wu, R. L. Prior; *Journal of Agricultural and Food Chemistry*, 53 (2005), 3101-3113.
5. J. Lachman, P. Martinek, Z. Kotíková, M. Orsák, M. Šulc; *Journal of Cereal Science*, 74 (2017), 145-154.

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).

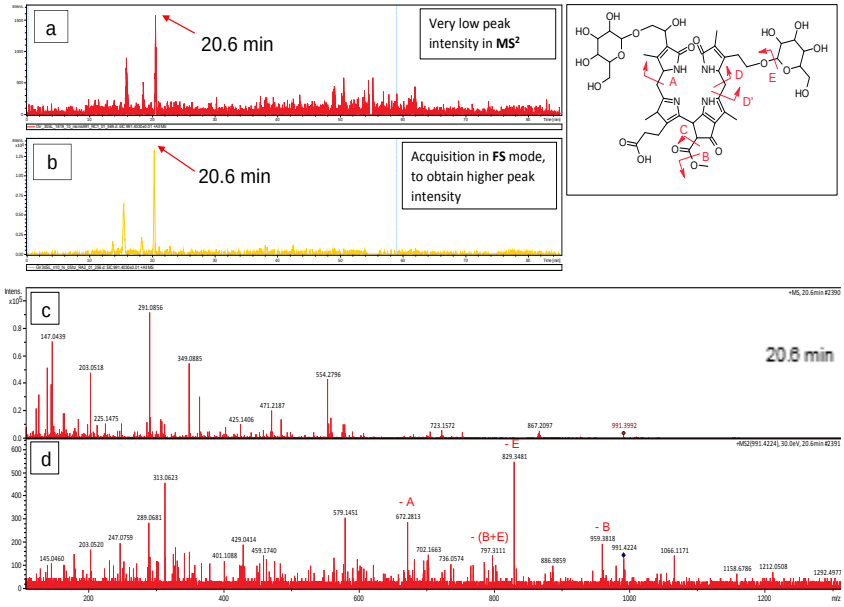

***Figure 1**. Extracted Ion Chromatogram's (EIC's) of DNCC-990 acquired with high-resolution mass spectrometry (a: obtained in data dependent method, b: obtained in full scan (FS) mode, c: FS mass spectra and d: MS^2 spectra are shown). The (tentative) constitutional formula and the fragmentation sites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

References

1. Hörtensteiner, S., Hauenstein, M., Kräutler, B. *Advances in Botanical Research*, 90 (2019), 213–271
2. Mittelberger, C., Yalcinkaya, H., Pichler, C., Gasser, J., Scherzer, G., Erhart, T., Schumacher, S., Holzner, B., Janik, K., Robatscher, P., Müller, T., Kräutler, B., Oberhuber, M. *Journal of Agricultural and Food Chemistry*, 65(2017), 2651–2660

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.

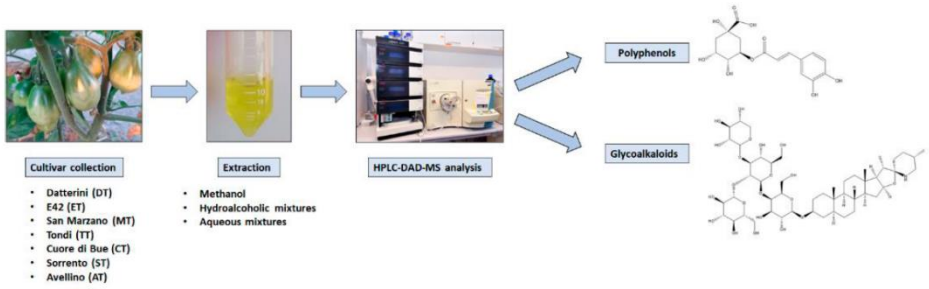

***Figure 1**. Workflow of the analysis of glycoalkaloids and polyphenols*

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.

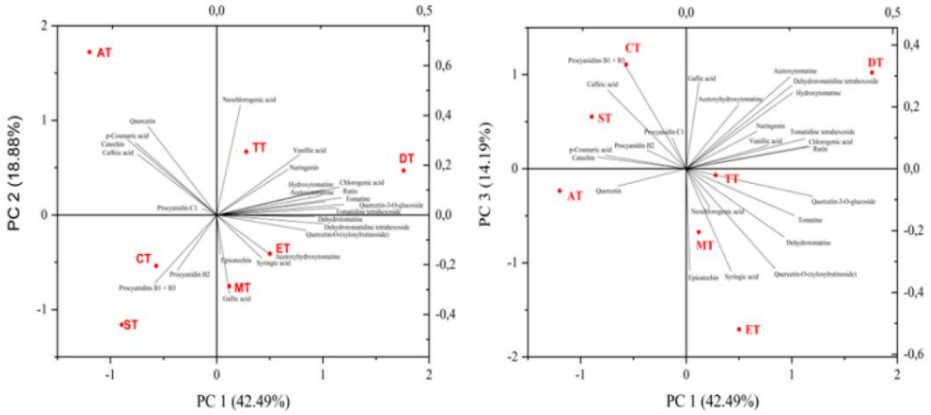

***Figure 2**. Biplot of the principal component analysis (PCA) of 7 unripe tomato cultivars and 24 quantified compounds*

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.

References

1. Matei, E.; Râpă, M.; Predescu, A.M.; Țurcanu, A.A.; Vidu, R.; Predescu, C.; Bobirica, C.; Bobirica, L.; Orbeci, C.; *Materials (Basel)*, 14 (2021), 221-232.
2. Rick, C.M.; Uhlig, J.W.; Jones, A.D. *Proc. Natl. Acad. Sci. U. S. A*., 91 (1994), 12877-12881.
3. Agency, E.M. ICH Topic Q 2 (R1), 2 (2006), 1-15.

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).

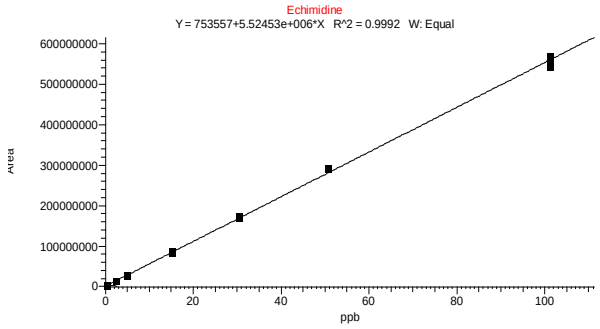

Figure 1. *Overall calibration curve for Echimidine (PA)*

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.

References

1. T Bessaire, M Ernest, N Christinat, B Carrères, A Panchaud, F Badoud; *Food Additives & Contaminants* (2021), Part A, 38:6, 978-996.
2. Q Baslé, C Mujahid, T Bessaire; *Food Additives & Contaminants* (2020): Part A, 37:10, 1744-1754
3. European Food Safety Authority (2011), *EFSA Journal.* 9:2406.
4. European Food Safety Authority (2013). *EFSA Journal*. 11:3386.
5. Commission Regulation (EU) No 2020/2040 *Off J. Eur. Union*, L420 (2020), 1-4.
6. Commission Regulation (EU) No 2021/1408 *Off J. Eur. Union*, L304 (2021), 1-4.
7. Dzuman Z, Jonatova P, Stranska-Zachariasova M, Prusova N, Fenclova M, Hajslova J; *Analytical and Bioanalytical Chemistry*, (2020); 412(26):7155-7167.
8. Martinello M, Borin A, Stella R, Bovo D, Biancotto G, Gallina A, Mutinelli F; *Food Chemistry* 2017; 234:295–302

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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.

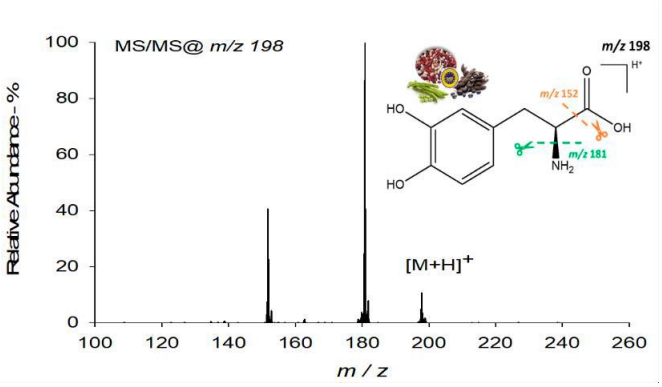

***Figure 1**. L-dopa CID-MS/MS spectra of Vicia faba L. sample, relative collision energy 19% was applied*

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.

References

1. R. Balestrino, A.H.V. Schapira; *European Journal of Neurology* 27 (2020), 27-42.
2. A. G. Miranda-Díaz, A. García-Sánchez and E.G. Cardona-Muñoz; *Oxidative Medicine and Cellular Longevity*, Hindawi 2020, 1-17.
3. S. B. Dhull, M. K. Kidwai, R. Noor, P. Chawla, P. K. Rose; *Legume Science,* Wiley Periodicals 2021, 1-13.
4. J. Benfica, E. S. Morais, J. S. Miranda, M. G. Freire, R. C. S. de Sousa, J. A. P. Coutinho; *Separation and Purification Technology* 274 (2021), 1-7.
5. K. Polanowska, R. M. Łukasik, M. Kuligowski, J. Nowak; *Molecules* 14 (2019), 1-15.

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.

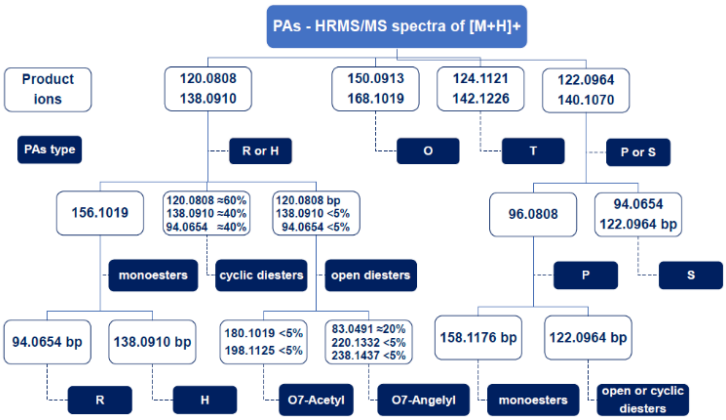

***Figure 1**. PAs' subset of the strategy*

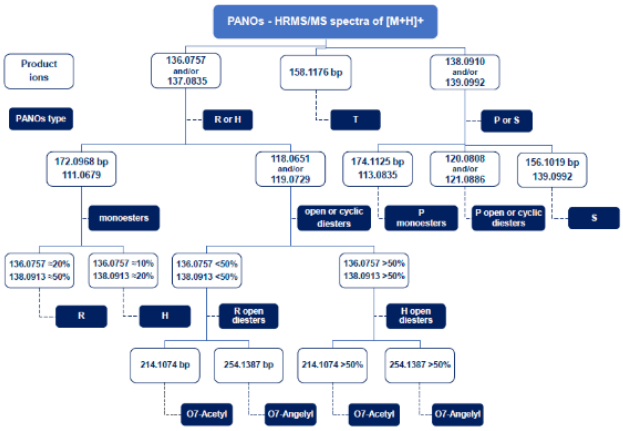

***Figure 2**. PANOs' subset of the strategy*

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.

References

1. EFSA CONTAM Panel (2016) *EFSA Journal* 14 (8), 4572.
2. EFSA CONTAM Panel (2017) *EFSA Journal* 15 (7), 4908.
3. Rizzo, S., Celano, R., Campone, L., Rastrelli, L., Piccinelli, A. L. (2022). *Journal of Food Composition and Analysis*, 108, 104457.
4. Commission Regulation (EU) 2020/2040.
5. Magnusson B., Örnemark U. (2014). *Eurachem Guide: The Fitness for Purpose of Analytical Methods.*

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.

References

1. K.Y. Christensen, M. Raymond, M. Blackowicz, Y. Liu, B.A. Thompson, H.A. Anderson, M. Turyk; *Environmental research*, 154 (2017), 145–151.
2. A.A. Jensen and H. Leffers; *International Journal of Andrology*, 31 (2008), 161–169.
3. S. Liu, R. Yang, N. Yin, F. Faiola; *Journal of Environmental Sciences*, 88 (2020), 187-199.
4. EFSA Panel on Contaminants in the Food Chain; *EFSA Journal*, 18(9) (2020), e06223.

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].

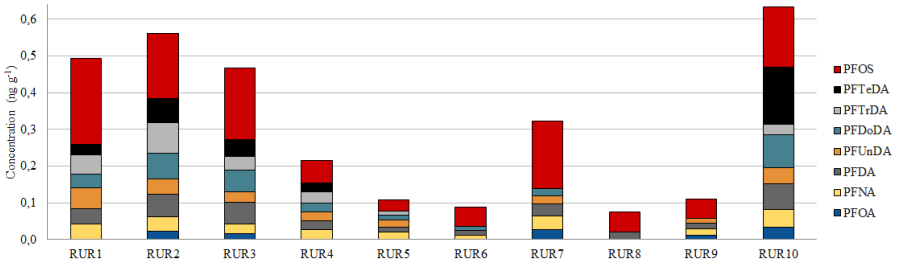

***Figure 1**. Concentrations (ng g^{-1}) and contributions of the detected PFASs in small-scale rural egg samples*

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.

References

1. EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel); *EFSA Journal*, 18 (2020) 6223.
2. A. Stramenga, T. Tavoloni, T. Stecconi, R. Galarini, M. Giannotti, M. Siracusa, M. Ciriaci, S. Bacchiocchi, A. Piersanti; *Journal of Chromatography B*, 1186 (2021) 123009.
3. T. Gazzotti, F. Sirri, E. Ghelli, E. Zironia, M. Zampiga, G. Pagliuca; *Food Chemistry*, 362 (2021) 130178.
4. E. Zafeiraki, D. Costopoulou, I. Vassiliadou, L. Leondiadis, E. Dassenakis, R. Hoogenboom, S.P.J. van Leeuwen; *Chemosphere*, 144 (2016) 2106-2112.

PL2

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

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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].

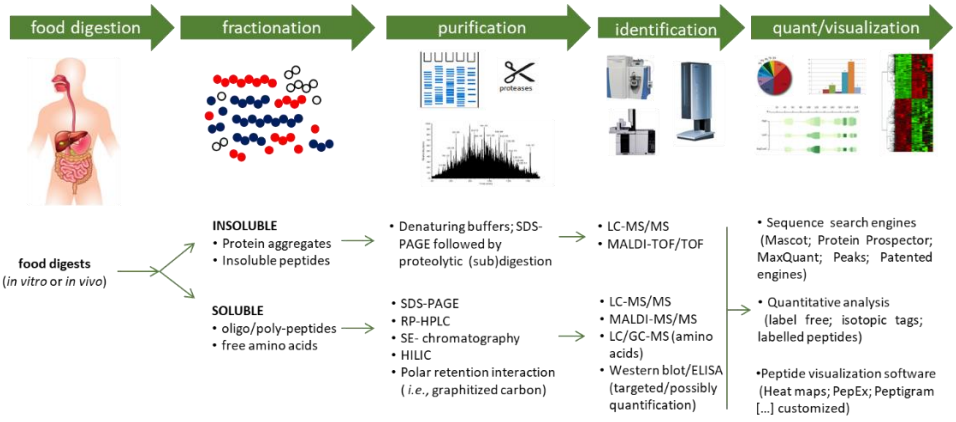

Figure 1. *General analytical workflow of proteomics and peptidomics in digestomics. Different options can be selected at each step, depending on the nature of the food matrix and on the experimental endpoint*

References

1. Brodkorb A et al. *Nature Protocol,* 2019;14(4):991-1014.
2. Picariello G, Addeo F, Ferranti P, Nocerino R, Paparo L, Passariello A, Dallas DC, Robinson RC, Barile D, Canani RB. *Food & Function*, 2016;7:3402-9.
3. Picariello G, De Cicco M, Nocerino R, Paparo L, Mamone G, Addeo F, Berni Canani R. *Frontiers in Nutrition*, 2019;6:25.
4. Palanski BA, Weng N, Zhang L, Hilmer AJ, Fall LA, Swaminathan K, Jabri B, Sousa C, Fernandez-Becker NQ, Khosla C, Elias JE. *Nature Communications* 2022;13:888.
5. Caira S, Pinto G, Picariello G, Vitaglione P, De Pascale S, Scaloni A, Addeo F. *Food Chemistry*, 2022;385:132663.

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].

References

1. J.Sun, I. Kato; Genes Dis, 2016, 3(2), 130-143.
2. H. Shamshoum, F. Vlavcheski, E. Tsiani; 2017, BioFactors, 43, 517-528.
3. J. Ruzzolini, S. Chioccioli, N. Monaco, S. Peppicelli, E. Andreucci, S. Urciuoli, A. Romani, L. Luceri, K. Tortora, L. Calorini, G. Caderni, C. Nediani, F. Bianchini; Antioxidants, 2021, 10, 1577.
4. T. Vezza, A. Rodríguez-Nogales, F. Algieri, J. Garrido-Mesa, M. Romero, M. Sánchez, M. Torale, J. Gálvez; 2019, Pharmacol Res., 150, 104487.
5. G. Bartolucci, M. Pallecchi, M. Menicatti, L. Moracci, S. Pucciarelli, M. Agostini, S. Crotti; J. Pharm. Biomed. Anal., 2022, 215.
6. F. Vitali, K. Tortora, M. Di Paola, G. Bartolucci, M. Menicatti, C. De Filippo, G. Caderni; 2022, Sci Rep., 2022 26, 12(1), 1432.

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.

References

1. Izzo L., Rodríguez-Carrasco Y., Gaspari A., Graziani G., Mañes J., Ritieni A. *Beverages 4 (2), 43, 2018.*
2. Luz C., Izzo Rodríguez-Carrasco Y., Castaldo L., Gaspari A., Graziani G., Ritieni A. *LWT-Food Science and Technology 99, 34-42, 2019.*
3. Izzo L., Rodríguez-Carrasco Y., Tolosa J., Graziani G., Gaspari A., Ritieni A. *Journal of Dairy Science 103 (2), 1250-1260, 2020.*
4. Narváez A., Rodríguez-Carrasco Y. Castaldo L., Izzo L., Ritieni A. *Toxins 12 (2), 114, 2020.*
5. Rodríguez-Carrasco Y., Narváez A., Izzo L., Gaspari A., Graziani G., Ritieni A. *Toxins 2020, 12, 415.*
6. Narváez A., Rodríguez-Carrasco Y., Castaldo L., Izzo L., Graziani G., Ritieni A. *Metabolites 10 (9), 344, 2020.*
7. Tolosa J., Graziani G., Gaspari A., Chianese D., Ferrer E., Mañes J., Ritieni A. *Toxins 2021, 13, 305.*
8. Narváez A., Castaldo L., Carrasco Y.R., Ritieni A*. Food Control 132, 2021.*
9. Narváez A., Izzo L., Pallarés N., Castaldo L., Rodríguez-Carrasco Y., Ritieni A. *Toxins 13, 869, 2021.*
10. Izzo L., Narváez A., Castaldo L., Gaspari A., Rodríguez-Carrasco Y. Grosso M., Ritieni A. *Journal of Dairy Science 105, 4, 2022.*
11. Narváez A., Rodríguez-Carrasco Y., Ritieni A, Manes J. *Journal of Chromatography B 1191 (2022) 123117.*
12. Izzo L., Mikusova P., Sluyok M., Ritieni A. *Toxins 14, 134, 2022.*

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.

References

1. EFSA. 'Scientific Opinion on Mineral Oil Hydrocarbons in Food', *EFSA Journal*, 10(6), 2012.
2. S. Bratinova, E. Hoekstra. EUR 29666 EN, Publications Office of the European Union, 2019.
3. L. Menegoz Ursol, C. Conchione, A. Srbinovska, S. Moret; *Food Chemistry*, 2022, 370, 130966.
4. T. Populin, M. Biedermann, K. Grob, S. Moret, L. Conte; *Food Additives and Contaminants*, 21, 893-904, 2004.
5. L. Menegoz Ursol, C. Conchione, D. Peroni, A. Carretta, S. Moret; (paper in preparation).

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.

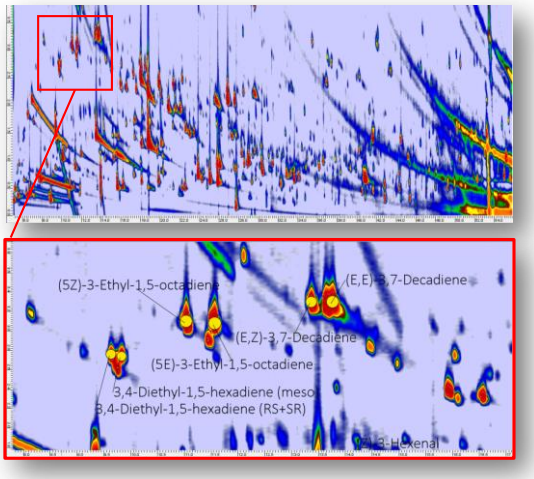

***Figure 1**. pattern of unsaturated alkenes detected in an Italian EVO sample*

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.

References

1. Peterson R. Nat Chem Biol. 2008; 4:635–8
2. https://www.ibm.com/topics/computer-vision
3. Nicolotti L, Mall V, Schieberle P. J. Agric. Food Chem. 2019;67:4011–22
4. Stilo, F., Liberto, E., Reichenbach, S.E., Tao, Q., Bicchi, C., Cordero, C., J. Agric. Food Chem., 2019. 67:5289–5302.

OR14

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

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

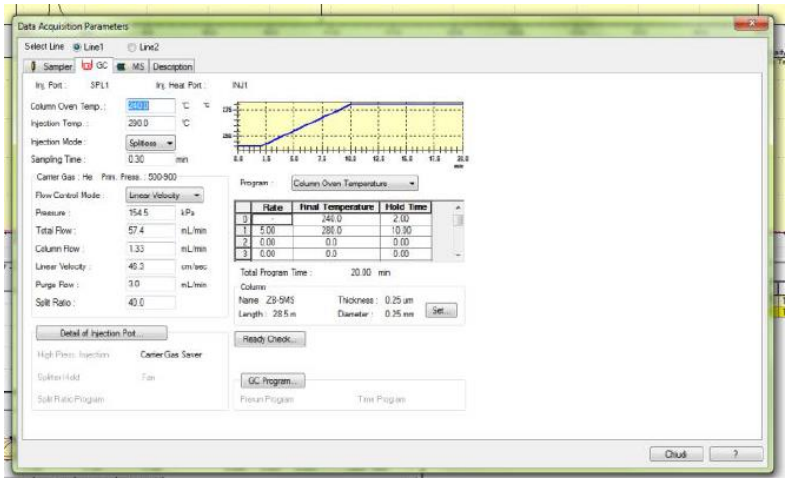
Introduction

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.

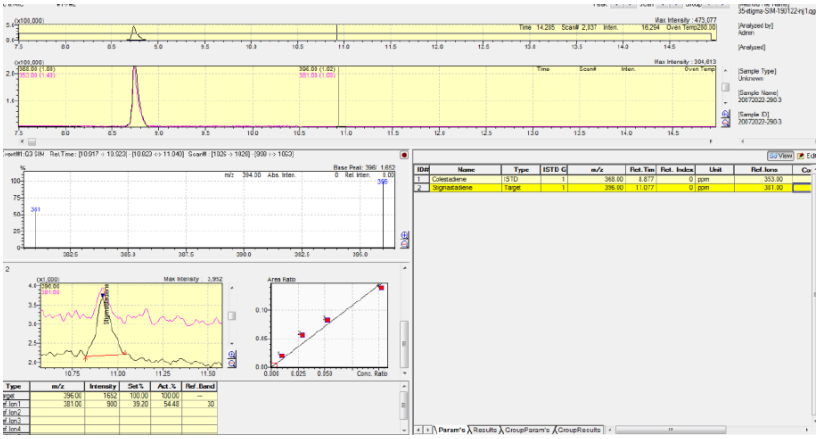
Experimental

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.

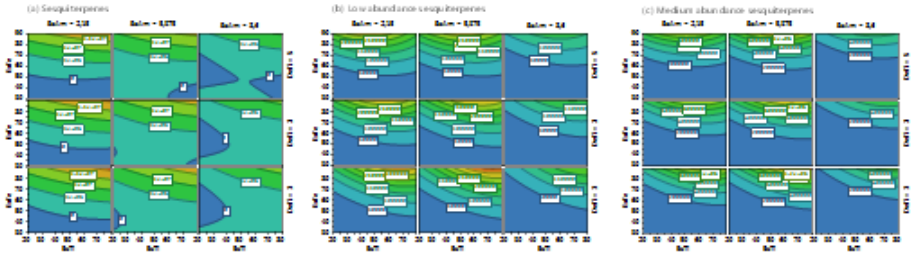

***Figure 1**. Contour plots for the responses of (a) Sesquiterpenes; (b) LA sesquiterpenes; (c) MAsesquiterpenes*

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.

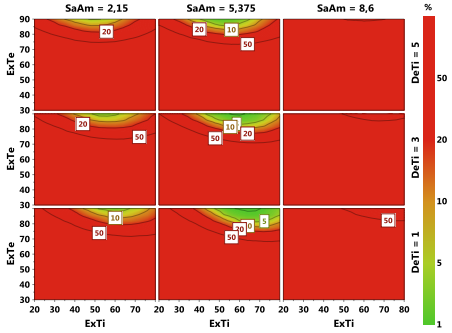

***Figure 2**. Probability maps obtained by plotting ExTe, vs. ExTi, at 3 values of SaAm and DeTi. 1, 3, 5 min. The MODR is the green zone included in the 10% isoprobability line*

References

1. L. Cecchi, M. Migliorini, N. Mulinacci, *J. Agric. Food Chem*, 69 (2021), 2013-2040
2. S. Vichi, N. Cortes-Francisco, A. Romero, J. Caixach, J. *Food Chem*, 149 (2014), 313-318
3. L. Cecchi, M., Migliorini, … Cane, N. Mulinacci, F. Melani, F. *Food Control,* 112 (2020), 107156
4. B. Torres-Cobos, B., Quintanilla-Casas, …., F. Guardiola, A. Tres, S. Vichi.

Food Control, (2021)128, 108200
5. F. Angerosa, L. Camera, N. d'Alessandro, G. Mellerio. *J. Agric. Food Chem*., 46 (1998), 648−653
6. R. Deidda, S. Orlandini, P. Hubert, C. Hubert. *J. Pharm. Biomed. Anal*., 161 (2018), 110-121
7. L. Eriksson, E. Johansson, N,… S. Wold. Design of Experiments - Principles and Applications, (2008). Umeå: MKS Umetrics AB, Sweden

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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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%.

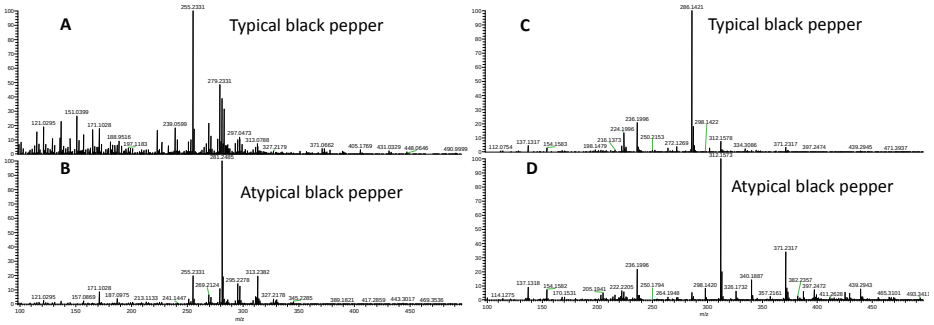

***Figure 1**. Representative (+/-) TD-DART-HRMS spectra of authentic (A and C) and adulterated (B and D) black pepper spiked with 30% of papaya seeds*

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.

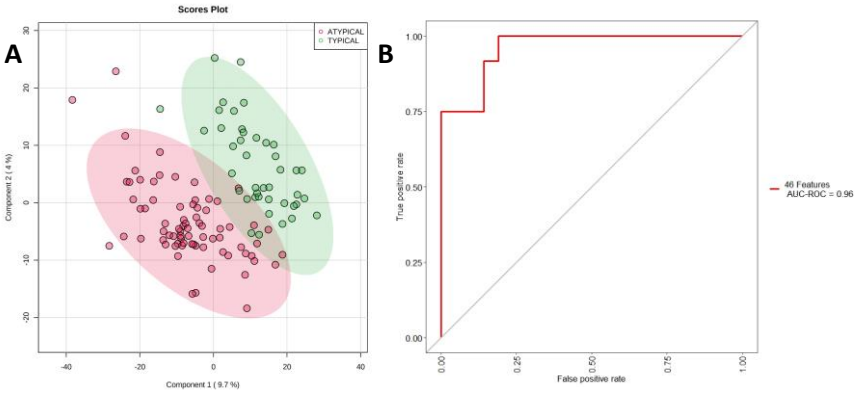

***Figure 2**. A) PLS-DA score plot. A tendency to discrimination of authentic (typical) and adulterated (atypical) samples can be observed. B) ROC curve obtained from the validation of LASSO classifier on the test set*

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.

References

1. Silvis ICJ, van Ruth SM, van der Fels-Klerx HJ, Luning PA. *Food Control*, 18 (2017),80-87.
2. A. Maquet, A. Lievens, V. Paracchini, G. Kaklamanos, M.B. De La Calle Guntinas, L. Garlant, et al. 2021. Contract No.: EUR 30877 EN.

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).

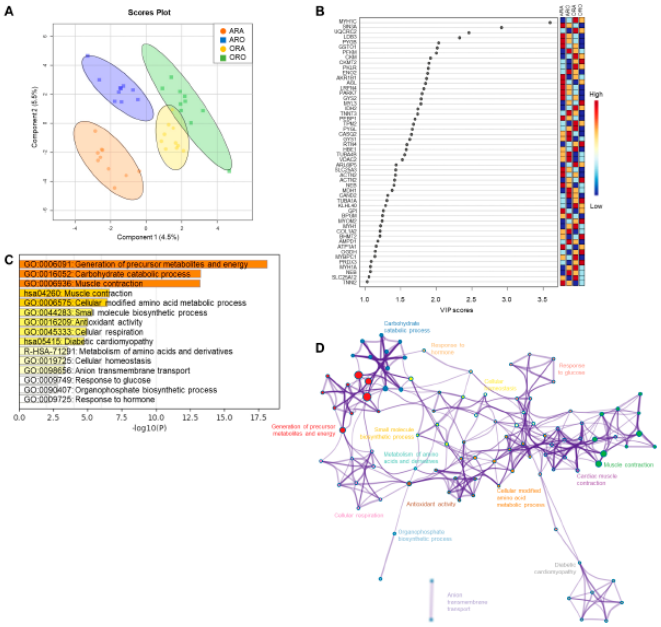

Figure 1. *A) Partial least squares-discriminant analysis (PLS-DA) score plot of meat proteome distribution according to chicken strain and production system. B) Variable Importance in Projection (VIP) plot obtained from meat samples classified as ARA, ARO, ORO and ORA. VIP cut-off of 1.0 has been used. 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).

References

1. Xing, T., Zhao, Z., Zhao, X., Zhuang, S., Xu, X. (2020). *Food Chemistry*, 319, 126531.
2. El-Deek, A., El-Sabrout, K. (2019). *World's Poultry Science Journal*, 75(1), 105-114.
3. Zhu, Y., Gagaoua, M., Mullen, A. M., Viala, D., Rai, D. K., Kelly, A. L., Sheehan, D., Hamill, R. M. (2021). *Meat Science*, vol. 176, p. 108488
4. Chantada-Vázquez, M. D. P., García Vence, M., Serna, A., Núñez, C., Bravo, S. B. (2021). *Shotgun Proteomics* (pp. 105-141). Humana, New York, NY.
5. Gagaoua, M., Terlouw, E. M. C., Mullen, A. M., Franco, D., Warner, R. D., Lorenzo, J. M., Purslow, P. P., Gerrard, D., Hopkins, D. L., Troy, D., Picard, B. (2021). *Meat Science*, 172, 108311.

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.

References

1. N. Cardullo, V. Muccilli, V. Di Stefano, S. Bonacci, L. Sollima, M.G. Molecules 2022, 27, 2482.
2. F. Sciacca, M. Palumbo, A. Pagliaro, V. Di Stefano, S. Scandurra, N. Virzì, M.G. Melilli, *Cyta – Journal of Food 2021, Vol. 19, No. 1, 96–104.*

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].

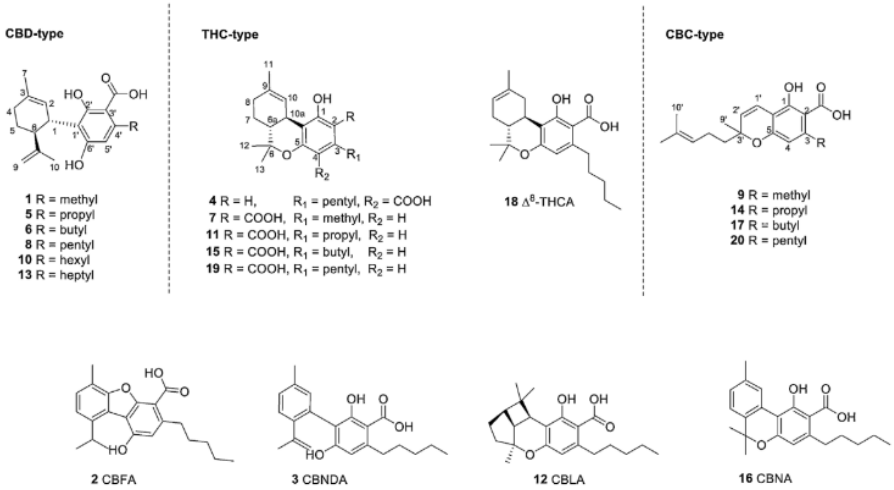

***Figure 1**. Structures of the pre-cannabinoids tentatively identified in the hemp pollen extract under study*

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.

References

1. H.P.V. Rupasinghe, A. Davis, S.K. Kumar, B. Murray, V.D. Zheljazkov; *Molecules*, 25 (2020) 4078.
2. G. Appendino, G. Chianese, O. Taglialatela-Scafati; *Current Medicinal Chemistry*, 18 (2011), 1085–1099.
3. S. Piccolella, M. Formato, M.T. Pecoraro, G. Crescente, S. Pacifico; *Journal of Pharmaceutical and Biomedical Analysis*, 201 (2021), 114125.

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]

References

1. Clifford, M. N.; Knight, S.; Kuhnert, N. Discriminating between the Six Isomers of Dicaffeoylquinic Acid by LC-MS^N. *J. Agric. Food Chem.* **2005**, *53* (10). https://doi.org/10.1021/jf050046h.
2. Jaiswal, R.; Matei, M. F.; Golon, A.; Witt, M.; Kuhnert, N. Understanding the Fate of Chlorogenic Acids in Coffee Roasting Using Mass Spectrometry Based Targeted and Non-Targeted Analytical Strategies. *Food Funct.* **2012**, *3* (9), 976–984. https://doi.org/10.1039/c2fo10260a.
3. Badmos, S.; Fu, M.; Granato, D.; Kuhnert, N. Classification of Brazilian Roasted Coffees from Different Geographical Origins and Farming Practices Based on Chlorogenic Acid Profiles. *Food Res. Int.* **2020**, *134*. https://doi.org/10.1016/j.foodres.2020.109218.
4. Schmidt, D.; Hakeem Said, I.; Ohl, N.; Sharifii, M.; Cotrell, P.; Kuhnert, N. Investigating the Interaction between Dietary Polyphenols, the SARS CoV-2 Spike Protein and the ACE-2 Receptor. *Food Funct.* **2022**. https://doi.org/10.1039/d2fo00394e.

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).

References

1. A. Farah, J. De P. Lima, The Royal Society of Chemistry, *Coffee,* London (2019) pp 584–610
2. M.N. Clifford, W. Wu, J. Kirkpatrick, R. Jaiswal and N. Kuhnert, *Rapid Commun. Mass Spectrom,* 24 (2010), 3109–3120
3. S. Badmos, S.-Hun Lee, N. Kuhnert, *Food Research International*, 126 (2019), 108544
4. S. Colomban, E. Guercia and L. Navarini, *J Mass Spectrom, 55* (2020), e4634
5. R.Jaiswal, M.A. Patras, P. J. Eravuchira and N. Kuhnert, *J. Agric. Food Chem.* 58 (2010), 8722–8737
6. G. Baeza, B. Sarriá, L. Bravo and R. Mateos, *J. Agric. Food Chem.* 64 (2016), 9963-9674

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.

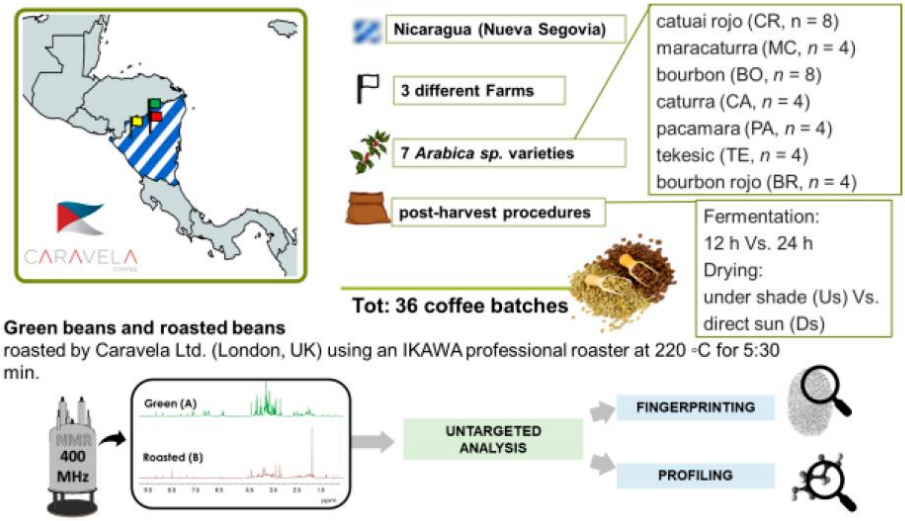

Figure 1. *Experimental scheme*

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.

References

1. Vegro, C.L.R.; de Almeida, L.F; Chapter 1—Global Coffee Market: Socio-Economic and Cultural Dynamics. In *Coffee Consumption and Industry Strategies in Brazil*; De Almeida, L.F., Spers, E.E., Eds.; Woodhead Publishing Series in Consumer Sci & Strat Market; Woodhead Publishing: Sawston, UK, (2020); pp. 3–19. ISBN 978-0-12-814721-4
2. Samoggia, A.; Riedel, B. *Nutrients* (2019), *11*, 653
3. Hameed, A.; Hussain, S.A.; Ijaz, M.U.; Ullah, S.; Pasha, I.; Suleria, H.A.R. *Compr. Rev. Food Sci. Food Saf.* (2018), *17*, 1184–1237
4. Worku, M.; de Meulenaer, B.; Duchateau, L.; Boeckx, P. *Food Res. Int.* (2018), *105*, 278–285
5. De Bruyn, F.; Zhang, S.J.; Pothakos, V.; Torres, J.; Lambot, C.; Moroni, A.V.; Callanan, M.; Sybesma, W.; Weckx, S.; De Vuyst, L. *Appl. Environ. Microbiol.* (2017), *83*, e02398-16
6. Guizellini, F.C.; Marcheafave, G.G.; Rakocevic, M.; Bruns, R.E.; Scarminio, I.S.; Soares, P.K. *Food Res. Int.* (2018), *113*, 9–17
7. Kwon, D.-J.; Jeong, H.-J.; Moon, H.; Kim, H.-N.; Cho, J.-H.; Lee, J.-E.; Hong, K.S.; Hong, Y.-S. *Food Res. Int.* (2015), *67*, 175–182

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*

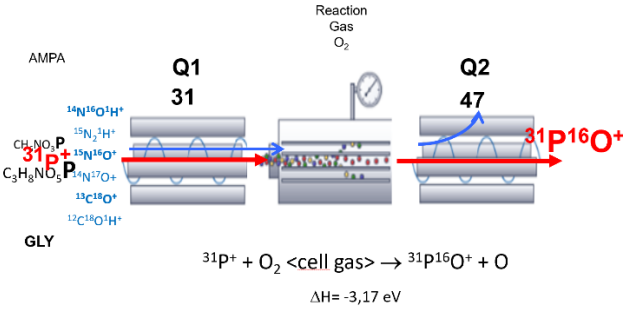
Keywords: *LC-ICP-MS/MS – Gliphosate - Rice*

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.

References

1. Glyphosate detection: methods, needs and challenge A. L. Valle · F. C. C. Mello · R. P. Alves-Balved L. P. Rodrigues· L. R. Goulart · Environmental Chemistry Letters (2019) 17:291–317
2. Direct speciation analysis of organophosphorus environmental pollutants in water by HPLC-ICPMS/MS – B. Lajin, W. Goessler - TalantaVolume 196, 1 May 2019, Pages 357-361
3. Quantification of Glyphosate and AMPA by HPLC-ICP-MS/MS and HPLC-DAD: A Comparative Study – E. M. Pimenta, F F. da Silva, É. S. Barbosa, A. P. Cacique,D. L. Cassimiro, Gevany P. de Pinhoa, F. O. Silvério - J. Braz. Chem. Soc., Vol. 31, No. 2, 298-304, 2020

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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References
1. J. Beauchamp; *Dynamic Flavor: Capturing Aroma Using Real-Time Mass Spectrometry*, Ed. J. Beauchamp, American Chemical Society, vol. 1402 (2021), pp 33-50.
2. J. Beauchamp, F. Biasioli, P. Silcock; *Mass Spectrometry for Food Analysis*, Ed. H. Koolen, Springer (2022), pp 307-320.
3. P. Silcock, M. Alothman, E. Zardin, S. Heenan, C. Siefarth, P. Bremer, J. Beauchamp; *Food Packaging and Shelf Life*, 2 (2014), 81-90.
4. E. Zardin, P. Silcock, C. Siefarth, P. Bremer, J. Beauchamp; *International Dairy Journal*, 62 (2016) 35-8.
5. V. Capozzi, S. Yener, I. Khomenko, B. Farneti, L. Cappellin, F. Gasperi, M. Scampicchio, F. Biasioli; *Journal of Visual Experimentation*, (2017), e54075

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.

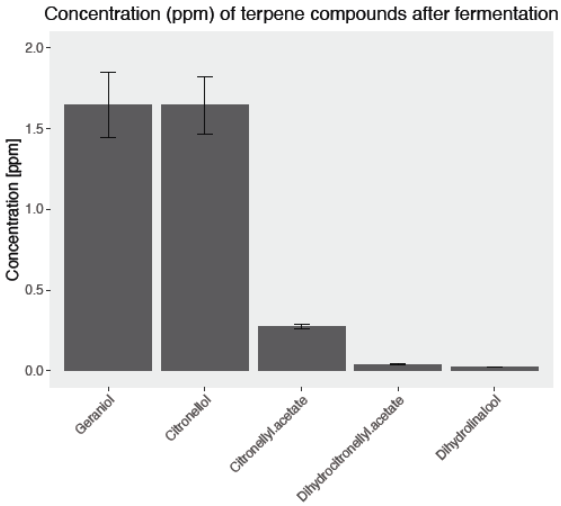
Method and materials

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*

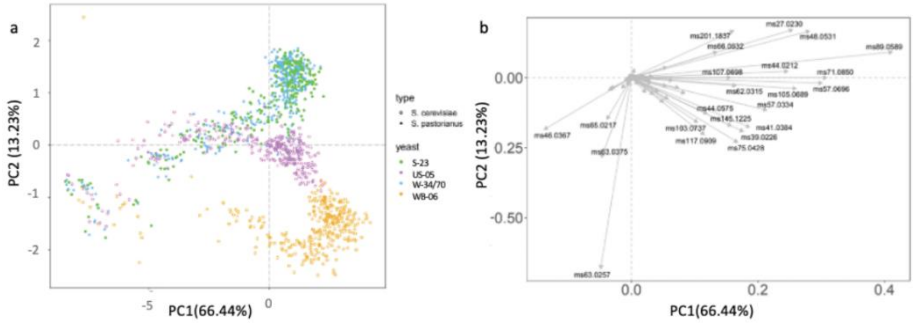


***Figure 2**. Score plot of the principal component analysis (PCA) of volatile organic compound (VOC) (a) and loading plot of PCA of the mass peaks (ms) (b) for yeast: S. cerevisiae (US-05 and WB-06) and S. pastorianus (W-34/70 and S-23). Samples were measured every 6 hours over a 5-day incubation at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

References

1. N. Rettberg, M. Martin and G. Leif-Alexander; *Hop Aroma and Hoppy Beer Flavor: Chemical Backgrounds and Analytical Tools—A Review* (2018), pp 1-20.
2. A. Forster and A. Gahr; *On the fate of certain hop substances during dry hopping* (2013), pp.94-103.
3. A. King and R. Dickinson *Biotransformation of hop aroma terpenoids by ale and lager yeasts* (2003), pp. 53-62.
4. D. Slaghenaufi et al; *Fate of Grape-Derived Terpenoids in Model Systems Containing Active Yeast Cells* (2020), pp 13294-13301.
5. M. Butnariu; *Plants as Source of Essential Oils and Perfumery Applications* (2021), pp 261-292.
6. K. Takoi et al; *Biotransformation of Hop-Derived Monoterpene Alcohols by Lager Yeast and Their Contribution to the Flavor of Hopped Beer (*2010), pp 5050-5058.
7. A. Paiva and L. Hantao; *Exploring a public database to evaluate consumer preference and aroma profile of lager beers by comprehensive two-dimensional gas chromatography and partial least squares regression discriminant analysis* (2020), pp 461529 .

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.

References

1. V. Capozzi, M. Fragasso, F. Bimbo, Fermentation 7 (2021) 54.
2. M.L. Marco, *et al*. Nat Rev Gastroenterol Hepatol 18 (2021) 196–208.
3. J.P. Tamang, K. Watanabe, W.H. Holzapfel, Frontiers in Microbiology 7 (2016).
4. A. Romano, V. Capozzi, G. Spano, F. Biasioli, Appl Microbiol Biotechnol 99 (2015) 3787–3795.
5. V. Capozzi, M. Fragasso, I. Khomenko, P. Silcock, F. Biasioli, in: Dynamic Flavor: Capturing Aroma Using Real-Time Mass Spectrometry, American Chemical Society, 2021, pp. 123–138.
6. F. Biasioli, F. Gasperi, C. Yeretzian, T.D. Märk, TrAC Trends in Analytical Chemistry 30 (2011) 968–977.
7. V. Capozzi, *et al*. J Vis Exp (2017) 54075.
8. S. Makhoul, *et al*. J Mass Spectrom 49 (2014) 850–859.
9. E. Benozzi, *et al*. Food Research International 76 (2015) 682–688.
10. S. Makhoul, *et al*. Food Bioprocess Technol 8 (2015) 1925–1937.
11. H. Campbell-Sills, *et al*. International Journal of Mass Spectrometry 397–398 (2016) 42–51.
12. T.M. Richter, *et al*. Food Research International 111 (2018) 582–589.
13. C. Berbegal, I. Khomenko, P. Russo, G. Spano, M. Fragasso, F. Biasioli, V. Capozzi, Fermentation 6 (2020) 55.
14. A. Yépez, *et al*. Food Microbiology 77 (2019) 61–68.

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.

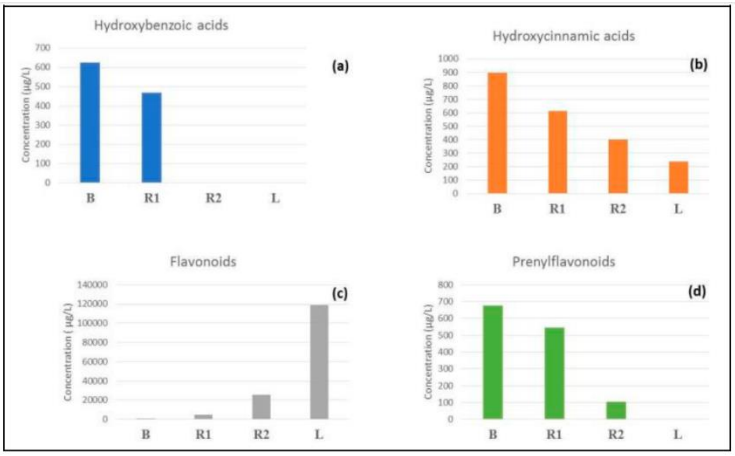

***Figure 1**. Phenolic content in the analyzed beverages (B, R1, R2 and L), assembled as total hydroxybenzoic acids (a), hydroxycinnamic acids (b), flavonoids (c) and prenylflavonoids (d)*

***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.

References

1. Petrucci R., Di Matteo P., De Francesco G., Mattiello L., Perretti G., Russo P.; *J. Agric. Food Chem.* 68 (2020) 7984-7994.
2. Petrucci R., Di Matteo P., Sobolev A.P., Liguori L., Albanese D., Proietti N., Bortolami M., Russo P.; *J. Agric. Food Chem.* 69 (2021) 4816-4826.
3. Di Matteo P., Stoller M., Petrucci R., Russo P.; *Chem. Eng. Trans.* 87 (2021) 13-18.
4. Di Matteo P., Bortolami M., Di Virgilio L., Petrucci R.; *J. Food Sci Technol*. Jun 26 (2021) 1-10.

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.

References

1. R. Flamini, M. De Rosso Mirko, A. Panighel, A. Dalla Vedova, F. De Marchi, L. Bavaresco. *J. Mass Spectrom.* 49 (2014) 1214–1222.
2. A. Calò, A. Costacurta, S. Cancellier, M. Crespan, N. Milani, R. Carraro, M. Giust, E. Sartori, F. Anaclerio, R. Forti, L. Ciprian, R. Di Stefano, R. Pigella, S. Bottero, N. Gentilini. Libra Edizioni, *Delle viti Prosecche ovvero della distinzione fra Prosecco tondo e Prosecco lungo*, Pordenone 2000, Italy.

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)

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

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Summary: *The aim of this work was to evaluate whether three cultivars of Italian olives (Carolea, Nocellara Messinese and Leccino), widely used to produce extra virgin olive oil, could be transformed into table olives. Olives were analysed for phenolic and sugar content before and after their transformation with the Spanish-style method.*

Keywords: *Table olives; Phenolic and sugars determination; Mass spectrometry*

Introduction

All table olive producers require innovative techniques that improve performance and industrial sustainability, as well as the development of new products that specifically respond to increasingly demanding consumers. The aim of this study was the valorisation as table olives of some Italian olive cultivars widely used for extra virgin olive oil production. The need to standardise and industrialise the processing best suited to each cultivar could economically improve the regional productions. The olives of the cultivars *Carolea* (from Calabria), *Leccino* (from Tuscany, but now ubiquitous in Italy) and *Nocellara Messinese* (from Sicily) [1] were analysed for their phenolic and sugar content as soon as they were harvested and after being transformed into table olives with the Spanish-style method, which consists of a treatment with alkaline lye (1.8-2.5%, w/v NaOH) to obtain olive debittering, followed by a washing step to remove the excess alkali. Then, the olives are brined (10-13% (w/v) NaCl) and a spontaneous fermentation take place.[2] In Italian market, *Carolea* and *Nocellara Messinese* are mostly consumed as green table olives,[3-4] while *Leccino* is usually known as black table olive.[5]

Experimental

The olives of *Leccino*, *Carolea* and *Nocellara Messinese* varieties were harvested in October 2020, supplied by organic farms in the regions of origin i.e. Tuscany, Calabria and Sicily, and processed with the Spanish-style method. Olives were analyzed for their content of phenols and sugars as soon as they were harvested and after being transformed into table olives with the Spanish method. Singol phenols and sugars were determined by following extraction procedures optimised at CREA laboratory. The analyses were done by mean of a MSD Sciex API 4000 Q-Trap mass spectrometer. In particular, the crude

methanol extracts containing the phenolic fraction were analyzed in negative ion mode using multiple reaction monitoring (MRM). The analytes were separated on an Eclipse XDB-C8-A HPLC column (5 µm particle size, 150 mm length and 4.6 mm i.d.) at a low rate of 250 µL/min. A binary mobile phase made up of 0.1% aqueous formic acid (A) and methanol (B) was gradient programmed. Sugars were determined in positive ion mode using MRM. For each analyte, the transition of the deprotonated molecular ion $[Sugar\text{-}H]^+$ was scanned on the first quadrupole and its main fragments on the third one $[Sugar\text{-}Cs]^+$. The analytes were separated on a Chromegabond carbohydrate column [(5 µm particle size, 15 cm length and 2.1 mm i.d.) at a flow rate of 300 mL/min. A binary mobile phase consisted of acetonitrile (A) and CsCl H_2O 54 µM (B). Quantitative analyses were achieved by external calibration curves built using a least-squares linear regression analysis with correlation coefficients between 0.9996 and 0.9999.

Results and Conclusions

The concentrations of oleuropein decrease as fermentation progresses. Sodium hydroxide, in fact, cleaves this compound resulting in an increase of hydroxytyrosol concentrations. The richest cv in oleuropein was *Carolea* (325 mg/kg), followed by *Leccino* (104 mg/kg) and *Nocellara Messinese* (72 mg/kg). At the end of the transformation, the values were 3, 0, and 4 mg/kg, respectively. By the contrary, tyrosol increses: from 115.73 to 268.78 mg/kg for *Carolea*; 107.92 to 189.21 mg/kg for *Leccino*; 52.51 to 118.91 mg/kg for Nocellara Messinese. Hydroxytyrosol increses in *Nocellara Messinese* (251.85 to 414.83 mg/kg) only. For *Carolea* and *Leccino* the obtained values were from 488.56 to 403.19 mg/kg and from 326.36 to 52.39 mg/kg, respectively. The concentrations of apigenin, luteolin, diosmetin, luteolin-O-glucoside, verbascoside and p-coumaric acid decrease from the start to the end of the process. The highest content of total sugars, sum of mannose, fructose, glucose and galactose, was recorded in *Leccino* fresh olives (23.7 g/100 g dry weight (DW)) followed by the variety of *Nocellara Messinese* (14.7 g/100g DW) and *Carolea* (12.3 g/100 g DW). At the end of the transformation into table olives, these values were greatly reduced (1 g/100 g DW for *Leccino,* 0.9 g/100 g DW for *Nocellara Messinese* and 0.6 g/100 g DW for *Carolea*).

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References

1. I. Muzzalupo; *Olive germplasm - Italian catalogue of olive varieties, 1st ed.; InTech*: Croatia, 2012; pp. 61–62; 171–172; 223–224.
2. M. Aponte et *al*.; *Food Microbiol*. (2012), DOI: 10.1016/j.fm.2011.10.005.
3. A. Piscopo et *al*.; *Eur. J. Lipid Sci. Technol.* (2016), DOI: 10.1002/ejlt.201500338.
4. A. De Bruno et *al*.; *Emir. J. Food Agr.* (2019), DOI: 10.9755/ejfa.2019.v31.i8.1985.
5. G. Ciafardini and B.A. Zullo, B.A; *Foods* (2020), DOI:10.3390/foods9070941.

P2

Volatile compounds evolution in vegetable oils subjected to mild thermal stress

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Summary: *Volatile compounds, forming during oxidation, are often responsible of the sensory deterioration (rancid odour) of oils. This study evaluates the evolution of the volatile component of different vegetable oils subjected to mild thermal stress for different times using a combination of solid phase micro extraction, gas chromatography and mass spectrometry.*

Keywords: *volatile compounds, vegetable oils, SPME-GC-MS*

Introduction

In view of the ever-increasing consumption of vegetable oils, the attribution of safe, rapid, and efficient qualitative markers are a necessity that cannot be neglected. Nowadays it is possible to find on the shelves of supermarkets many types of refined and non-refined oils, which often (except for oils of perceived superior quality, such as extra-virgin olive oil) are treated by large retailers and by the final consumer with little regard about proper temperature control, lighting conditions stocking etc. This work aims to specifically study the evolution of the volatile fraction of vegetable oils, to possibly identify qualitative parameters capable of effectively identifying specific conditions of abuse for each oil, under conditions of not extreme abuse of temperature and time. In this research 4 oils were evaluated (3 refined and 1 non-refined) under the same abuse conditions.

Experimental

10 g of 4 different oils (extra-virgin olive oil, high oleic sunflower oil, low oleic sunflower oil and grapeseed oil) were placed in 100 ml glass bottles and heated at 100 ° C in a thermostatic oven for different times: 0, 30, 60, 90, 120, 180 and 300 minutes. About 1 g of each treated oil was weighted into a 10 mL vial and by a solid-phase micro extraction (SPME) the volatile fraction was extracted and then injected into a gas-chromatograph equipped with mass spectrometry (GC-MS).

Results

During the heat treatment, , ketones assumed a dominant character within the volatile fraction of all refined oils (high oleic sunflower oil, low oleic sunflower oil and grapeseed oil) ; on the other hand, extra-virgin olive oil, showed aldehydes as predominant group of volatile compounds. High oleic sunflower oil and grapeseed oil presented clear growing trends of development, of aldehydes and ketones, respectively. In accordance with literature [1,2] hexanal, propanone, 2-heptanone, 2-heptenal, and nonanal were the most widespread molecules in all oil samples, showing an increase during time of thermal stress.

Conclusions

By observing the data of this study it is important to note how the development of volatile compounds is not an exclusive factor of the unsaturation degree of the fatty acid composition of the oils e.g. high and low oleic sunflower oil). Besides, this work point out how, even under mild conditions of temperature and time, evolutionary phenomena can be identified in each oil, and some specific volatile compounds, in particular: hexanal, nonanal, 2-heptanone and 2-heptanal could be useful to evaluate oxidative stress in vegetable oils.

References

1. Petersen, K. D.; Kleeberg, K. K.; Jahreis, G.; Fritsche, J. *Int. J. Food Sci. Nutr.* 2012, 63 (2),160–169. https://doi.org/10.3109/09637486.2011.609158.
2. Morales, M. T.; Rios, J. J.; Aparicio, R. *J. Agric. Food Chem.* 1997, 45 (7), 2666–2673. https://doi.org/10.1021/jf960585+.

P3

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

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Summary: *The objective of our work was to determine the concentrations of polyphenols, through the use of LC/HR-MS, in different samples of extra virgin olive oil. Polyphenols contribute to the healthy nutritional benefits attributed to the oil by referring to the food claims which qualify the oil as a functional food.*

Keywords: *Polyphenols, HPLC/ MS, extra virgin olive oil*

Introduction

The "Mediterranean Diet" includes the traditional dietary regimes of the Mediterranean basin and represents a real model of a healthy and sustainable diet. It is based on the prevalent consumption of foods of plant origin such as cereals and derivatives, legumes, fruit, vegetables and extra virgin olive oil (EVOO) and on a moderate consumption of products of animal origin such as meat, dairy products and fish. Thanks to its health properties, EVOO is considered to all intents and purposes a "nutraceutical". The word "nutraceutical" is a combination of nutrition and pharmaceuticals and is a food that provides health benefits [1]. The fundamental constituents of olive oil are triglycerides (98-99%) which represent the saponifiable fraction; while 1-2% of the oil is characterized by the unsaponifiable component: hydrocarbons, phytosterols, fat-soluble vitamins, pigments, aliphatic and triterpene alcohols and polyphenols. [2-4]

Numerous studies have shown how a diet rich in extra virgin olive oil leads to healthy nutritional effects mainly due to the phenolic compound content of EVOO. In fact, phenolic compounds have numerous characteristics:

- contribute to the stability of virgin olive oils;
- the capacity to prevent damage to cells and tissues;
- They have anti-inflammatory and antiviral properties;
- They contribute to the organoleptic properties.

Thanks to these characteristics, the European Food Safety Authority (EFSA) has allowed health recognition based on bio-phenols in olive oil, with reference to food claims, present on the label and summarized in CE Regulation 432/2012, which qualify the oil as a functional food [3].

The concentration and profile of polyphenols depend on many factors, such as the variety of olive cultivars, the degree of ripeness, the climatic conditions, the soil, the irrigation, the technical process for separating the oil and the time and storage conditions [4].

The objective of this study was to determine, through the use of HPLC/MS, the polyphenol content in extra virgin olive oils obtained from different cultivars, from

the use of different pressing methods and delivered in 3 different periods of the year (the first half of November, the second half of November and December) and at the end of selecting, referring to health claims, the oil samples useful to be tested in future clinical trails.
This research was developed with the "Trial: food, nutraceuticals, and health" project, funded by the Production Activities Department of the Sicilian Region as part of the FESR 2014-2020. Scientific partner is the “PROMISE” department flanked by the “ATeN Center"both of the University of Palermo. The project leader is "Manfredi Barbera e Figli" a Sicilian company that deals with the production and marketing of extra virgin olive oil, and "Nuova Farmaceutica" is the partner company in charge of developing some nutraceutical compounds aimed at contrasting four different clinical pathologies.

Experimental
48 samples of extra virgin olive oil (EVOO) arising from the three cultivars (*Nocellara, Biancolilla* e *Cerasuola*) using four different pressing methods were analysed. For each oil sample 2 g of oil were weighed and added to 5 ml of a solution MeOH/H2O 80:20 (v/v). The solution was agitated in vortex for 1 min, placed in ultrasonic bath for 15 min at room temperature and, then, centrifuged at 5000 rpm, at 20°C for 25 min. Supernatant was filtered with PTFE filter (0,45µm)[5] adding 20 µl of internal standard solution (ethyl gallate 1000 ppm) [6][7].
For each analysed analyte a calibration curve was constructed at different concentrations.
Phenolic compounds were identified by ultra-high performance liquid chromatography, heated electrospray and mass spectrometry (UHPLC-HESI-MS).

Results
From the analyses carried out for the determination of polyphenols, with reference to the total of the calculated claims (According to EU Reg. 432/2012, oils must have a polyphenol content: hydroxytyrosol and its derivatives, such as oleuropein and tyrosol, at least equal to 5 mg for 20 g of olive oil)[3], three different samples of EVOO oil were selected: a sample of oil obtained from the Nocellara cultivar with disc pressing method, a sample of oil obtained from the Biancolilla cultivar with the use of the hammer pressing method and finally a sample of oil obtained from the Cerasuola cultivar with the use of the disc pressing method.The first two oil samples from the first delivery (the first half of November) while the sample obtained from the Cerasuola cultivar from the third delivery (December).

Conclusions
Extra virgin olive oil, thanks to the high content of polyphenols that characterizes it, is considered a food with important health characteristics. After selecting the EVOO oil samples based on the total polyphenol content, the next step of this project is to administer them to volunteer patients withvarious pathologies and carry out a real clinical trial.

References

1. D.Bongiorno, V.Di Stefano, S. Indelicato, G. Avellone, L. Ceraulo; *Mass Spec Rev. 2022;e21744*
2. M. Servili, B. Sordini, S. Esposto, S. Urbani G. Veneziani, I. Di Maio, R. Selvaggini, A. Taticchi; *Antioxidants 2014, 3,1-23*
3. Commission Regulation (EU) 432/2012. Off J Eur Union 136:1–40
4. R. Ghanbari, F. Anwar, K. M. Alkharfy, A-M. Gilani, N. Saari; *Int. J. Sci. 2012, 13, 3291-3340*
5. F. Grilo, M.E. Novara, M.C. D'Oca, S. Rubino, R. Lo Bianco, V. Di Stefano; *International Journal of foodSciences and Nutrition*
6. L. Dugo, M. Russo, F. Cacciola, F. Mandolfino, F. Salafia, A. Vilmercati, C. Fanali, M. Casale, L. De Gara, P. Dugo, L. Mondello, F. Rigano; *Food Analytical Methods (2020) 13:1027–1041*
7. A.Rózanska, M. Russo, F. Cacciola, F. Salafia, Z. Polkowska, P. Dugo, L. Mondello; *Foods 2020, 9, 1120*

P4

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

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Summary: *Despite the extensive research some minor components of the extra virgin olive oil (EVOO) polar fraction remain unassigned. A previously undescribed compound, which was detected at varying abundance by HPLC-UV (320 nm) in ~ 75% EVOO samples, was tentatively identified as 3-(4-methylpentyl)-isocoumarin by high resolution-tandem mass spectrometry.*

Keywords: *extra-virgin olive oil; isocumarin derivatives; high-resolution mass spectrometry*

Introduction

Minor components of extra-virgin olive oil (EVOO) have been largely investigated, especially because they include several classes of phenols, which are responsible of chemical stability of oil and health-promoting properties. EVOO minor compounds change both qualitatively and quantitatively depending on many biotic, abiotic, technological and storage factors.
Generally, EVOO (poly)phenols are extracted in hydroalcoholic solutions (*e.g.*, 80% methanol, v/v) and determined by HPLC-UV (280 nm) [1]. This analytical method is used to assess the compliance of EVOO samples with the EFSA recommendation, which allows labelling EVOO with a specific health claim, *i.e.*, "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress", if it contains at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of olive oil [2]. Despite the extensive research, the chemical structure of some minor components of the EVOO "polar" fraction still remains unknown [3].

Experimental

The 80% methanol extracts from more than 100 EVOO samples, including oils obtained at local (Southern Italy) olive mills and some commercial Italian and foreign samples, all collected during the olive oil campaigns 2017-2021, were analyzed by HPLC, adapting the International Olive Council (IOC) method [1]. *HPLC chromatographer*: HP 1100 (Agilent, Palo Alto, CA, USA) equipped with a diode array detector (DAD). *Stationary phase*: C18 reversed-phase column 250 × 2.1 mm i.d., 4 µm particle diameter (Jupiter Phenomenex, Torrance, CA, USA), kept at a 40 °C. Linear gradient: 5% B (0.1 % in TFA acetonitrile) for 5 min and then 5–65% B in 5–65 min, at a 0.2 mL min^{-1} flow rate. *Eluent A* was 0.1% TFA in HPLC-grade water. Monitoring: λ = 254, 280, 320, and 340 nm wavelengths. Selected samples were analyzed using an Ultimate 3000 nanoflow HPLC equipped with a capillary C18 EASY spray column (250 mm x 75 µm i.d.) on-line coupled with a Q Exactive Orbitrap mass spectrometer (Thermo

Scientifics/Dionex, San Jose, CA, USA). *Gradient*: 2-45% B (0.1% formic acid in acetonitrile) in 65 min. *Eluent A*: 0.1% formic acid in HPLC-MS- grade water. Spectra were acquired at 70.000 FWHM resolution in both positive and negative ion modes. The purified unknown molecule was analyzed also by off-line electrospray ionization (ESI)-MS/MS (Q Exactive Orbitrap) and by GC-MS (Agilent 7890A).

Results

HPLC chromatograms at 320 nm of the polar extracts from ~75% of the analyzed EVOO samples contained a previously undescribed component eluting as a sharp peak at high retention time, with variable intensity. This component exhibits a strong UV absorbance max at 315 nm, and in some EVOO samples it was by far the most intense peak detected in the chromatograms at 320 nm (Fig. 1, upper panel). The UV spectrum would suggest a hydroxycinnamic acid-like structure, which was not consistent with its low ionization efficiency in the negative ion mode. The molecular formula of this compounds was $C_{15}H_{18}O_2$ (Δmass = - 0.89 ppm) as inferred by high accuracy measurement of the molecular weight (MH^+ = 231.1383). The MS/MS spectra (both electrospray and electron ionization at 70 eV) contained diagnostic ion fragments of the coumarin (2*H*-chromen-2-one)/isocoumarin (1*H*-isochromen-1-one) derivatives (Fig. 1, lower panel).

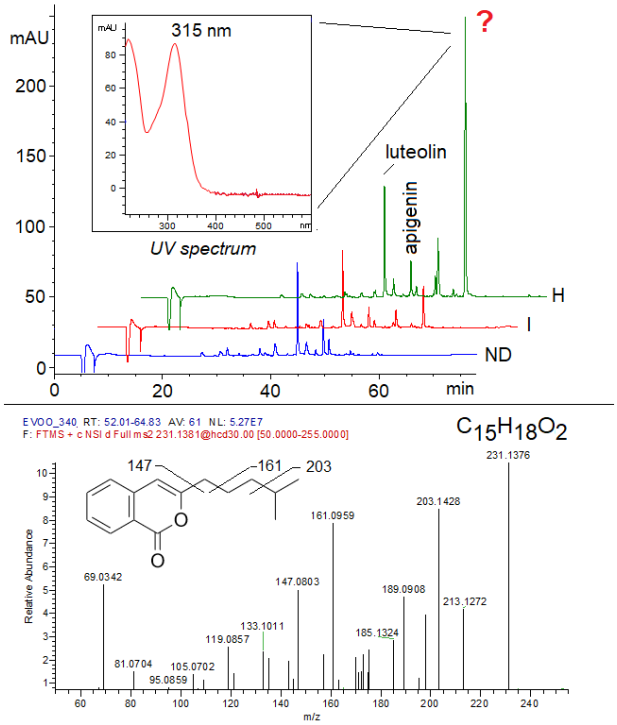

Figure 1. *Upper panel: RP-HPLC chromatogram (320 nm) of EVOO samples containing high (H, green line) intermediate (I, red line) and no detectable (ND, blue line) levels of the unknown. The inset shows the UV spectrum. Lower panel: high resolution ESI MS/MS spectrum of the unknown, with calculated molecular formula and assigned fragments*

Combining spectroscopic (UV) and spectrometric data, the unknown was tentatively identified as 3-(4-methylpentyl)-isocoumarin [*i.e.*, 3-(4-methylpentyl)-1*H*-isochromen-1-one]. The experimental MS/MS spectra of the unknown were compatible with the theoretical fragmentation spectra of 3-(4-methylpentyl)-isocoumarin at varying collision energies (obtained with the Competitive Fragmentation Modeling for Metabolite Identification, software CFM-ID vers. 4.0).

Conclusions

A new isocoumarin derivative has been isolated from several EVOO samples and tentatively identified as 3-(4-methylpentyl)-isocoumarin. Some 3-alkyl-isocoumarin derivatives, such as dihydroartemidin (3-butyl-isocoumarin) and structurally related compounds already described in the lipophilic extracts from plants of the Asteraceae-Anthemidae families, exhibit remarkable antifungal and anti-infective properties [4]. Large-scale purification of the unknown aiming at the definitive structural elucidation by NMR is underway.

References

1. IOC/T.20/Doc No. 29, Official method of analysis. Determination of biophenols in olive oil by HPLC. International Olive Council, Madrid (2009)
2. European Commission Regulation EC No. 432/2012. *Official Journal of European Union* L136 (2012) 1–40.
3. A.L. Capriotti, C. Cavaliere, C. Crescenzi, P. Foglia, R. Nescatelli, R. Samperi, A. Laganà. *Food Chemistry*, 158 (2014) 392-400
4. D. Engelmeier, F. Hadacek, O. Hofer, G. Lutz-Kutschera, M. Nagl, G. Wurz, H. Greger *Journal of Natural Products*, 67 (2004) 19–25.

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

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Summary: *This work explores the extra virgin olive oil volatilome by GC×GC-MS/FID and evaluates the effectiveness of chromatographic fingerprinting in highlighting patterns characteristic for cultivar and cultivation practices (organic vs. conventional). Moreover, by accurate quantification of key-aroma compounds, aroma blueprint is unraveled completing the identitation process.*

Keywords: *comprehensive two-dimensional gas chromatography; untargeted/targeted UT fingerprinting, extra-virgin olive oil; organic cultivation, aroma blueprint.*

Introduction

Extra virgin olive oil (EVOO) represents the premium subcategory of virgin oils. It is obtained from the fruit of the olive tree exclusively by mechanical or other physical processes under conditions that do not compromise its quality and/or induce alterations. In recent years, olive cultivation has undergone a transition to cultivation with less impact on the ecosystem and/or following organic practices. To assess the possible impact of different olive trees cultivation methods on the aroma profile, comprehensive two-dimensional gas chromatography coupled with parallel mass spectrometry and flame ionization detection (GC×GC-MS/FID) was chosen. In fact, GC×GC-MS/FID leads to a high-performance analysis strategy capable of fully exploit the information encrypted on the volatile fraction including also those key-analytes responsible of the EVOOs aroma blueprint.
Moreover, the complementary characteristics of MS and FID open the possibility of performing multi-target quantitative profiling by predicted relative response factors with great accuracy [1].

Experimental

Samples were provided by University of Sevilla and consisted of EVOOs from Spanish (n=16) 2020 harvest year. Olives were from two different cultivars (*Picual* and *Hojiblanca*) and followed different cultivation methodologies (organic

vs. conventional).
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 60 min on a 0.100 g of EVOO in a 20 mL HS vial. Untargeted/targeted fingerprinting (*UT* 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 [2].

Results
The volatilome of high quality extra virgin olive oil encrypts information regarding the *cultivar,* the pedo-climatic conditions of the harvesting region and soil composition, the ripening stage of the olives, the cultivation methods and processing technologies used to obtain the oils, and the storage conditions. A total of 189 reliable peaks (including targeted and untargeted features) were found in the samples volatilome, 84 of which were identified through the use of Van den Dool and Kratz retention indices and EI-MS spectral similarity with reference in commercial and in-house databases. An initial unsupervised statistical analysis, *i.e.* Principal Component Analysis (PCA) on a single olive *cultivar* showed a natural clustering according to cultivation methodologies (Fig. 1).

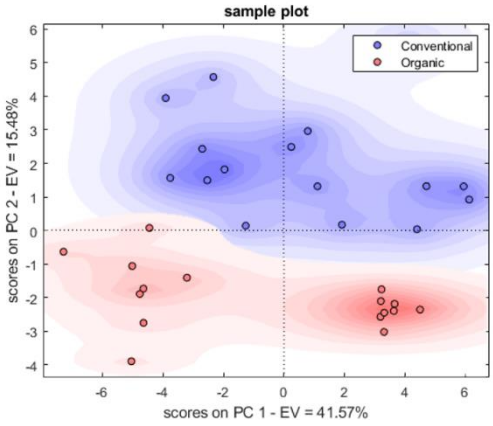

Figure 1*. PCA on Organic vs. Conventional Picual extra-virgin olive oils*

By supervised statistics, Partial Least Squares Discriminant Analysis (PLS-DA) compounds with greater discriminating ability were screened. Sixtheen compounds obtained a Variable Importance on the Projections (VIPs) score greater than 1, thus considered significant by the classification algorithm; among these are 2-octanone, 2-heptanone and butanoic acid, whose relative distribtion

illustrated by box-plot on raw 2D volumes is shown in Fig. 2.

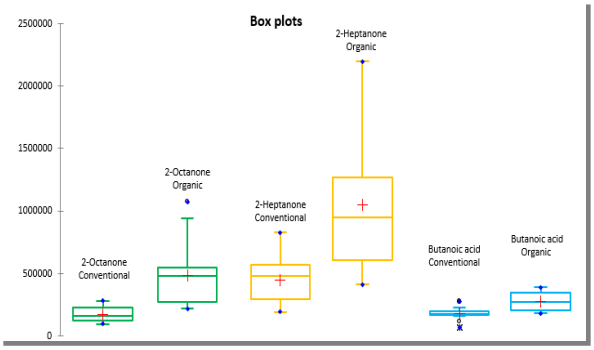

Figure 2. *Raw volumes of 2-Octanone, 2-Heptanone and Butanoic acid in Picual Organic and Conventional*

The last step of the stydy consisted in the definition of samples aroma blueprint. Specifically, 42 key odorants were accurately quantified; in order to understand the impact of each compound on the general flavour sensory maps obtained from Odor Activity Values (OAV) were created [3]. As shown in Fig. 3, the two cultivation methodologies did not impact on the overall aroma, despite the fact that specific chemical compounds such as eucalyptol (1,8-cineol - *herbal*, *minty*), α-pinene (*herbal*) and (E,E)-2,4-hexadienal (*fatty waxy*) were more abundant in the organic samples.

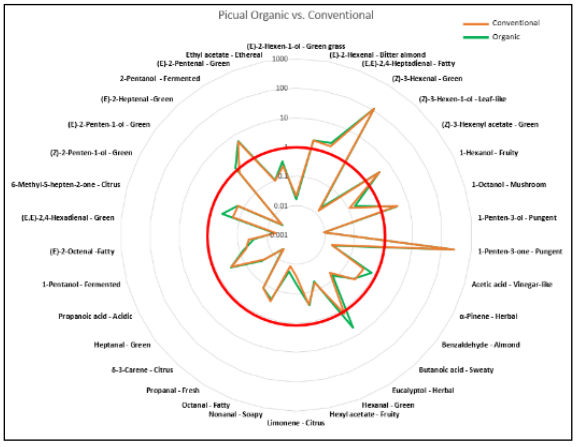

Figure 3. *Sensory map of Picual Organic vs. Conventional extra-virgin olive oils*

Conclusions

HS-SPME combined to GC×GC-MS/FID and accurate quantification by predicted RFF resulted to be a great tool in the quality assessment of EVOO samples. By effective exploration of the information encrypted in EVOOs

volatilome the impact of functional variables (e.g., cultivar and cultivation methodologies) is reliably correlated to diagnostic patterns with great classification and *identitation* [5] attitudes. By the accurate quantification of key-odorants an Artificial Intelligence smelling machine is realized with peculiar comparative possibilities for EVOOs aroma qualities.

References

1. F. Silo, M.d.P. Segura Borrego, C. Bicchi, S. Battaglino, R.M. Callejòn Fernandez, M.L. Morales, S.E. Reichenbach, J. Mccurry, D. Peroni, C. Cordero; *Journal of Chromatography A*, 1650 (2021), 462232
2. F. Stilo, E. Liberto, S.E. Reichenbach, Q. Tao, C. Bicchi, C. Cordero, *J. Agric. Food Chem*., 69 (2019), 8874-8889.
3. Neugebauer, A.; Granvogl, M.; Schieberle, P. *J. Agric. Food Chem*. 68 (2020), 5927–5937
4. Vichi, S.; Pizzale, L.; Conte, L.S.; Buxaderas, S.; López-Tamames, E. *J. Agric. Food Chem*. 51 (2003), 6572–6577.
5. Cuadros-Rodríguez, L.; Ruiz-Samblás, C.; Valverde-Som, L.; Pérez-Castaño, E.; González-Casado, A. *Anal. Chim. Acta* 909 (2016) 9–2

P6

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

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Summary: *In this work a new method based on LC-HRMS was developed in order to characterize the biophenolic profile of extravirgin olive oil and to study the effect of storage on its composition.*

Keywords: *olive oil, secoiridoids, LC-HRMS*

Introduction

The beneficial effects of Mediterranean diet on human health have been mainly attributed to the high consumption of extra virgin olive oil (EVOO), which is an important bioactive food for the nutraceutical properties of its antioxidant components. The main antioxidants in EVOO are lipophilic phenols, such as tocopherols, and hydrophilic phenols, including flavonoids, phenolic alcohols and acids, secoiridoids and their metabolites. Phenolic alcohols and acids, and secoiridoids are known as biophenols. Recently, the beneficial effects of EVOO on human health have been attributed to biophenols, showing antioxidant, anti-inflammatory and antithrombotic activities in humans. In this regard, the European Food Safety Authority (EFSA) approved a health claim by which the cardiovascular protective effects of some olive oil phenols are recognized: *"Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress. The claim may only be used for olive oil, containing at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and thyrosol) for 20 g of olive oil. In order to bear the claim, the consumer should be informed that the beneficial effect is obtained by the daily intake of 20 g of olive oil"* (EFSA claim No. 432/2012) [1]. This ealth claim refers to polyphenols containing tyrosol (p-hydroxy-phenylethyl alcohol, HPEA, Tyr) and/or hydroxytyrosol (3,4-dihydroxy-phenylethyl alcohol, 3,4-DHPEA, Htyr) in their structure, which are the most abundant phenolic alcohols found in olives together with their secoiridoids derivatives. Secoiridoids are present in EVOO mainly as oleacin, oleocanthal, oleuropein and ligstroside aglycones, while in olive fruits and leaves they appear as glycosilated forms of oleuropein, dimethyloleuropein, ligstroside and verbascoside [2] (Fig. 1). Considering that an official method for assessing secoiridoids composition of EVOO is not available, the possibility to obtain these pure molecules would allow to develop a new method in which single components may be used as analytical standard to define oil biophenolic profile.

Experimental

The present work has been focused on the development of an analytical method to characterise the biophenolic profile of two monovarietal olive oils produced in Calabria region (12 samples Nocellara di Belice, NB, and 12 samples Dolce di Rossano, DR). In addition, the second purpose of the work was to verify the stability of their secoiridoids content over a period of one year, by carrying out a

monthly control, in order to assess the preservation of secoiridoids during storage and to demonstrate the compliance with the EFSA claim. The identification and quantification of biophenols was performed by ultra-high performance liquid chromatography (UHPLC) coupled to electrospray high resolution mass spectrometry (ESI-HRMS), using analytical standards, synthesized in house [3; 4], for calibration of each single EVOO biophenols.

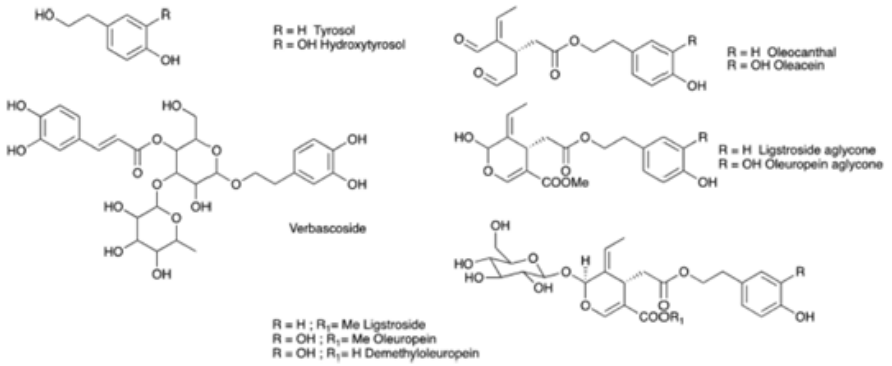

Figure 1. *Chemical structure of main secoiridoids and secoiridoid metabolites according to EFSA claim*

Results

As expected, in both monovarietal oils analysed, major secoiridoids founded were oleacin, oleocanthal and oleuropein aglycone, while oleuropein and verbascoside were not detected. Htyr concentration increases gradually over the year, as consequence of normal degradation of its derivatives; while concentrations of others secoiridoids quantified were constant over time. In DR monovarietal oil, compared to NB oil, a higher concentration of oleuropein aglycone was observed, while oleacin and oleocanthal were founded at lower concentration.

Conclusions

In conclusion, our results demonstrated that both oils are in line with the EFSA claim during the year of analysis, considering that their biophenols concentration is almost five times higher than required. This method is non-invasive and can be a useful tool for producers to recognize the claim on the label, thus giving added value to products. Thereafter, additional analysis will be necessary to finalize the method.

References

1. European Community, Council Regulation No. 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk, to children's development, health, Off. J. Eur. Union, 2012, L136,1
2. Oliverio M, Nardi M, Di Gioia ML, Costanzo P, Bonacci S, Mancuso S, Procopio A. *Nat Prod Rep.* 2021 Mar 1;38(3): 444-469. doi: 10.1039/d0np00084a. Epub 2020 Dec 10

3. Mancuso S, Costanzo P, Bonacci S, Nardi M, Oliverio M, Procopio A. *ACS Sust Chem & Eng*, 2021; 9, 12614-12622 DOI: 10.1021/acssuschemeng.1c04030
4. Costanzo P, Bonacci S, Cariati L, Nardi M, Oliverio M, Procopio A. *Food Chemistry,* 2018; 245, 410-414. https://doi.org/10.1016/j.foodchem.2017.10.097

P7

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

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Summary: *The aim of this work was the validation of an optimized analytical method suitable to the analysis of pesticides from different chemical classes in vegetable oils using an easy extraction and clean-up procedure followed by a selective, repeatable and robust instrumental method, such as gas and liquid chromatography coupled with triple quadrupole mass spectrometry detection.*

Keywords: *Pesticides, Vegetable Oil, Gas and Liquid Chromatography, Mass Spectrometry*

Introduction

Vegetable oils, obtained from fatty fruits and oil seed crops, are rich in saturated and unsaturated fatty acids, triglycerides, antioxidants, and other fat-soluble vitamins. Thanks to their composition, vegetable oils represent an important source for human nutrition and therefore they are widely used for cooking and in food industry [1]. Within the group of vegetable oils, in Europe olive oil is the most demanded by consumers, whereas peanut oil and soybean oils are largely consumed in other parts of the world. Pesticides are chemicals extensively used in agriculture worldwide. They belong to different chemical classes and perform various functions, such as herbicides, fungicides, insecticides. Although pesticide employment represents an important tool to preserve plants health, their entrance into the food chain carries potential risks in terms of food safety. In the literature, several studies are reported concerning the toxic effects of high levels or prolonged exposure to pesticides, such as cancer, neurodegeneration, reproductive and endocrine disorders. For this reason, competent authorities have developed legislation to regulate pesticide usage and maximum residue levels (MRLs) accepted in food [2]. Therefore, official European laboratories for food safety are required to develop analytical methods fit for verifying that pesticide residues in food are in compliance with the prescribed limits and for research purposes also.

The aim of this work was the optimization and validation of an analytical method suitable for the detection of more than 150 different pesticides belonging to several chemical classes using Gas and Liquid Chromatography coupled with Triple Quadrupole Mass Spectrometry (GC-MS/MS and LC-MS/MS) in vegetable oils according to the criteria established by SANTE/11312/2021 [3] taking into account maximum residue levels (MRL) indicated by Regulation (EC) No 396/2005[2]. The optimized method was characterized by fast, simple and repeatable extraction and clean-up methods and by good robustness, be

suitable for routine analysis in an Official Laboratory.

Experimental

Starting from the indications described in the QuOil method [4], the laboratory optimized the method for the determination of more than 150 pesticides. Two different commodity groups from SANTE/11312/2021 Annex A [3] (4a-high oil content and very low water content; 4b-high oil content and intermediate water content) were taken into account during the method optimization and validation. Olive oil was used as reference matrix to perform optimization and validation experiments. Accurate amounts of labelled internal standards were added to each sample to take under control analytical procedure and extraction efficiency. Moreover, some labelled internal standards were used for the quantification by internal calibration curve for some pesticides. For the extraction, an amount of 2.5 g of oil sample was weighed into a 50 mL polypropylene tube; then 2.5 mL of n-hexane were added and shaken manually for 30 seconds. A volume of 10 mL of acetonitrile was added, the tube was placed on an orbital shaker for an extraction time of 10 minutes and then it was centrifuged at 1000 rpm for 5 min. After centrifugation, the supernatant phase was cleaned up using two different procedures depending on the specific instrumental analysis used for the quantification phase. For GC-MS/MS analysis, a pass-through clean-up process was performed using Oasis PRIME HLB cartridge (3cc-150 mg). At first, 1 mL of extracted solution was applied to the SPE cartridge for conditioning the stationary phase; successively 1.5 mL of extracted solution were applied on the cartridge, eluted dropwise under atmospheric pressure and they were collected and transferred into a glass vial, ready for the Gas Chromatography injection. For LC-MS/MS the extract clean-up process was performed using dSPE for Fruits and Vegetables with Fats and Waxes, containing 400 mg PSA, 400 mg C18, 1200 mg $MgSO_4$. A volume of 6 mL of extracted solution was transferred into a 15 mL polypropylene tube containing the cleaning up phase; the tube was manually shaken for 30 seconds; then it was centrifuged at 4000 rpm for 5 min. Finally, the purified extract was transferred into a glass vial, ready for the Liquid Chromatography injection. The GC-MS/MS instrumental analysis was carried out with a 7890B GC coupled with 7010 triple quadrupole (Agilent Technologies). The separation was performed using a column ZB-MultiResidue-1 (30 m, 0.25 mm ID, 0.25 µm film - Phenomenex). The LC-MS/MS instrumental analysis was carried out with a 1290 UPLC coupled with 6470 triple quadrupole (Agilent Technologies). The separation was performed using a column Acquity UPLC HSS T3 (100 mm, 2.1 mm, 1.8 µm - Waters) equipped with a pre-column Acquity UPLC HSS T3 (5 mm, 2.1 mm, 1.8 µm - Waters).

Results

All parameters required by SANTE/11312/2021 [3] were considered in validation study. Specificity was studied through the response obtained from reagents blank and blank control samples: the absence of significant interferences around the characteristic retention times for each pesticide was verified. Blank oil samples were spiked at different levels and processed both to identify appropriate quantification limits (LOQ), and to determine recovery (trueness), precision and accuracy of the method according with requirements of reference

document [3]. For the most analytes, quantitative analysis was carried out using external matrix-matched calibration curve (1/x regression line), whereas for some pesticides an internal matrix-matched calibration curve was used to improve the trueness.
During validation study, precision and trueness were checked at LOQ, 5xLOQ and safety levels; mean recoveries (trueness) ranged between 60 and 120% and Relative Standard Deviation (RSD) was below 20%, in repeatability condition. Accuracy was also estimated analysing different samples coming from inter-laboratory tests obtaining acceptable results for different vegetable oils analysed in the same analytical batch.
Obtained results appeared very satisfying and coherent with criteria indicated in SANTE/11312/2021 Document [3].

Conclusions

The analytical method optimized and validated is suitable to the routinely application in an Official Laboratory, thanks to its simple and fast extraction and purification procedures and to the method accuracy and robustness. The method allows to quantify pesticides in vegetable oils belonging to several chemical classes to guarantee the conformity evaluation in relation to the maximum residue limits in current regulation [2].

References

1. Gunstone FD. Production and trade of vegetable oils. Vegetable oils in food technology: composition, properties and uses, second edition. Wiley-Blackwell; 2011.
2. Regulation EC/396/2005, (consolidated versions to February 2022). Regulation EC/396/2005 of the European Parliament and of the Council of February 23 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. Official Journal of the European Union.
3. Document No. SANTE/11312/2021. Analytical quality control and Method validation and procedures for pesticide residues analysis in food and feed. European Commission Directorate-General for Health and Food Safety. Implemented by 01/01/2022.
4. Food of plan origin – Multimethod for the determination of pesticide residues in vegetable oils by LC-MS/MS (QuOil).

P8

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

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Summary: *An oleuropein-rich leaf extract (ORLE) was administered at low-dose for one week to PIRC rats spontaneously developing multiple tumors in the colon and small intestine. The untargeted metabolomics approach allowed to check the impact of ORLE on plasma metabolome, together with evaluating the modifications of parent phenolic compounds.*

Keywords: *Oleuropein; Foodomics; UHPLC-HRMS*

Introduction

Plasma represents an ideal biological fluid to evaluate the physiologic effects of dietary exposures and major changes in metabolites composition [1]. Therefore, the aim of this study was to explore whether an oleuropein-rich leaf extracts (ORLE) potentially modulated the plasma metabolome of PIRC rats carrying a heterozygous germline mutation in the Apc gene. The APC mutation is the first event triggering colon carcinogenesis both in the majority of sporadic cases and in hereditary forms. PIRC rat spontaneously develops multiple tumors in the colon and small intestine, thus standing as a robust model to study the protective effect of ORLE on colon cancer progression [2]. We used an untargeted metabolomics approach to assess a potential modulation of plasma metabolome in Apc-mutant rats, following the intake of an ORLE-enriched diet. Also, the screening of phenolic metabolites was investigated using the same approach.

Experimental

Organic olive (Leccino cultivar) leaves (harvested in Tuscany, Vinci, Florence, Italy) were processed to obtain a powder extract [3] having a total phenolic content of 383 mg/g, of which oleuropein was 298.48 mg/g [4]. PIRC rats (F344/NTac-Apcam1137) aged 12 months were randomly assigned to an AIN-76 diet (Control group: four males, three females) and to the same diet containing

ORLE (2.7 g/kg of diet) (ORLE group: three males, three females), administering a dose of 100 mg ORLE/kg b.w. [2]. Plasma samples were then collected after one week of treatment. Then, 100 µL of plasma samples were extracted with 400 µL of methanol-acetonitrile (1:1, v/v) acidified with 3% formic acid [5] and analyzed for ultra-high-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) analysis, based on a Q-Exactive™ Focus Hybrid Quadrupole-Orbitrap mass spectrometer [6]. Finally, multivariate statistical analysis was done using two different softwares, namely SIMCA 16.0 and MetaboAnalyst 5.0 [7].

Results

Untargeted metabolomics allowed to structurally identify 271 metabolites in plasma sample against the comprehensive database Mass Bank of North America (MoNA). The most represented classes were amino acids, peptides, glycerophosphocholines, fatty acids and derivatives, prenol lipids, and glycerophosphoethanolamines. Multivariate statistics based on orthogonal projections to latent structures discriminant analysis (OPLS-DA) clearly separated the plasma samples as a function of the one week-low-dose treatment with ORLE- enriched diet (Fig. 1A). The discriminant model was characterized by excellent goodness parameters, being the Q^2 value (goodness of prediction) = 0.911. Looking at the OPLS-DA score plot, a secondary impact of sex (females *vs* males) on the modification of plasma metabolomic profile was detected. Thereafter, both enrichment and pathway analyses were done to evaluate the discriminant VIP compounds driving the separation observed. Overall, 165 compounds showed a significant prediction ability, with LysoPC (16:0) (LogFC = -1.89) and sperminidine (a potential cancer-related marker, having a LogFC = 2.32) being the most discriminant for the comparison ORLE *vs* Control. The most enriched metabolites belonged to aminoacids and diacylglycerophospho-ethanolamines, as showed in Fig. 1B. The pathway analysis (not shown) revealed a perturbation of four metabolic routes, namely glycerophospholipid metabolism, lysine degradation, phenylalanine metabolism, and retinol (vitamin A) metabolism. Overall, these discriminant pathways were coherent with the control diet administered to rats.

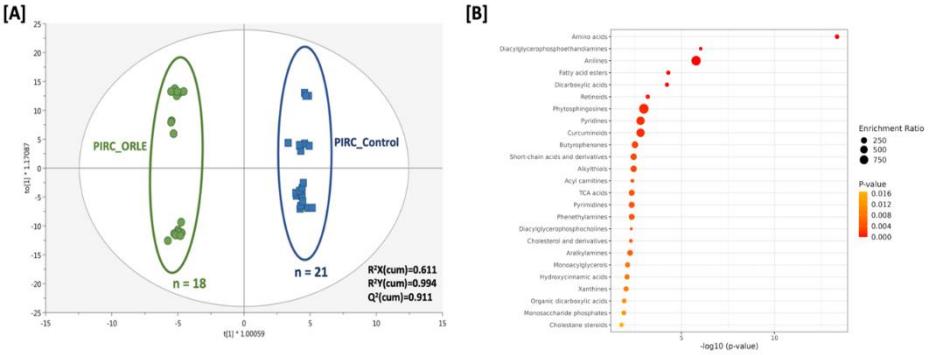

***Figure 1**. OPLS-DA score plot built considering the metabolomic profile of plasma samples [A] and enrichment analysis showing the most represented classes of metabolites following ORLE administration [B]*

Regarding the phenolic profile of plasma samples, nine discriminant metabolites could be detected (Table 1). Overall, oleuropein-aglycone (a deglycosylation product of oleuropein) showed the highest LogFC values (3.60), being a marker of ORLE dietary group. Interestingly, we found also typical microbial metabolites of oleuropein, namely catechol (the final product of the hydrolysis and oxidation of oleuropein-aglycone), eriodictyol (derived from the catabolism of flavones, such as luteolin) and hippuric acid (the glycine conjugate of 4-hydroxybenzoic acid likely derived from oleuropein). Finally, typical phase-II metabolites of phenolics (i.e., glucuronides and sulfates, Table 1) showed a significant increase in PIRC_ORLE plasma samples, thus indicating a metabolization of the parent compounds characterized the raw ORLE [2].

Table 1. *VIP discriminant phenolic metabolites following the OPLS-DA prediction model reported with their VIP score and Log Fold-Change (FC) values for the comparison ORLE vs Control groups.*

VIP phenolic compound (OPLS-DA)	**VIP score (OPLS-DA)**	**Log FC (p < 0.05) [PIRC_ORLE *vs* PIRC_Control]**
Oleuropein-aglycone	1.38 ± 0.45	3.60
Luteolin	1.44 ± 0.61	2.76
Caffeic acid	1.47 ± 0.39	1.92
Luteolin 7-*O*-glucuronide	0.87 ± 0.37	1.88
Hippuric acid	1.21 ± 0.38	1.78
Caffeic acid 3-*O*-glucuronide	0.90 ± 0.59	1.48
Eriodictyol	1.57 ± 0.64	1.46
Caffeic acid 4-sulfate	1.37 ± 0.78	1.02
Catechol	1.24 ± 0.74	0.83

Conclusions

The present study assessed whether one week-low-dose treatment with an ORLE- enriched diet significantly affected the plasma metabolome and phenolic profiling of PIRC rats. Several phenolics metabolites of oleuropein were detected in plasma samples, potentially involved in anti-inflammatory mechanisms. However, few differences were observed when considering the modulation of plasma metabolome, with some potentially cancer-related compounds showing not expected LogFC values.

References

1. I. Zarei, B.A. Baxter, R.C. Oppel, *et al*. *Cancer Prevention Research*, 14 (2021), 497-508.
2. J. Ruzzolini, S. Chioccioli, N. Monaco, *et al*. *Antioxidants*, 10 (2021), 1577.
3. A. Romani, M. Campo, S. Urciuoli, *et al*. *Frontiers in Nutrition*, 7 (2020), 120.
4. G. Rocchetti, M.L. Callegari, A. Senizza, *et al*. Food Chemistry, 380 (2022), 132187.
5. X. Zhang, B. Hu, G. Sun, *et al*. *Journal of Pharmaceutical and Biomedical Analysis*, 211 (2022), 114590.
6. G. Rocchetti, S. Michelini, V. Pizzamiglio, *et al*. *Food Research International*, 149 (2021), 110654.
7. Z. Phang, G. Zhou, J. Ewald, *et al*. *Nature Protocols*, (2022), doi.org/10.1038/s41596-022-00710-w.

P9

Spectrometric and spectroscopic metabolomic profile of *Coffea arabica* leaves

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Summary: *Coffea arabica leaves were extracted using ultrasound assisted extraction, the extracts were characterized using NMR and untargeted data independent HPLC-MS^n approaches. A total of 39 compounds were identified and confirmed through comparison with standards and MS^n databases. The identified bioactive compounds proved the strong potential of C. arabica leaves as functional food.*

Keywords: *Coffee leaves; tandem mass spectrometry*

Introduction

Coffea arabica leaves represent a viable alternative to traditional matrices used for preparing beverages as tea leaves and grounded coffee beans. Coffee leaves infusions are rich in antioxidant phenolic compounds, along with a lower concentration of caffeine [1]. Considering the promising beneficial effects on human health and the growing interest in the applications of coffee leaves, it is necessary to fully understand the secondary metabolites profile and all factors affecting the phytochemical composition [2]. The present study aims to characterize coffee leaves extracts from *Coffea arabica* cultivar to empower the knowledge on antioxidant compounds present in coffee leaves using un-targeted high throughput techniques as tandem mass spectrometry coupled with high performance liquid chromatography (HPLC-MS^n) and nuclear magnetic resonance (NMR).

Experimental

The leaves were collected in Colombia, in the Department of Huila at Garzon at an altitude of about 1700 m asl on a cultivation of *Coffea arabica* "Castillo" variety. The leaves were freeze-dried, cold crushed and then extracted. The NMR analysis were performed using a Bruker DRX-600 Avance spectrometer operating at 600.13 MHz for ^{1}H, equipped with an xyz gradient unit. To characterize the extracts a data dependent untargeted approach was used to collect information on the structure and the fragmentation of the various analytes. In each MS scan both in positive and negative mode, the most abundant ion was fragmented through CID (collision induced dissociation), generating a MS^2 spectra, the first and second most abundant ions were then fragmented again in two separate steps, generating two different MS^3 spectra. The generated

fragmentation tree was used to preliminary identify the constituents of the extracts. The MS full scan analysis were in the range of m/z 100-1000. The collision energies were 35 nCE (normalized collision energy) and 45 nCE respectively for MS^2 and MS^3 fragmentation steps. The chromatographic elution was optimized on a biphenyl column using gradients composed of H_2O (A) and CH_3CN (B) both acified by 0.1% of formic acid.

Results

NMR Spectroscopy

NMR spectroscopy was used to characterize the main components of the extract without any further purification, the acquired ^1H spectrum for the coffee leaves extract reconstituted in deuterated methanol is reported in Fig. 1, the signals reported in the spectrum were analyzed and compared with the available standards, already published papers [3] and databases (HMDB, BMRB).

NMR analysis allowed the identification of 17 compounds, including alkaloids (trigonelline and caffeine), flavonoids (rutin, mangiferin), hydroxycinnamic acids derivatives (chlorogenic acids) and primary metabolites (aminoacids and organic acids).

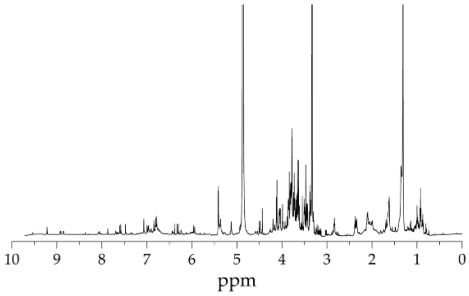

Figure 1. *^1H NMR spectrum of C. arabica leaves extract*

HPLC-MS^n

Two chromatographic gradients were optimized for extracts characterization, the data dependent approach allowed for the identification of 39 compounds. In the ESI negative chromatogram reported in Fig. 2, five chlorogenic acids derivatives were identified in total, and three compounds characterized as xantones were (mangiferin, isomangiferin and 6-O-(p-hydroxybenzoyl) mangiferin). Flavonoids were constituting a large part of compounds observed, together with procyanidins [4]. In addition, two lignans isomers were identified.

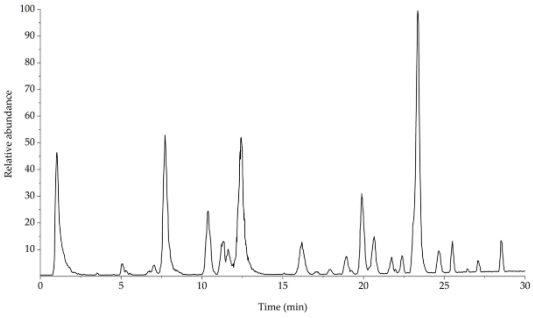

Figure 2. *HPLC-ESI(-) chromatogram of C. arabica leaves extract*

For the identification of alkaloids, the ESI positive mode was used to acquire the mass chromatogram. Two main alkaloids were identified as trigonelline and caffeine (Fig. 3) [4].

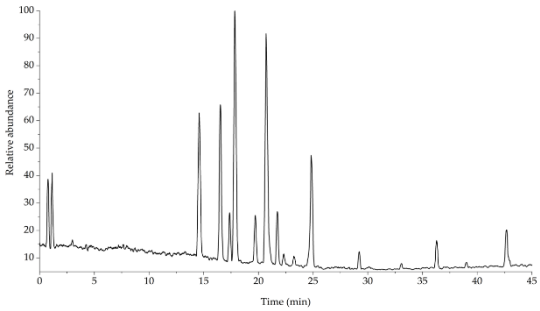

***Figure 3**. HPLC-ESI(+) chromatogram of C. arabica leaves extract*

The main components of the *Coffea arabica* leaves extracts were quantified in both positive and negative mode. Single Ion Monitoring (SIM) method was used for the quantitation of all caffeoylquinic derivatives, mangiferin and alkaloids. The concentration data are comparable with the amounts reported in literature, only the concentration of 5-CGA differ from the data reported in other studies with slightly lower values [5], a suggested explanation could be addressed to important variability factors such as fluctuations in environmental, atmospheric and agronomic treatments.

Conclusions

A combined NMR and HPLC-MS^n approach has been developed with the aim to separate, identify and quantify bioactive components in plant matrices belonging to the *Coffea arabica* family. These molecules are important components of the pool of natural compounds responsible for the main beneficial effects of coffee leaves, as regards the antioxidant activity. The results emphasize how coffee leaves represent an important source of bioactive compounds as functional foods. The combined use of two powerful techniques, chromatography coupled with tandem mass spectrometry and NMR spectroscopy allowed the characterization of secondary metabolites.

References

1. Chen, X. (2019). *Critical reviews in food science and nutrition*, *59*(6), 1008-1025.
2. Campa, C., Mondolot, L., Rakotondravao, A., Bidel, L. P., Gargadennec, A., Couturon, E., ... Davis, A. P. (2012). *Annals of Botany*, *110*(3), 595-613.
3. Gogna, N., Hamid, N., Dorai, K. (2015). *Journal of pharmaceutical and biomedical analysis*, *115*, 74-85.
4. Mesquita Júnior, G. A. D., da Costa, Y. F. G., Mello, V. D., Costa, F. F., Rodarte, M. P., Costa, J. D. C. D., ... Vilela, F. M. P. (2022). *Phytochemical Analysis*.
5. Monteiro, Â., Colomban, S., Azinheira, H. G., Guerra-Guimarães, L., Do Céu Silva, M., Navarini, L., Resmini, M. (2019). *Antioxidants*, *9*(1), 6.

P10

The chemistry of the temporal evolution of coffee flavor quality

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Summary: *Coffee is a beverage becoming an asset of socialization. It is appreciated, above all, for its taste and aroma, which confer a unique qualitative feature. Even if coffee is a stable food product, it could undergo to a series of chemical and physical reactions, bringing to a change in sensorial quality and acceptability.*

Keywords: *packaging, aroma and taste*

Introduction

There isn't anything so delicious and tantalizing as the smell of freshly brewed coffee. This is due to the potent flavour and aroma given off by coffee during the roasting process. Due to coffee sensory properties and its important financial value, it is essential to preserve and maintain its quality [1]. Coffee quality is a multidimensional attribute, influenced by a series of chemical precursors, environmental factors and above all technological processes, such as roasting, and storage conditions, involving sensory and chemical properties [2,3]. The storage, of whole or ground coffee beans, is strongly influenced by different environmental conditions. Humidity, temperature and oxygen are the basic dynamic forces that play a fundamental role in a serious of deteriorative processes, like volatilization of odorant molecules (VOCs), CO2 release, oxidative reactions with the formation of off-notes and rancidity development [2,4-6]. Coffee staling, due to the loss of aroma freshness with the aging, has been studied extensively, since it determines the consumer's acceptability and delineate the shelf-life of the product [7]. In industries, the sensory tests are a daily routine analysis used to evaluate the quality, the effect of the environment on the storage conditions and the packaging effects on the food products. The sensory techniques presented some limitations, such as low repeatability, reproducibility, time and costs [8-9]. For these reasons, nowadays, we are looking for potential alignment with the analytical instrumental measurements, capable to sample, separate, identify and extrapolate chemical information in a complex food matrix [2, 10-11]. In particular, gas chromatography coupled with mass spectrometry is used in foodomics for the quality control, authentication and characterization of the products of interest. This work aims to study the chemical changes of coffee flavour investigating the contemporary evolution over time of the coffee volatilome and of its lipid fraction.

Experimental

Different commercial coffee capsules in diverse packaging (standard and eco-caps), lot (3 lots each) and blend (P and B, 100% Arabica and I 50/50% Arabica/Robusta) were analysed. Samples were stored in stress condition

(65%UR and 45°C and monitoring over time from T0 to T3 (90 days). A target fingerprinting method, by HS-SPME-GC-MS for the volatile fraction was used. In addition, free (FFA) and esterified fatty acids (EFA) were analysed by GC-MS; acidity, peroxide value and p-anisidine were evaluated by a spectrophotometric analysis. Sensory tests were carried out from an industrial panel. According to sensory evaluation, the eco-caps were considered not sensorial good at T2, while the standard caps were still acceptable at T3.

Results

The analytical data of the normalized responses for eco and standard caps-report a different behaviour for the two types of packaging, as shown by Principal component analysis (PCA) (Fig. 1), that shows for the eco-caps (Fig 1a) a discrimination in time along the F1. Volatilome of standard caps (Fig 1b) presents a discrimination of blends along the F1, while the F2 describes the time and highlights the major stability of this pack to the stress conditions.

A series of volatile compounds emerged in the oxidative samples and, among them, we highlighted 3 VOCs that presented pungent, rancid and acid notes and that arise in sensory not acceptable samples independently from blends, lots and packaging.

The trend of free fatty acids (FFAs) has been correlated to the colorimetric values (peroxides, p-anisidine and acidity), pH and UR% measurements and the VOCs. The results showed that the different blends behave differently over time, under stress conditions and in the different packaging.

B eco-caps present a FFAs decrease due to oxidative reactions, while p-anisidine and peroxide values and volatile acids increased. This behaviour highlights a rancidity event where the formation of oxidative secondary products and the continuous increase of peroxides are a consequence of a high oxidative stress on samples. The other two blends (P and I) present a common trend in which the FFAs increases together with the peroxides. What distinguishes the two blends is the different behaviour of p-anisidine. In P blend, p-anisidine decreased indicating an initial catalytic phase of peroxides formation, while in I the p-anisidine increases as a consequence of an initial formation of secondary oxidative products.

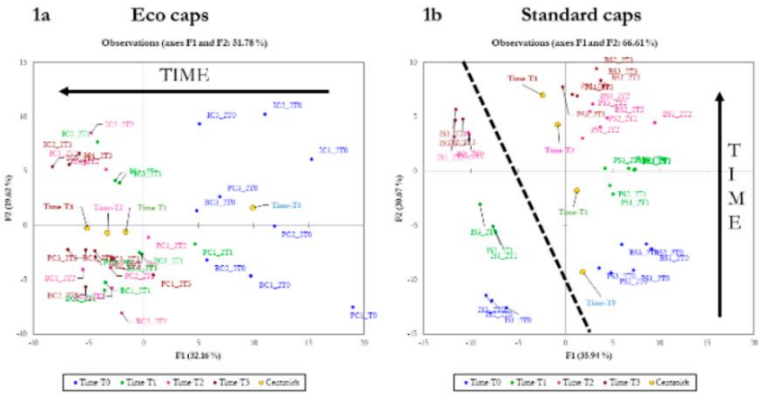

Figure 1. *PCA score plots of the coffee capsules: 1a) shows the eco-caps;1b) the standard caps*

In standard capsules, FFAs in the three blends slowly increase over time due to the possible TAG hydrolyses with a contemporary increase in acidity. This behaviour could explain the higher stability of standard caps compared to the eco-caps. The behaviour of p-anisidine is different for the three blends. While p-anisidine increase in P and I blends because of the initial phenomenon of secondary products formation, in B it decreases with a contemporary increase of volatile acids, indicating an initial rancidity.

Conclusions

This project investigated the sample volatile and non-volatile fractions. Among the volatiles constituting the coffee aroma, some markers describing the coffee aging independently from blends and packaging have been identified and also monitored over time in stress conditions. About the lipid fraction, it was evidenced the significant role played by the FFAs degradation and their derivatives on aging of samples. In any case, a different behaviour between blends and packaging was observed.

References

1. N. Bhumiratana, K. Adhikari, and E. Chambers. (2011). LWT - Food Science and Technology 44(10): 2185–92.
2. B. Folmer. (2016). The Craft and Science of Coffee. Elsevier.
3. W. B. Sunarharum, D. J. Williams, and H.E. Smyth. (2014) Food Research International 62: 315–25.
4. F. Kong, and R. P. Singh. (2016) The Stability and Shelf Life of Food Chemical Deterioration and Physical Instability of Foods and Beverages. Elsevier Ltd.
5. L. Manzocco, S. Calligaris, M. Anese, and M. C. Nicoli. (2016) The Stability and Shelf Life of Food Elsevier Ltd.
6. C. F. Ross, K. Pecka, and K. Weller. (2006). Journal of Food Quality 29(2006): 596–606.
7. I. Flament - Coffee Flavor Chemistry (2001), Wiley.
8. J. M. Murray, C. M. Delahunty, and I. A. Baxter. (2001). Food Research International 34(6): 461–71.
9. J.Regueiro, N. Negreira, and J. Simal-Gándara. (2017). Critical Reviews in Food Science and Nutrition 57(10): 2112-2127.
10. D. Bressanello, E. Liberto, C. Cordero, B. Sgorbini, P. Rubiolo, G. Pellegrino, M. Ruosi, C. Bicchi. (2018) Journal of Agricultural and Food Chemisty. 66(27): 7096–7109
11. G. Strocchi, E. Bagnulo, M. R. Ruosi, G. Ravaioli, F. Trapani, C. Bicchi, G. Pellegrino, E. Liberto, Food Chemistry submitted

P11

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

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Summary: *UHPLC-ESI-QqTOF-MS/MS was used to characterize a set of minor di-acyl quinic acid isomers in green Coffea canephora Pierre ex Froehner (Robusta coffee). The characterized Robusta extract may represent a reliable "surrogate" standard to study the occurrence of minor di-acyl quinic acid isomers in Coffea species not yet commercially exploited.*

Keywords: *Coffea canephora, UHPLC-ESI-QqTOF-MS/MS, di-acyl quinic acids*

Introduction

Chlorogenic acids are relevant coffee quality markers, taste, and aroma precursors as well as important bioactive compounds. A number of mono-acyl, di-acyl, and tri-acyl quinic acid isomers were found in green coffee beans, being mono-caffeoyl (CQA), mono-feruloyl (FQA), mono- *p*-coumaroyl (*p*CoQA), and di-caffeoyl quinic acid (diCQA) isomers considered as quantitatively major compounds. Several quantitatively minor di-acyl quinic acid isomers such as di-feruloylquinic (di-FQA), di-p-coumaroylquinic (di-*p*CoQA), di-sinapoylquinic (di-SiQA), di-methoxy cinnamoylquinic including hetero di-acyl quinic acid isomers such as caffeoylferuloylquinic acid isomers and many others have been detected in green coffee [1, 2]. These minor compounds are much less investigated than the others and the literature is still rather scarce in reporting their MS data as well as their occurrence in botanical *Coffea* species not yet commercially exploited. In facts, on one hand, chlorogenic acids are partially degraded by the roasting process, and this thermal degradation may be particularly critical as far as minor compounds survival in roasted coffee is concerned. Due to the considerable importance in characterizing roasted coffee compared to green coffee it is not surprising the lack of data on minor chlorogenic acids. On the other hand, information on the occurrence of chlorogenic acids in coffee species not yet commercially exploited is limited to the major compounds. In the present preliminary study, green *Coffea canephora* Pierre × Froehner, well known as Robusta coffee, in view of its relative abundance of di-acyl quinic acid isomers in respect to the other commercially exploited coffee species (*Coffea arabica* L.), after proper extraction has been characterized by UHPLC-ESI-QqTOF-MS/MS. Due to the lack of commercially available standards of the great majority of di-acyl quinic acid isomers, the present study represents the opportunity to propose Robusta extract as a "surrogate standard" to characterize other *Coffea* species not yet investigated from this point of view.

Experimental

Green coffee beans of *Coffea canephora* from Vietnam were finely grounded. Five grams of coffee was defatted with *n*-hexane by 2×30 mL extraction under stirring for 30 minutes at room temperature. The sample was filtered, and the residue was then extracted with 15 mL of H_2O/CH_3OH 20:80 (v/v) under stirring overnight. After centrifugation 5000 g for 15 minutes, the sample was filtered by 0.22-µm filter (RC membrane) and collected in a vial for LC-MS analysis. System used was composed by Agilent UHPLC 1290 Infinity ultrahigh performance-liquid chromatography system coupled to Agilent 1290 Infinity Autosampler (G4226A) and Agilent 6540 accurate-mass Q-TOF Mass Spectrometer (resolving power 40000) and Dual Jet Stream Ionization source (Agilent Technologies, Santa Clara, CA). Chromatographic conditions: column Zorbax RP column RRHD SB-C18 3×150 mm, 1.8 µm (Agilent Technologies), mobile phase composed of A) 0.1% (v/v) aqueous formic acid and B) acetonitrile/0.1% formic acid (v/v). Gradient elution program: 5% B isocratic for 8 min, from 5% to 45% B in 10 min, from 45% to 65% B in 5 min, from 65% to 90% in 4 min, 90% B isocratic for 10 min. Flow rate 0.4 mL/min; column temperature 35 °C; volume sample injection 5 µL. ESI-QqTOF-MS/MS conditions: sheath gas nitrogen 10 L/min at 400 °C; drying gas nitrogen 8 L/min at 350 °C; nebulizer pressure 60 psi, nozzle voltage 0 kV, capillary voltage 3.5 kV. Signals recorded in the *m/z* 100-1700 range. MS/MS experiments were performed by using a collision energy ramp from 20 to 60 eV. Precursor ions fragmented had MW included in the range *m/z* 100 to 1700. Acquisition rate 2 spectra/s.

Results

All data of chlorogenic acids presented in this paper use the recommended IUPAC numbering system [3]. Several minor di-acyl quinic acid isomers were identified in the analyzed green Robusta coffee sample: putative *p*-coumaroyl-caffeoylquinic acid, putative caffeoyl sinapoylquinic acid, putative *p*-coumaroyl-feruloylquinic acid isomers, putative caffeoyl-dimethoxycinnamoyl quinic acids and putative sinapoyl-feruloylquinic acids.

Conclusions

Several di-acyl quinic acid isomers were identified in a proper extract of Robusta coffee. In particular, HR-MS/MS provided putative identification of a number of minor compounds. The use of the characterized extract as a “surrogate standard” is explored by investigating *Coffea* species not yet commercially exploited.

References

1. M.N. Clifford, *J. Sci. Food Agric*. 80 (7) (2000), 1033−1043.
2. R. Jaiswal, M. A. Patras, P. J. Eravuchira and N. Kuhnert. *J. Agric. Food Chem.* 58 (15) (2010) 8722–8737.
3. IUPAC. *Biochem. J*. (153) 1976, 23−31.

P12

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

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Summary: *VOCs non-targeted fingerprinting of wet-processed green Arabica coffee samples from 4 different geographical origins was obtained comparing SPME-GCxGC-TOF with HS-GC-IMS. Both analytical approaches permitted the clustering of the samples, leading information about aroma precursors. Moreover, HS-GC-IMS permitted the direct rapid analysis of green beans in vials, reducing sample pretreatment.*

Keywords: *Coffea arabica, VOCs, HS-GC-IMS, GCxGC-MS*

Introduction

The overall quality of the green coffee (Coffea spp) is mainly related to germplasm quality (genetic profile, variety), post-harvest treatments e.g. microbial activity during fermentation in the case of wet processing and drying. Geographical origin and quality of green coffee can be established applying non-targeted methods by mean of different analytical approaches, followed by post-analytical chemometric treatment of data, often linked to the Artificial Intelligence-based methods. The quality of roasted coffee can be predicted considering the overall fingerprint of green coffee beans. Multidimensional comprehensive GCxGC-MS was used in the recent past to cluster green and roasted coffee beans [1]. Few data are available for green coffee VOCs analysis by HS-GC-IMS, particularly regarding the evaluation of VOCS during storage [2]. Main aim of this work was to evaluate the volatile fraction of Arabica wet processed green coffee beans from different geographical origin, sampled by headspace-solid phase microextraction (GCxGC-TOF) as well as directly analysed (head space) with HS-GC-IMS. Non supervised chemometric methods were used to cluster samples, obtaining interesting results regarding the capability to identify coffee origin/ecotype.

Experimental

Samples: Nine different green wet-processed coffee samples (C. arabica L., commercial lots) from 4 different geographical origins (Ethiopia (4), Brazil (3), Nicaragua (1) and Guatemala (1)) were supplied by illycaffè S.p.A (Trieste (Italy)). The samples with zero primary and secondary defects, were selected on the basis of standard internal procedures of sorting and visual aspect, moisture content, screen size, and cup quality.

HS-GC-IMS: Headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS) (FlavourSpec®, G.A.S., Dortmund, Germany) was used to assess the

volatile composition with an untargeted fingerprinting approach. A 20 mL glass vial was filled with 1.0 g of the sample. Then samples were treated for 5 minutes at 50 °C at 500 rpm. Then, in splitless mode, a 300 µL headspace sample was automatically delivered through a 70 °C heated syringe. Using an MXT-5 column (15 m × 0.53 mm i.d., 1 µm film thickness; Restek Corporation, Bellefonte, PA, USA), the volatile chemicals were separated at 40 °C. As the carrier gas, 99.999 percent pure nitrogen was employed, and the flow rate program was configured as follows: 2 mL/min for 3 minutes, followed by a 17-minute rise to 25 mL/min and a 5-minute hold. A 3H ionization source ionized the eluted analytes before driving them to a drift tube, which was run at a constant temperature of 45 °C and voltage of 5 kV.

GCxGC-TOF: A two-dimensional GC coupled to MS was used to characterize volatile molecules using headspace-solid phase microextraction analysis (SPME). A Pegasus BT 4D GCxGC-TOFMS instrument (Leco Corp., St. Josef, MI, USA) equipped with a LECO dual stage quad jet thermal modulator was used. 2.0 g of sample was placed into a 20-mL glass vials with septa and equilibrated at 50 °C under continuous stirring for 5 min. The volatiles were extracted by SPME, using a 50/35 µm DVB/CAR/PDMS fiber (Supelco, Inc., Bellfonte, PA, USA). The SPME fiber was preconditioned for 30 minutes at 270 °C and reconditioned between each run to minimize carry over effects.

The first dimension column was a Stabilwax-DA (Restek Corp., Bellefonte, PA) MS capillary column, with an internal diameter of 0.25 mm and a stationary phase film thickness of 0.25 mm, while the second dimension chromatographic columns was a 2 m Rxi-17Sil MS (Restek Corp., Bellefonte, PA) with the same diameter and thickness of the first one. High-purity helium (99,9999%) was used as the carrier gas with a flow rate of 1.4 mL/min. The temperature program of the oven was as follows: the initial temperature was set at 50 °C for 5 min, then ramped at 4 °C/min up to 250 °C for 5 min. The secondary column was maintained at +15 °C relative to the GC oven temperature of the first column. Electron impact ionization was applied (70 eV). The ion source temperature was set at 250 C, the mass range was 35-550 m/z with an extraction frequency of 32 kHz. The acquisition rates were 200 spectra/s. The modulation periods for both programs were 4s for the entire run.

Statistical analysis: principal component analysis and partial least square discriminant analysis were performed using MetaboAnalyst 5.0 software.

Results

As well known, climatic factors directly impact the volatile organic compounds fingerprint in green Arabica coffee bean as well as coffee beverage quality [3]. Geographical origin is strictly correlated with different macro and micro-climatic conditions, so representing a critical parameter for coffee quality. The traceability of green coffee can be obtained using different analytical approaches, often completed by appropriate supervised or unsupervised statistical processing of data. The analysis of volatile profile of green coffee is of great interest particularly regarding the evaluation of the fermentation process. The aim of this work was to establish (and compare) the usefulness of two hyphenated analytical techniques applied to fingerprint green coffee volatile compounds, evaluating the capacity to cluster specific samples from specific geographical origins.

Results demonstrate the ability of GC×GC-MS to explore in depth the complexity of green coffee samples (e.g. the targeted identification of some key bioactive compounds within the aroma, as the putative presence of raspberry ketone). The application of this analytical method permitted to clearly identify some key odorants of green coffee including methoxypyrazines, aldehydes, alcohols and hydrocarbons. The putative presence of raspberry-ketone (previously indicated as molecular marker of interest in green coffee, and currently largely suggested as key ingredient in food supplements for weight loss) was not confirmed in these samples; contrary, it was clearly identified in some raspberry samples, used as positive control.
Moreover, the rapid application of HS-GC-IMS on green beans (here proposed a screening rapid method compared to GCxGC-TOF) was functional to clearly recognize clusters of samples. HS-GC-IMS provided 2D chromatogram useful to quickly obtain very clear 2D patterns, avoiding any kind of sample pre-analytical handlings and processing. Also using this method, the presence of raspberry ketone was not evidenced in the samples. The comparison of these approaches, processing the overall data (and a selection of the most representative compounds) with principal component analysis, partial least square discriminant analysis and hierarchical clustering heat-map allowed us to obtain comparable clustering, clearly permitting to identify the geographical origin of the samples.

Conclusions

These approaches emphasizes the usefulness of the hyphenated multi-platform approach as analytical tool, preliminary to data mining. Both GCxGC-TOF and HS-GC-IMS analysis allowed to identify specific clusters of samples of known geographical origin, also permitting some considerations regarding the precursor of aroma. HS-GC-IMS, particularly, reduced the time of analysis permitting the direct rapid analysis of green beans in vials, opening new perspectives for green coffee quality control.

References

1. C. Cordero, E. Liberto, C. Bicchi, P. Rubiolo, S.E. Reichenbach, X. Tian, and Q. Tao; *Journal of chromatographic science*, 48(4) (2010) 251-261.
2. C. Min, M. Bivi, L. Jianneng, L. Yimin, L. Yijun and C. Long, *RSC advances*, 12(24) (2022) 15534-15542.
3. B. Bertrand et al. *Food Chemistry*, 135(4) (2012) 2575-2583.

P13

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

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Summary: *The flavour of a coffee beverage brewed from an individual coffee can be directly influenced by the parameters of its extraction, i.e., pressure, temperature and flow rate. In this work, we used PTR-TOFMS to explore this influence on qualitative and quantitative changes in the aroma composition of different extracts of coffee.*

Keywords: *coffee extraction, PTR-TOFMS, aroma compounds*

Influence of coffee extraction variables on flavour

Coffee is one of the most popular beverages worldwide, with an annual production of 10.5 billion kg beans [1]. Its popularity has been associated with its stimulating effect, as well as its attractive flavour characteristics. The sensory attributes of the coffee beverage is driven by the composition of its constituent aroma compounds – whereby only up to 50 of more than 1000 constituent volatiles are considered key aroma compounds [2] – and non-volatile compounds, such as acids, bitter tastants and matrix compounds that influence the taste and mouthfeel. The coffee flavour is influenced throughout the entire production chain, starting with the choice of the crop, the cultivation and harvesting conditions, as well as further parameters, such as roasting, storage, packaging and grinding, and eventually the coffee extraction process itself. While coffee producers monitor most of these stages, the latter two steps are usually determined by the end-user/consumer.

There are many different ways to brew coffee, with the espresso (pressure) method being the most frequently studied. Investigations have evaluated the impact of different extraction variables, such as temperature, extraction time, pressure, coffee/water ratio, water quality and grinding on the physicochemical characteristics and flavour compounds of the ensuing brew [2]. However, evaluating the impact of a single factor is difficult due to the interdependency of the process parameters. For example, the grind size distribution affects the brewing pressure, which subsequently influences the flow rate and extraction time. For the latter, it has been shown that compounds that are more soluble are efficiently extracted within the first seconds of the process, while for the less soluble compounds more water or a longer extraction time is needed, respectively [2]. Further, a higher extraction temperature increases the kinetic energy, which facilitates the extraction of all compounds from the coffee grounds,

but this can also cause a higher degree of evaporation of aroma compounds, thereby affecting the sensory perception of the final beverage [2].

Quantification of aroma compounds using PTR-TOFMS

The examination of aroma compounds in coffee is most commonly made using gas chromatography (GC) combined with mass spectrometry (MS), flame ionisation detection (FID) and/or olfactometry, coupled to a variety of sample preparation techniques that include headspace (HS)-solid phase micro-extraction (SPME) or static HS [2, 3]. An alternative approach for analysing aroma compounds in coffee is proton transfer reaction MS, often coupled to time-of-flight MS (PTR-TOFMS), which allows for direct headspace analysis and real-time measurements. Coffee analysis was one of the first and key applications of PTR-MS when it was developed in the mid-1990s [4], and has been subsequently used for on-line monitoring of aroma compounds during coffee extraction, amongst other coffee-related applications [5, 6]. Compared to GC methods, which are often time consuming in terms of both sample preparation and analysis, PTR-MS enables a direct analysis of volatile compounds in samples and a high sample throughput, albeit without separation of isomeric compounds. A recently published application for PTR-MS analysis was the coupling of the instrument to a liquid calibration unit (LCU) [7], whereby the latter was supplied with an aqueous sample that is vaporised, thereby allowing constituent volatiles to be analysed directly by PTR-MS, as has been demonstrated for whisky [8]. This approach was used in the present work for coffee samples. Diluted and filtered coffee samples were vaporised and directly analysed using an LCU coupled to PTR-TOFMS to explore qualitative and quantitative changes in the aroma composition of coffee in relation to brewing parameters.

This talk reports on the influence of different process variables during coffee extraction on the aroma of the coffee beverage, evaluated by means of PTR-MS, with a focus on the novel use of an LCU system for direct quantitation of coffee volatiles.

References

1. International Coffee Organization, 2021. Crop year production by country. Available from: https://www.ico.org/prices/po-production.pdf (assessed on 06.07.2022).
2. Cordoba, N.; Fernandez-Alduenda, M.; Moreno, F. L.; Ruiz, Y., *Trends Food Sci. Technol.* 2020, 96, 45-60.
3. Pua, A.; Goh, R. M. V.; Huang, Y.; Tang, V. C. Y.; Ee, K.-H.; Cornuz, M.; Liu, S. Q.; Lassabliere, B.; Yu, B., *Food Chem* 2022, 132971.
4. Beauchamp J. D., Pushing the boundaries of dynamic flavor analysis with PTR-MS. *In Dynamic Flavor: Capturing Aroma Using Real-Time Mass Spectrometry*, American Chemical Society: 2021; pp. 33-50.
5. Sánchez-López, J. A.; Wellinger, M.; Gloess, A. N.; Zimmermann, R.; Yeretzian, C*., Int J Mass Spectrom* 2016, 401, 22-30.
6. Sánchez-López, J. A.; Zimmermann, R.; Yeretzian, C., *Anal. Chem* 2014, 86, (23), 11696-11704.
7. Fischer, L.; Klinger, A.; Herbig, J.; Winkler, K.; Gutmann, R.; Hansel, A. In

The LCU: versatile trace gas calibration, CONFERENCE SERIES, 2013; p. 192.
8. Beauchamp, J.; Biberacher, S.; Gao, S., Rapid quantitation of phenolic compounds in Islay single malt Scotch whiskies by direct injection mass spectrometry. *In Sex, Smoke, and Spirits: The Role of Chemistry*, American Chemical Society: 2019; pp. 117-124.

P14

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

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Summary: *LC/MS enables screening & quantification of bioactive human serum metabolites of a uniquely formulated whole coffee fruit extract*

Keywords: *Phytochemicals, Bioavailability, Tandem mass spectrometry*

Introduction

Coffee is a dietary source of bioactive compounds such as antioxidants with potential health benefits for humans [1,2]. It contains highly abundant chlorogenic acids (CGAs), which are implicated in health benefits including reducing risk of cardiovascular disease, anti-bacterial & anti-inflammatory activities. In the current study we investigated the phytochemical contents and bioavailability of *Coffeeberry*® Energy, a commercially available whole coffee fruit caffeine extract, by tandem mass spectrometry (LC-MS/MS) approach.

Experimental

For phytochemicals screening, 20 mg of the extract was dissolved in 1 ml of 50% methanol/water. The extract was then centrifuged at 14000 rpm for 10 min & a portions of the supernatant transferred to MS vial for phytochemicals identification. For bioavailability study, serum samples were collected in duplicate from four human subjects before and after 1, 2 and 3 h after ingestion of 150 mg of the extract. Protein precipitation and extraction of bioactive compounds were carried out using methanol. Identification of intact compounds and metabolites were performed using Q-Exactive Orbitrap MS coupled to Dionex uHPLC and Compound Discover 3.0 software program for database search & data processing.

Results

This study identified more than 170 phytochemicals including caffeine, diterpenoids, organic, phenolic, and chlorogenic acids in the extract. As expected, caffeine & chlorogenic acids make up most of the identifications. For the serum samples, we identified 176 manually confirmed intact compounds and metabolites in all the subjects. Of these, 32 metabolites exhibited greater 2-fold increase in serum concentration 1 h after ingestion of the extract. Among the metabolites that showed the highest increase are caffeic acid-4-sulfate, 1,3,7-trimethyluric acid, iso-ferulic-4-O-sulfate, caffeine, & uric acid derivatives. We also observed a dramatic increase in the concentration of three iso-feruloylquinic acid isoforms (3-iFQA, 4-iFQA & 5-FQA). Interestingly, these isoforms were not detected in our phytochemical screening of the extract suggesting their possible formation during the digestion and/or absorption process in the gut.

Conclusions

This study provides evidence of the potential of in vivo metabolites of a uniquely formulated whole coffee fruit extract. This formula generated a district serum profile of health promoting phenolic compounds demonstrated by the more than 32 metabolites that exhibited a dramatic increase in concentration in circulation one hour after ingestion.

References

1. A. Stalmach, W. Mullen, D. Barron, K. Uchida, T. Yokota, C. Cavin, H. Steiling, G. Williamson and A. Crozier, *Drug Metab. Dispos*., 2009, 37, 1749–1758.
2. M.N. Clifford, I. Jaganath, I.A. Ludwig and A. Crozier, *Nat. Prod. Rep*., 2017, 34, 1391

P15

Authentication of coffee: target screening of markers

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Summary: *Coffee is one of the most frequently traded commodities. Due to high prices and big consumer demands, there is still an effort to adulterate coffee. For example, the Arabica is replaced by the cheaper Robusta. This work is focused on the development of a suitable method for detecting this adulteration.*

Keywords: *authenticity of coffee; Arabica and Robusta; UHPLC-HRMS/MS*

Introduction

The *Coffea* genus includes more than 100 species, nevertheless, among the best known and most important are *C. arabica* (Arabica) and *C. canephora* (Robusta) [1-3]. Arabica, which is known for its milder and more aromatic taste with lower caffeine content, is one of the most valued commodities on the world market [4]. Compared to Robusta coffee, Arabica is much more prone to diseases and requires more demanding growing conditions [1]. Due to the price difference between these two species, Arabica is often adulterated with the addition of cheaper Robusta [2]. However, detecting such fraud is not an easy task as the chemical composition of the two species is very similar. According to the German standard method DIN 10779:2011, a suitable method for detecting this counterfeiting is high-performance liquid chromatography coupled to the UV detector (HPLC-UV) [5]. This method is used to determine the amount of 16-O-methylcafestol (16-OMC), the known Robusta marker [2, 6]. However, extraction of the samples is very time-consuming and complicated, because this marker occurs in coffee mainly in the esterified form and hydrolysis is a necessary step. Moreover, the variability of 16-OMC in different Robusta samples is very large, and, therefore, this method does not always provide reliable results [7, 8].
This presented study aimed to investigate the potential of metabolomic non-target UHPLC-HRMS/MS strategy to enable the identification of ´markers´ applicable for distinguishing the presence of Robusta in Arabica. The target screening of these unique substances was used to verify the declared composition of coffee samples. The final method was compared with authentication based on the quantitative determination of 16-OMC.

Experimental

Non-target analysis: The samples of roasted Arabica (n = 41) and Robusta (n = 21) coffee beans from different countries were analyzed. The 16 samples of Arabica and 4 samples of Robusta were obtained from Czech roasters, who buy green beans directly from farmers in the country of origin. Other samples were purchased from other Czech roasters declaring a transparent supply chain. Ultra-high reverse-phase liquid chromatography coupled to tandem high-resolution mass spectrometry (UHPLC-HRMS/MS) was used for the analysis of

methanolic extracts. The characteristics markers of Arabica and Robusta were searched by multivariate statistical analysis, specifically, principal components analysis (PCA) followed by partial least squares-discriminant analysis (PLS-DA). The internal database of characteristic markers was created. The prepared mixtures with a known ratio of Arabica and Robusta were also analyzed to determine the lowest addition of Robusta to Arabica which is possible to detect. For the preparation of these mixtures, all previously analyzed samples of Arabica and Robusta were mixed separately, so that the variability of the coffee samples was captured in the prepared "calibration".
Target screening: In addition to the samples mentioned above, 6 Arabica blends and 22 different samples declared as mixtures of Arabica and Robusta were analyzed by target screening. All these samples were bought from the Czech market. UHPLC-HRMS/MS was used for the target screening of markers from an internal database in acetonitrile extracts. Based on the comparison of the content of these markers in prepared mixtures with a known ratio of Arabica and Robusta and in the bought coffee samples, the content of Arabica and Robusta in them was calculated. The prepared mixtures were utilized as a "calibration curve".
Target analysis of 16-O-methylcafestol: The content of 16-OMC, a ´common´ Robusta marker was also monitored in the acetonitrile extracts of all above-mentioned samples of coffee. The extracts and 16-OMC matrix calibration were analyzed by the validated UHPLC-HRMS/MS method.

Results
Based on statistical processing of data generated by the non-target analysis (metabolomic fingerprinting) of ground roasted coffee beans, it was possible to distinguish Arabica and Robusta samples, moreover, an addition of 15 % (w/w) addition of Robusta to Arabica could be detected. 18 unique marker ions were found in Arabica and 9 in Robusta, approx 50 % of respective compounds were identified. The variability in analyzed Arabica and Robusta samples was documented. Interestingly, esters of 16-OMC (variability: 15 - 21 %) had approximately three times lower variability in different analyzed Robusta samples than the 16-OMC (variability: 57 %). The same situation was also for kahweol (variability: 44 %) and his esters (variability: 9 – 23 %) in different analyzed Arabica samples.
The following experiments dealing with the optimization of the extraction method for target screening of markers showed, that most of the monitored unique substances had a higher intensity in acetonitrile extracts of coffee compared to methanolic ones. The target screening of markers in the acetonitrile extracts enabled to detection of up to 1-2 % weight addition of Robusta to Arabica. As far as only the presence of 16-OMC was monitored, then the detectable addition of Robusta to Arabica was higher, 5 % (w/w).
Furthermore, it was found, that the most reliable results were provided by markers from the positive electrospray ionization, which were identified as 16-OMC, kahweol and their esters. In experiments dealing with the calculation of the ratio of Arabica and Robusta in coffee samples, it was sufficient to compare the content of 8 markers from positive electrospray ionization mode in the unknown sample and the prepared "calibration" with a known ratio of Arabica

and Robusta. The analysis and evaluation of samples were very quick and easy. This method was applied to check the ratio of Arabica and Robusta in samples from the Czech market. The results showed, that 5 out of 28 samples were probably countrified, i.e. the Robusta content was higher than label declaration, in two cases even by 40-50 %.
The last part of this study was devoted to the quantitative determination of 16-OMC, which is commonly used to verify the authenticity of coffee. Since mass spectrometry is a more sensitive/selective technique than the commonly used UV detection, it was not necessary to perform hydrolysis of the sample and acetonitrile extracts were used for the analysis. The performance characteristics such as linearity and limit of detection/quantification were determined through validation. A hundred times lower quantification limit (0.5 mg/kg) was achieved compared to the standard DIN method used HPLC-UV [5] and forty times lower compared to NMR [6]. The determined concentration of 16-OMC in the unknown samples was compared with the concentration of this analyte in the prepared mixtures of a known ratio of Arabica and Robusta. This method has been found to give both false positive and false negative results. Two samples were wrongly marked as falsified and two samples, that were adulterated, were not detected by this method.

Conclusions

In this study, a set of markers enabling distinguishing Robusta and Arabica ground roasted coffee beans has been discovered by UHPLC-HRMS/MS based metabolomic fingerprinting. Using these unique ´markers´ for target screening of samples significantly simplifies authenticity control under routine conditions, moreover, increases reliability achieved compared to the commonly used method based on the determination only of 16-OMC. Furthermore, the addition of Robusta at the amount as low as 1-2 % (w/w) to Arabica is possible to detect by this new analytical approach.

References

1. J.-F. Morin, M. Lees, *Food Integrity Handbook: A Guide to Food Authenticity Issues and Analytical Solutions*, Eurofins Analytics France, (2018). pp 295-314.
2. Y. Gunning, M. Defernez, A.D. Watson, et al.; *Food Chemistry*, 248, (2018) 52-60.
3. R.S. Singhal, P.R. Kulkarni, D.V. Rege, *Handbook of Indices of Food Quality and Authenticity*, Woodhead Publishing, (1997). pp 457-488.
4. R.M.N. de Souza, M.T. Benassi; 23, (2012), pp 1347-1354.
5. German Institute for Standardization (DIN); *DIN 10779:2011 Analysis Of Coffee And Coffee Products - Determination of 16-O-Methyl Cafestol Content of Roasted Coffee - HPLC-Method*, (2011).
6. E. Schievano, C. Finotello, E. De Angelis, et al.; *Journal of Agricultural and Food Chemistry*, 62, (2014) 12309-12314.
7. A.L.B. Mori, D.L. Kalschne, M.A.G. Ferrão, et al.; *Journal of Food Composition and Analysis*, 52, (2016) 52-57.
8. R. Zanin, C. Kitzberger, M. Benassi; *Brazilian Archives of Biology and Technology*, 63, (2020) 1-8.

P16

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

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Summary: *The aim of this study was to quantify acrylamide by HPLC-MS/MS and furanic compounds by a new HS-SPME GC-MS method, in ground coffee samples to find a possible correlation between the concentration of these compounds, the coffee variety (Arabica/Robusta), the post-harvesting treatments (dried/wet) and the roasting degree.*

Keywords: *acrylamide, furanic compounds, coffee ground, roasting degree, post-haversting methods, HPLC-MS/MS, HS-SPME GC-MS.*

Introduction

The interest in studying coffee quality is increasing to evaluate the flavour and aroma but also to assess its potential toxicological effects. Acrylamide and furanic compounds, formed mainly through the Maillard reaction, are some of the most studied compounds as acrylamide has been classified in the group 2A as a substance “probably carcinogenic to humans”, furan has been classified in the group 2B as a substance “possibly carcinogenic to humans, based on sufficient evidence in experimental animals” and furfuryl alcohol (FFA) has been classified as group 2B and defined as “possibly carcinogenic to humans, based on sufficient evidence of carcinogenicity in experimental animals and no data or inadequate evidence in human beings” by the International Agency for Research on Cancer (IARC). This study had the aim of studying the possible influence of the coffee variety, the post-harvesting treatments and the roasting degree on the concentration of these compounds in coffee grounds.

Experimental

Arabica (Coffea arabica L.) and Robusta (Coffea canephora var. Robusta) coffee cherries used in this study were roasted at five different roasting degrees (Cannella, City, Full-city, Italiana and Napoletana) and underwent two different post-harvesting treatments (dry and wet). The quantification of acrylamide in different coffee samples has been performed following a previous developed and validated procedure [1] by (HPLC-MS/MS) with a triple quadrupole equipped with an electrospray ionization (ESI) source operating in positive ionization mode. The acquisition was performed in “Selected Reaction Monitoring” (SRM) mode (Table 1). Furanic compounds concentration was determined using HS-SPME GC-MS, using a DVB/C-WR/PDMS fiber and a gas chromatograph and a mass spectrometer equipped with an electron ionization source (EI). The acquisitions

were carried out in 'Selected Ion Monitoring' (SIM) mode and detection was divided into time windows to enhance the sensitivity (Table 2). Data were processed by One-way Anova analysis. The chosen level of significance was 0.05.

Table 1. *HPLC-MS/MS acquisition parameters optimized for acrylamide quantitation.*

Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Fragmentor (V)	Collision Energy (V)	Retention Time (min)	Polarity
Acrylamide	72	55	45	8	1.52	Positive
Acrylamide-d_3	75	58	45	8	1.52	Positive

Table 2. *GC-MS parameters used for the analysis.*

Compound	Time window (min)	Ion (*m/z*)	Rt (min)
3-methylbutanal	0-12.20	58[a], 44, 71	8.5
2-methylpentanal	0-12.20	58[a], 71, 43	11.0
Hexanal	12.20-20.00	56[a], 44, 72	13.2
Furfural	20.00-25.20	96[a], 95, 39	24.3
Furfuryl Acetate	25.20-26.60	81[a], 98, 140	26.2
5-methylfurfural	26.60-28.20	110[a], 109, 53	27.2
Furfuryl alcohol	28.20-35.00	98[a], 81, 41	29.1
5-HMF	35.00-	97[a], 126, 41	43.3

Results

Acrylamide content is related to the presence of its two main precursors: free asparagine and sucrose. In contrast with earlier findings [2,3], in this study Arabica samples showed a significantly higher acrylamide content than Robusta ones, probably due to a different reduced sugars content in green coffee beans. Wet-processed samples demonstrated a significantly lower acrylamide content than dry-processed ones, as previously reported. In addition, the dry-processed Arabica samples showed a higher acrylamide content than the other samples. Acrylamide content decreases with the increase the roasting temperature, as previously reported [1,2,4-6], and it was higher under low-temperature and short-time roasting conditions (Cannella and City, for example). As the roasting temperature and time increase, the level of acrylamide decreases until no longer detected. This is due to the fact that asparagine is thermally degraded very quickly without further transformation [2]. The concentration of furanic compounds in dried arabica coffee samples was higher with respect to dried robusta and wet-processed coffee samples, as reported by Gonzalez-Rios et al. (2007) [7]. In dried arabica coffee samples the concentration of furanic compounds increased when passing from light to medium light roasting degree and then decreased in dark roasted samples, reaching the lowest concentration in the very dark roasted coffee samples, with statistically significative differences for all compounds monitored, as previously reported by Vignoli et al. (2014) [8]. In dried robusta coffee samples the concentration of furanic compounds increased and decreased with stronger roasting conditions, reaching the lowest values for very dark roasted coffee samples, with statistically significative differences for furfural and 5-HMF. When comparing dried and wet arabica and

robusta samples, data showed that furanic compound content was higher in arabica coffee with respect to robusta ones, especially for furfural and 5-HMF, with statistically significative differences. In conclusion, the concentration of furanic compounds was higher in dried ground arabica coffee samples with respect to other coffee samples. In all wet samples the concentration of all monitored compounds was lower with respect to dried ones.

Conclusions

Data obtained in this study showed that the concentration of acrylamide was higher in arabica coffee samples with respect to robusta with a concentration that decreased with the increase in the roasting temperature. Furanic compounds concentrations had an oscillatory trend with stronger roasting conditions, globally decreasing when passing from light to dark roasting conditions for most of them. Furanic compounds concentration was higher for dried arabica ground coffee samples with respect to all other samples.
Moreover, wet-processing resulted to reduce the concentration of all monitored compounds in this study.

References

1. Schouten, M. A., Tappi, S., Angeloni, S., Cortese, M., Caprioli, G., Vittori, S., Romani, S. (2021). *Food Chemistry*, 343, 128514.
2. Bagdonaite, K., Derler, K., Murkovic, M. (2008). *Journal of Agricultural and Food Chemistry*, *56*(15), 6081–6086.
3. Bertuzzi, T., Martinelli, E., Mulazzi, A., Rastelli, S. (2020). *Food Chemistry*, *303*, 125372.
4. Budryn, G., Nebesny, E., Oracz, J. (2015*). International Journal of Food Properties*, 18(2), 290–302.
5. Esposito, F., Fasano, E., De Vivo, A., Velotto, S., Sarghini, F., Cirillo, T. (2020). Food Chemistry, 319, 1–7.
6. Lantz, I., Ternité, R., Wilkens, J., Hoenicke, K., Guenther, H., Van Der Stegen, G. H. D. (2006). *Molecular Nutrition and Food Research*, *50*(11), 1039–1046.
7. Gonzalez-Rios, O., Suarez-Quiroz, M. L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J. P., Schorr-Galindo, S. (2007). *Journal of Food Composition and Analysis*, 20(3-4), 297-307.
8. Vignoli, J. A., Viegas, M. C., Bassoli, D. G., de Toledo Benassi, M. (2014). *Food Research International*, 61, 279-285

P17

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

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Summary: *This study aims to characterize four techniques of filter coffee preparation; the classical French Press, V60, and AeroPress were compared with the newly developed Pure Brew at three different degrees of roasting, i.e., light, medium and dark, by HS-SPME/GC-MS, UHPLC-MS/MS, and sensory analysis.*

Keywords: *Brewing methods, GC-MS, UHPLC-MS/MS*

Introduction

Nowadays, among the different brewing methods used for specialty coffee applications, drip methods (using a coffee cone and paper filter), such as immersion methods (ground coffee is immersed in water) have been proposed [1]. More recently, V60, French Press (FP) and AeroPress, have been the most commercial extraction methods for the development of filter coffee [2]. To give a comprehensive overview of the filter coffee world, it was chosen to compare the newly developed Pure Brew to these three extraction methods. Pure Brew represents a real innovation: it produces a fast filter coffee using an espresso machine, without the need for additional equipment. Therefore, the aim of this study was to investigate the differences between the newly developed filter coffee extraction method, Pure Brew, in comparison with traditional ones (V60, French Press and AeroPress) in terms of volatile profile, bioactive compounds, and sensory aspects. To the best of our knowledge, this is the first study about these parameters for filter coffee produced with the Pure Brew technique.

Experimental

For all extractions, coffee with three different degrees of roasting were used. Beans were ground using a professional grinder (Atom Brew Pro - Eureka). Samples were fitted using the same commercial water brand, Nerea, chosen for its mineral content. A specific routine was used for each of the four brewing methods. These extractions were then assessed through a sensory evaluation process, according to the SCA protocol, with SCA cupping form.
From analytical point of view, UHPLC-MS/MS triple quadrupole (Agilent Technologies) equipped with an electrospray ionization (ESI) source operating in positive and in negative polarity was chosen for the 13 bioactive compounds quantitation. The method was validated (Table 1). The acquisition was performed in Dynamic “multiple reaction monitoring” (Dynamic-MRM) mode (Table 2). The separation was achieved using a Kinetex PFP analytical column (100 × 2.10 mm i.d., 2.6 µm) using a binary gradient of water and methanol, both

with 0.1% of formic acid. Coffee samples were centrifuged at 15000 rpm for 5 min and filtered before analysis. On the other hand, volatile compounds were scanned with HS-SPME/GC-MS. A gas chromatography/mass selective detector (GC/MSD with PAL3) (Agilent, Santa Clara) was used. The column used for separation was DB- WAX (0.25 mm × 60 m, 0.25 um) (Agilent 122-7062, CA, USA) and the flow rate (He) was 1.2 ml min-1 under spitless mode. Fiber assembly was from Supelco (Bellefonte, PA, USA) and had a 50/30 µm divinylbenzene/carboxy/polydimethylsiloxane (DVB/CAR/PDMS).

Table 1. *UHPLC-MS/MS method validation parameters: regression equation, linearity (R2), limits of detection (LODs), limits of quantification (LOQs) and reproducibility for the thirteen monitored compounds.*

No.	Compounds	Concentration range (µg ml^{-1})	Regression Equation	R^2	LOQs[a]	LODs[b]	Reproducibility (%RSD)	
							Intraday	Interday
1	Gallic acid	0.01-5	y = 13243x - 1329.5	0.993	0.05	0.02	2.1	6.8
2	3-Caffeylquinic acid	0.01-5	y = 16814x - 1057.5	0.997	0.01	0.003	3.6	8.6
3	(+)Catechin	0.01-5	y = 6481.3x + 31.934	1	0.01	0.003	5.6	7.6
4	5-Caffeylquinic acid	0.1-5	y = 4483.2x - 433.35	0.998	0.1	0.03	4.1	7.8
5	Caffeine	0.005-5	y = 135892x + 1311.1	1	0.004	0.001	0.4	4.0
6	Vanillic acid	0.05-5	y = 857.52x - 19.701	0.999	0.05	0.02	5.6	14.2
7	Caffeic acid	0.01-5	y = 23436x - 537.21	0.999	0.01	0.003	2.2	13.0
8	(-)Epicatechin	0.01-5	y = 6524.8x + 151.77	1	0.01	0.003	2.6	4.7
9	Syringic acid	0.005-5	y = 2125.6x - 154.2	0.995	0.005	0.002	6.2	15.2
10	P-coumaric acid	0.005-5	y = 28093x - 2.4157	0.999	0.005	0.002	3.1	10.3
11	Ferulic acid	0.01-5	y = 5853.5x - 113.69	0.999	0.01	0.003	6.0	13.5
12	3.5-Dicaffeoylquinic acid	0.005-5	y = 22621x - 1430.1	0.996	0.005	0.002	1.4	10.6
13	Trans-Cinnamic acid	0.005-5	y = 54199x + 1479.1	0.999	0.05	0.02	4.3	1.0

Table 2. *UHPLC-MS/MS acquisition parameters (Dynamic MRM mode) used for the analysis of the 13 bioactive compounds.*

No.	Compounds	Retention time (Rt)	ΔRt	Precursor ion (m\z)	Product ion (m\z)	Fragmentor	Collision Energy	Polarity
1	Gallic acid	2.37	3	169	125	92	12	Negative
2	3-Caffeoylquinic	3.58	3	353	191	102	12	Negative
3	acid (+) Catechin	5.48	3	289	245	121	8	Negative
4	5-Caffeoylquinic	6.22	3	353	191	92	12	Negative
5	acid Caffeine	6.5	3	195	138	107	20	Positive
6	Vanillic acid	6.7	3	167	108	78	16	Negative
7	Caffeic acid	6.87	3	179	135	87	12	Negative
8	(-) Epicatechin	7.03	3	289	245	126	8	Negative
9	Syringic acid	7.48	3	197	182	92	8	Negative
10	p-Cumaric acid	8.47	3	163	119	83	12	Negative
11	Ferulic acid	9.16	3	193	134	88	12	Negative
12	3,5 Dicaffeoylquinic acid	9.82	3	515	353	117	8	Negative
13	Trans Cinnamic acid	10.79	3	150	131	44	8	Positive

Results

By UHPLC-MS/MS analysis, PB filter coffee showed the highest levels of caffeine in medium (598.28 µg/mL) and dark (556.13 µg/mL) roasted coffee, compared to the other brewing methods, while for light roasting the highest level of caffeine was detected in FP method (734.72 µg/mL). PB filter coffee at medium (1726.8 µg/mL) and dark (1407.89 µg/mL) roasting degree exhibited

also the more abundant total content of bioactive compounds. These data are in agreement with other studies [3]. A total of 71 volatile compounds was identified by HS-SPME/GC-MS, representing from 77.09 to 96.88% of the total headspace composition. At medium roast the most olfactometrically impactful molecules of the study were discovered: 5-Methyl 2- furancarboxaldehyde, Furfural and 2-Furanmethanol, which were connected with positive notes, associated to almond, sweet and caramel; those were higher in V60, followed by Pure Brew, as confirmed by the panel test's higher acidity values.

Conclusions

From the obtained results, the new extraction method, Pure Brew, has shown performances similar or even better than the known filter coffee preparation methods already on the market (i.e., V60, French Press and AeroPress); it performed better, as for sensory evaluation and for bioactive compounds content (for medium and dark roast). The evaluation of these analyses could contribute to the commercial development of this innovative way for obtaining filter coffee (Pure Brew), which today is prepared mainly with the traditional techniques considered in our study.

References

1. Córdoba, N., Moreno, F. L., Osorio, C., Velásquez, S., Ruiz, Y. (2021). *Food Research International*, *141*, 110141.
2. Stanek, N., Zarębska, M., Biłos, Ł., Barabosz, K., Nowakowska-Bogdan, E., Semeniuk, I., ... Szkutnik, K. (2021). *Scientific reports*, *11*(1), 1-13.

Angeloni, G., Guerrini, L., Masella, P., Bellumori, M., Daluiso, S., Parenti, A., Innocenti, M. (2019). *Food Research International*, *116*, 1327-1335.

P18

Identification of potential aroma markers of coffee oxidized note

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Summary: *This study aims to investigate the whole volatilome of good and oxidized coffees from the two commercial coffee species (i.e. Arabica, Robusta) and from different packaging (i.e. vacuum-packed and Eco-caps) in order to find a potential fingerprint describing the oxidized note of coffees independently from coffees and packaging.*

Keywords: *Coffee volatilome, HS-SPME-GC-MS/FPD, machine learning*

Introduction

Coffee is considered a stable product with a long shelf-life, although, after roasting, it is still an effective 'chemical reactor' because of the high reactivity of its components.
During storage, roasted coffee undergoes chemical and physical changes that can affect its quality. The changes in sensory properties are generally attributed to the loss of volatile compounds that are characteristic of the aroma of roasted coffee, and the appearance of oxidation products that can cause off-flavours.
Storage conditions are closely related to these sensory changes. Several authors have shown that storage temperature is a fundamental parameter for maintaining the quality of the product over time [1-5].
The presence of oxygen, which is, in turn, related to packaging technology, and moisture are another important elements to consider in the deterioration/ alteration of coffee aroma. Despite their number, the studies in this field have always focused on one or two compounds in the expression of coffee staling and not on the synergism between the components of the whole coffee volatilome [2]. Due to the complexity and dynamics of the chemistry involved, coffee oxidation studies have mainly been conducted on a single species, package or condition.
This study investigates the volatilome of good quality and oxidised coffee from different packaging (i.e. standard with metallic barrier and Eco-caps) by combining HS-SPME-GC-MS/FPD with a machine learning approach to define a potential fingerprint that can describe the oxidised note of roasted coffee.

Experimental

Coffee samples: Samples included 30 R&G coffees for moka preparation from 3 lots packed under vacuum in a multilayer film with a metallic barrier (M samples). Also included were a set of Eco caps in modified atmosphere for espresso coffee 5 caps from different lots of different commercial blends named B and P (100% Arabica of different origins) and I (50/50 Arabica and Robusta) for a total of 30 samples. A portion of the coffee samples was maintained at room temperature

and the other was submitted to accelerated ageing under stressed storage conditions in oven at 37°C and 50% relative humidity. The samples were classified as good (G) and oxidised (OX) by an industrial panel.
HS-SPME sampling: Volatiles were sampled by HS-SPME using combi-PAL AOC 5000 Autoinjector installed on a GC-MS system (Shimadzu - Milan, Italy) online integrated with GC-MS/FPD system Shimadzu QP-2010 (Shimadzu - Milan, Italy). 1,5 g of ground roasted coffee in a 20 mL vial were at 50 °C for 10 min. with a PDMS/DVB df 65 µm, 1 cm long (Supelco, Bellefonte, PA, USA).
Instrumental set-up: oven and injector temperature, 250 °C; injection mode, splitless; s helium; flow rate, 1 mL/min; fiber desorption time and reconditioning, 5 min. EI mode ionization at 70 eV; transfer line, 260°C, scan range 35-350 m/z. A SolGel-Wax column (100% polyethylene glycol) (30 m × 0.25 mm dc, 0.25 µm df) from Trajan (Melbourne, Australia) was used. The oven temperature program was: 40°C (1min) to 200°C at 3°C/min, then to 250°C (3min) at 10°C/min. FPD detector: 260 °C.
Statistical analysis: Chemometric analysis were performed with XLSTAT software ver. 2021.2.1 (Addinsoft, New York, NY USA).

Results
The analysed coffee samples were described by the 147 volatiles. Fig. 1 is an illustrative pattern of the chemical signatures of the good and oxidised moka samples visualized with a heat map of the normalized volatile responses versus the IS.

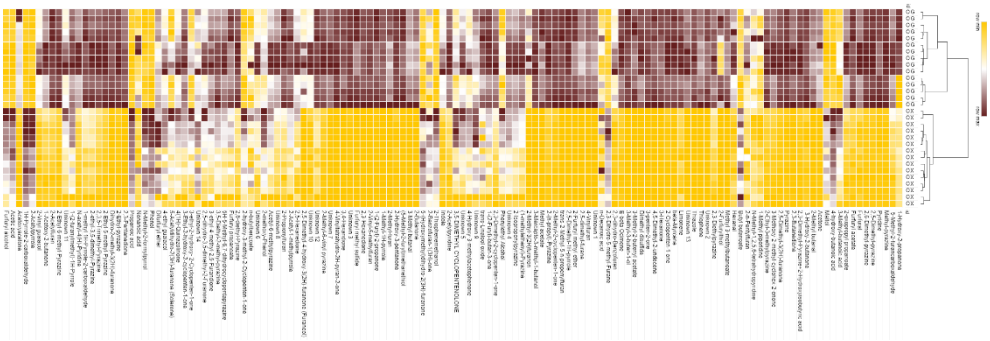

Figure 1*. Coffee volatilome heatmap of good and oxidized samples*

Supervised PLS-DA machine learning was applied to the whole sample set to extract informative volatiles that describe the oxidised coffee note. 71 significant volatiles, out 147, with a VIP (variable importance in projection) > 1 in describing the oxidised samples. Few of the volatiles that characterise the oxidised coffees have already been described as ageing markers in the literature. The components in all of the samples analysed that displayed behaviour over time that is similar to previous reports are: 2/3-methylbutanal (malty); 2,3-butandione (buttery, pungent); 2,3-pentandione (buttery, pungent); 2-furfurylthiol (roasty, coffee- like); 2,5-dimethyl-3(2H)-furanone (caramellic), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) (sweet, candy) [3-5] In this study, a number of components known to be related to ageing behave contrary to literature data in oxidised samples (i.e. 2-butanone, 2-methylfuran, hexanal, 2-acetylfuran,

dihydro-2-methyl-3(2H)-furanone), or do not significantly vary (CV% lower than 20%) in all of the investigated samples (1-methyl-1H-pyrrole, N-acetyl-4(H)-pyridine). Twenty five highly significant volatiles that describe the oxidised coffees and that show a similar trend in all of the investigated blends and packaging, and with a CV% of at least 20, were identified as potential markers of oxidised coffees.

Many are in present lower amounts in oxidised samples and a few have already been reported, in the literature, as decreasing over time [1-4], while others behave differently to previous studies. These components are all heterocycles and are highly reactive, in particular in the presence of moisture and oxygen, which may explain their decrease in oxidised coffees.

Four volatiles increase in all of the investigated samples, although to different extents depending on blend and packaging: acetic and propionic acids (pungent, sour, rancid); 1-H-pyrrole-2-carboxaldehyde (musty); and, 5-(Hydroxymethyl)-dihydro-2(3H)-furanone.

Conclusions

Twenty five target components of the coffee volatilome have been identified as markers of coffee oxidation because they present the same behaviour and statistical meaning in all of the investigated samples, independently of packaging and blend. All together, they have a synergistic role in the recognition of oxidised coffee and can be considered the fingerprint of the oxidize note. These data provide information on the oxidised note and demonstrate that artificial intelligence models can be successfully used to instrumentally define the evolution of the quality of coffee aroma over time.

References

1. Czerny, M., Schieberle, P. (2001). *19 th International Scientific Colloquium on Coffee*, Trieste, Italy.
2. Lytou, A. E., Panagou, E. Z., Nychas, G. J. E. (2019). Current Opinion in Food Science. Elsevier Evaud, Ä. P. D., Oodman, B. E. A. G. *Journal of Agricultural and Food Chemistry,* (2002), 8, 2356–2364.
3. Folmer, B. The craft and science of coffee. (2017). London, UK: Academic Press.
4. Glöss, A. N., Schönbächler, B., Rast, M., Deuber, L., Yeretzian, C. *Chimia*, (2014), 68(3),179–182.
5. Manzocco, L., Calligaris, S., Anese, M., Nicoli, M. C. In P. Subramaniam, P. Wareing (Eds.), The stability and shelf life of foods (2016) (pp. 375–396). Elsevier.

P19

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

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Summary: *The determination of residual solvents is essential for assessing the quality of food manufacturing processes in many productions such as tea and coffee. The work presents an innovative method for extraction of residual solvents, without losses during extraction, and a good solution for their quantification using a GCMS/MS system.*

Keywords: *New Approach, 7012B GC/MS Triple Quad, Residual Solvents*

Introduction

Residual solvents are unwanted volatile compounds that can be found as impurities in various food (vegetable oils, tea and coffee) Their presence is due to the industrial preparation and treatment processes such as extraction, drying or decaffeination. These solvents have a negative influence not only on the quality of food but also on human health. Methods developed in the past for the determination of residual solvents were distillation procedures coupled to GC with electron capture detector (ECD) and headspace coupled to GC with flame ionization detection (FID). Both procedures required specially fabricated equipment and are time consuming [1]. In our knowledge there are no articles in literature that extract residual solvents with a solid liquid extraction through the use of modified QuEChERS and analyze them with GC-MS/MS. So the aim of this work was to develop an innovative and rapid method which determined four residual solvents: Dichloromethane, Ethyl Acetate, Ethyl Methyl Ketone and Methyl Acetate in coffee and tea. The method showed higher sensitivity and lower LOQ than existing procedures and was validated in terms of accuracy, repeatability and linearity.

Experimental

Chemicals and Reagents: Dichloromethane 99.88% (HPC standard Gmbh); Ethyl Acetate 99.9% (HPC standard Gmbh); Methyl Acetate 99.5% (HPC standard Gmbh); 2-Butanone 99.97% also known as Ethyl Methyl Ketone (HPC standard Gmbh). Stock solution about 100 mg/L and working solution 10 mg/Kg of residual solvents were prepared in acetonitrile by dilution of pure standard by weighing on analytical balance.

During the extraction phase it was used a modified QuEChERS method which reduce potential interferences in analysis. The sample was finely ground with a blender for a few seconds at high speed using dry ice (sample to dry ice ratio 5:1). A total of 2,5 g of the sample was weighed in a 50 ml centrifuge tube, and 10 ml of pure water where added. After vortexing for a few minutes where added 10ml of acetonitrile and the sample was mechanical shaked for 20 minutes. QuEChERS was added and the tube was vortexed for 2 minutes and centrifuged for 5 minutes at 3000 rpm. The extract was cleaned up with dispersive SPE

containing GCB adsorbents to remove sugars, lipids, organic acid and others pigment. The extract was filtered (0,20 um) and injected into the GCMS/MS.
The extracts were analysed by Agilent 7890B GC System equipped with MMI injection system and coupled, through a transfer line (280°C), to an Agilent 7012B MS/MS with an Electron Impact Interface.
The column used was the Agilent J&W DB-Select 624 capillary gas chromatography designed for the analysis of volatile organic pollutants (30m x 0.25mm, 1.4 µm film thickness). In addition, a pre-column (1m) was used to prevent rapid degradation. The column temperature was initial set at 45°C and held for 3 min, then increased to 80°C at the rate of 12°C/min and held for 6 min. Finally, it was faster increased until to 260°C.
The MMI injection temperature was set at 90°C for 0,8 min. and ramped at 12°C/min until 250°C
One microliter of a standard or sample solution was injected in split mode (slit ratio of 1:10). Helium carrier gas pressure was set at of 4 psi and the temperature of the source was set at 280°C.
The study of the transitions was carried out in Multi Reaction Monitoring (MRM) mode in order to identify the best fragmentation conditions and the main m/z ratios (Table 1).

Results

When performing residual solvents analysis it is important to investigate the risk of losing these compounds during extraction phase. A comparative study using extractions at low temperatures and extractions at room temperatures highlighted the importance of using dry ice in this initial phase. The best quantity of water to be used in the first extraction phase has also been optimized in order to obtain the best recovery.
Finally, the correct extraction time to avoid losses of analytes and the best matrix amount to be analysed to prevent the signal suppression were identified.
The method was validated in coffee and tea studying repeatability, accuracy, linearity and quantification limit (LOQ).
Precision and accuracy were determined by spiking blank coffee and tea samples at different 3 fortification levels considered LOQ level, medium level and at Maximum Residue Limit (LMR)
For each level of validation were performed 10 tests in order to determine the relative standard deviation of each analyte for each level of fortification. Accuracy was determined by calculating the average recovery for each level for each analyte. For all tested matrix were achieved average recoveries between 80% and 120% and CV% well below 10%.
LOQ was estimated as the concentration of the analyte that gives a S/N equal at least to 10 and was calculated with a specific function of the Mass Hunter Agilent software (0.40 mg/Kg for all analytical residue).
The calibration was performed by matrix addition (7 levels) using a blank samples.
The added quantities correspond to finally spiking levels of 0.2 mg/Kg, 0.4 mg/Kg, 0.8 mg/Kg, 2 mg/kg, 4 mg/kg, and 20.0 mg/Kg. A dilution factor 4 is to be considered on finally results.
The matrix improves the shape of the peak and ensures good linearity. All the

calibration curves showed correlation coefficients R2 > 0.99 and Relative Residues < 20%.

Table 1. *MRM transitions of residual solvents.*

Residual solvents	MRM Transition Q1	MRM transition Q2	Collision energy (eV)
Dichloromethane	88/86	49/51	5
Ethyl Acetate	88	61/45	5
Ethyl Methyl Ketone	72	43/57	5
Methyl Acetate	74	43/31	5

Conclusions

This work presented an innovative and robust method for the extraction of residual solvents and for their identification and quantification using a GC-MS/MS system in MRM mode. In Comparison with others know methods, in this work it was described a very sensitive GC-MS/MS approach and a quick extraction procedure without any losing of analytes.
The method showed a good linearity in coffee and tea and it could be extended to other matrices.

References

1. David L. Heikes, *Journal of Association of Official Analytical Chemists,* 70 (1987) 176-180.
2. UNI EN 15662:2018
3. Sante 12682-2019 “Analitical quality control and method validation procedure for Pesticide Residue Analysis in Food and Feed”

P20

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

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Summary: *The metabolic profile of Agropyron repens rhizome herbal tea, the most used formulation, was investigated by tandem-mass spectrometry. The ESI-MS fingerprinting with diagnostic ions, and the phenolic profile by HPLC-PDA-ESI-MS/MS were provided. The essential amino acid tryptophan was identified for the first time, suggesting new perspectives of applications for Agropyron repens rhizome.*

Keywords: *tryptophan; phenolic acids; flavonoids.*

Introduction

A growing offer of products for herbal tea is currently available in herbalist shops and supermarket shelves, intended for a wide choice of different applications. But, in addition to the components with specific effects, what does really intake the consumer with a cup of herbal tea?

Agropyron repens (L.) P. Beauv. (couch grass) is a perennial rhizomatous plant widespread in the world, well-known for its infesting action for crops, and for some pharmacological properties. Used in traditional medicine mainly for urinary problems, received a scientific confirmation for hypoglycemic, hypolipidemic, anti-inflammatory and antidiabetic effects [1]. This must be why the research focused on the allelopathic compounds and anti-inflammatory molecules, mainly present in the essential oil. The *Agropyron repens* components, including phytotoxins and tryptophan derivatives growth regulator, were reviewed by the European Medicine Agency [2]. Conversely, the aqueous extracts were sparingly studied, although the herbal tea is the most used formulation in traditional medicine and herbalism. To fill this gap, our research focused on the *Agropyron repens* rhizome aqueous extracts at different temperatures, to investigate on the herbal tea chemical composition.

Experimental

Materials. Three commercial samples (AR1, AR2, AR3), purchased from different local herbal medicine shops, were characterized for water activity, absolute humidity and microbiological analysis, and used to prepare the rhizome herbal tea according to dose and procedure recommended by the Official Pharmacopoeia of the Italian Republic: 1g dry rhizome/10 mL boiling water, for 10 minutes. The supernatant was then filtered at 0.22 µm, diluted with the mobile phase, and analyzed. Aqueous extracts at 25 °C and 50 °C were also

investigated.
Targeted analysis. The samples were analyzed by a Waters system composed of a 1525µ HPLC, a PDA detector and a tandem MS/MS with an ESI source, using an XBridge C18 (150 × 2.1 mm i.d.) 5 µm analytical column, Milli-Q water/formic acid 5 mM (A) and acetonitrile/formic acid 5 mM (B) as mobile phase, flowing at 0.20 mL/min, applying a separation method previously developed [3,4,5]. Statistical analysis was applied.
Untargeted analysis. The same samples were analyzed by direct infusion into the ESI source, and spectral data acquired in full scan for 2 minutes in the mass range 80-800 Da, in both ESI- and ESI+. Fragmentation data were acquired in daughter mode in ESI- and/or ESI+, using argon as collision gas, by selecting each *m/z* value evidenced in the full scan as precursor ion, in turn.

Results
Targeted analysis. AR1, AR2 and AR3 showed similar chromatograms (TIC, Fig.1, a), regardless of extraction temperature, evidencing a few abundant peaks with characteristic absorption at λ 322-325 nm (EC 324 nm, Fig. 1, b), and one peak with λ=279 nm (EC 279 nm, Fig. 1, c). Among 29 phenolic compounds searched by SIR mode, at least 20 phenolic compounds representative of the *Agropyron repens* rhizome were identified and quantitated in most cases, among which caffeoyl and feruloyl quinic esters, caffeic and coumaric acids were the most abundant. Furthermore, tryptophan (λ=279 nm) was identified for the first time and quantitated in *Agropyron repens* rhizome.

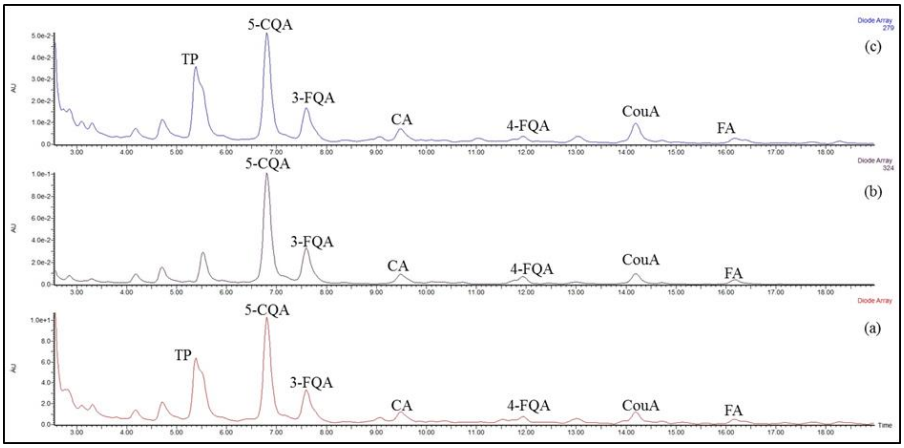

***Figure 1**. AR2 herbal tea diluted 1:10 with mobile phase (A:B, 95:5, v:v): (a) PDA chromatogram; (b) EC at λ = 324 nm; (c) EC at λ = 279 nm; TP: tryptophan; 5-CQA: 5-caffeoylquinic acid; 3-FQA: 3-feruloylquinic acid; CA: caffeic acid; 4-FQA: 4-feruloylquinic acid; CouA: coumaric acid; FA: ferulic acid*

Untargeted analysis. The ESI mass spectral fingerprinting of *Agropyron repens* rhizome herbal tea was first provided. Diagnostic ions for a di-hexose, di-hexose-pentose trisaccharide, eight small organic acids, and six amino acids were evidenced (ESI- and ESI+, Fig. 2, a and b, respectively). Some phytotoxins were

also investigated, and the presence of some of them could not be excluded even in aqueous extracts.

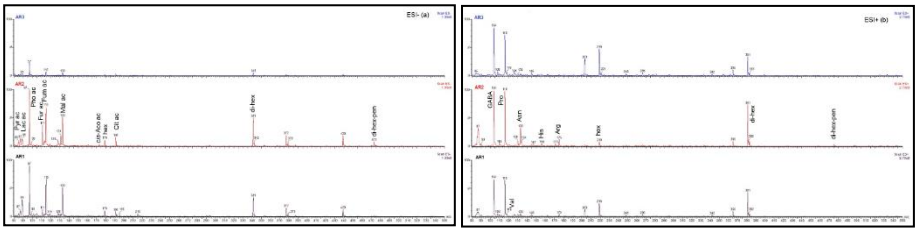

Figure 2. *ESI-MS/MS profile of Agropyron repens rhizome herbal tea (AR1, AR2, AR3): (a) ESI-; (b) ESI+. Signals are normalized to the highest one for a direct comparison among samples. (a): pyruvic acid, lactic acid, phosphoric acid, 2-furoic acid, fumaric acid, malic acid, cis-aconitic acid, hexose, citric acid, di-hexose, di-hexose-pentose. (b): γ-aminobutyric acid, proline, valine, asparagine, histidine, arginine, hexose, di-hexose, di-hexose-pentose*

Conclusions

Despite some differences likely due to geographical origin, collection period, drying process and/or storage of the AR1, AR2 and AR3 rhizomes, a comprehensive characterization of the metabolic profile of the *Agropyron repens* rhizome herbal tea was obtained. The added value of antioxidants and tryptophan in the herbal tea was evidenced, and new perspectives for AR rhizome uses were suggested. Last, diagnostic ions were provided, with useful application for fast and simple quality control analysis.

References

1. Al-Snafi, A.E.; *Res. J Pharmacol Toxicol*, 1(2) (2015) 37-41
2. EMA/HMPC/113793/2021, Committee on Herbal Medicinal Products (HMPC), 07 July 2021
3. Petrucci, R. et al.; *J Agric Food Chem,* 68 (2020) 7984-7994
4. Petrucci, R. et al.; *J Agric Food Chem,* 69 (2021) 4816–4826
5. Di Matteo, P. et al.; *J Food Sci Technol, (2022)* https://doi.org/10.1007/s13197-022-05536-8

P21

Mepiquat natural formation in cocoa commercial products

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Summary: *Mepiquat (MQ) is well-known products commonly used in agriculture as inhibitor of gibberellin biosynthesis. Several studies reported the natural formation of MQ in food heated under dry conditions. In this study cocoa products were analysed with IC-HQOMS to evaluate the presence of MQ.*

Keywords: *IC-HQOMS, Mepiquat, cocoa, chocolate*

Introduction

Chlormequat chloride (2-chloroethyltrimethylammonium chloride, CQ) and mepiquat chloride (1, 1′-dimethylbipyridinium chloride, MQ) are quaternary ammonium growth regulators usually used as chloride salt, which work on gibberellin synthesis inhibition. Several studies reported the natural formation of MQ in foods through a Maillard-type reaction that requires free lysine, a reducing sugar, and an alkylating agent, heated under dry conditions. Trigonelline, choline, and betaine have been identified as possible methylating agents [1,2]. Rarely, also the natural formation of CQ from choline is shown [3].
Theobroma cacao contains several alkaloids: mainly theobromine, caffeine and trigonelline. Moreover, also lysine is present in sufficient concentrations to be able to think that MQ can be produced during the roasting processes or the preparations of derivative products.

Experimental

In this study an IC-HQOMS method for a fast and sensitive evaluation of CQ and MQ was performed.
Cocoa powder and chocolate chips were cooked in oven at 160, 180 and 230 ◦C for 15 and 50 minutes, mimicking cakes, or biscuits cooking. These lab-cooked samples and other 24 representative cocoa commercial samples were extracted adapting the procedure of QuPPe-PO-methods and IC-HQOMS measured.

Results

The formation of MQ occurred both in cocoa and in chocolate drops starting from 180°C. At this temperature, the formation appears to be slow and visible only after 55 minutes of cooking (about 2 µg/kg), while at 230 ° C the formation is extremely faster and at 15 minutes there is already an amount almost equal to that detected at 55 minutes (about 8 µg/kg for cocoa and 7 µg/kg for chocolate drops).
The presence of either analyte was not detected in the commercial samples.

Conclusions

Cocoa can be a natural source of production of MQ, however temperatures and cooking times adopted in commercial processes are not sufficient to determine their formation. On the other hand, it would seem more plausible is the formation of MQ during the home cooking that most reflect what was operated in the laboratory.

References

1. Bessaire, T., Tarres, A., Stadler, R. H., Delatour, T.; *Food Additives & Contaminants: Part A,* (2014), 31, 1949–1958.
2. Yuan, Yuan, Y., Tarres, A., Bessaire, T., Rademacher, W., Stadler, R. H., Delatour, T.; *Food Chemistry* (2017), 228, 99–105.
3. Ekielski, A., Mishra, P. K., Żelaziński, T.; F*ood and Bioprocess Technology,* (2018), 11(6), 1177–1187.

P22

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

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Summary: *Cocoa shells are one of the main by-products of cocoa beans. In this work, a method for the recovery of methylxanthines from cocoa shells was optimized by enhancing it as a source of biomolecules. The extracts were also tested on colorectal and breast cancer cells for their effect on human health.*

Keywords: *Theobroma cacao, PHWE, chemometric analysis*

Introduction

Cocoa beans are widely used in the food, pharmaceutical and cosmetic industries given their high content of antioxidant compounds. World production of cocoa beans is estimated at some million tons, producing large amounts of waste. Cocoa shells are 12-20% of the cocoa beans and represent are one of the main by-products during the pre-roasting/roasting process with disposal costs and an impact on the environment[1-2]. However, some studies have shown that cocoa shells still contain active molecules such as phenols and methylxanthines[3-4]. Therefore, the valorization of this by-product could bring economic and environmental benefits. By-product valorization requires rapid green methods that increase extraction yields reducing environmental impact. Pressurized liquid extraction (PLE) is a green extraction technique widely used for the recovery of metabolites from food matrices[5-6]. Using high temperatures and pressures, PLE decreases extraction times and improves solvent extraction efficiency, often this technique is used with only water taking the name pressurized hot water extraction (PHWE)[5].
The aim of this work is to develop an environmentally friendly, fast, and inexpensive extraction method for the recovery of metabolites of nutraceutical, cosmetic and pharmaceutical interest from cocoa shells.

Result and Discussion

First, the chemical composition of cocoa shells was studied by high-resolution mass spectrometry analysis. The results show a low content of phenolic compounds, and the presence of caffeine and theobromine, two methylxanthines abundant in cocoa[7]. Therefore, a method was optimized in PHWE for methylxanthine recovery. The optimization was performed by a chemometric approach using experimental design. For optimization, 4 parameters (temperature, static time, number of cycles, and solvent composition) consider the influence on 2 variables: methylxanthine yield and antioxidant activity of the extract. The results show a significant influence of the number of cycles and temperature on the antioxidant power and methylxanthine yield. Considering the

response variables (caffeine, theobromine and AOC), the chemometric analysis suggested the flowing parameters in the optimized conditions: Temperature 90 °C, Cycles 5, EtOH 15% and Static Time 6 minutes with optimized desirability to 98.42%. Also, the PHWE extract increased the recovery of theobromine and caffeine by 156% and 160%, respectively, compared with ultrasound extraction. Finally, the PHWE extract was tested in vitro on cell viability of colorectal and breast cancer cell lines showed an interesting mechanism of sensitization of human cancer cells to the anti-cancer agent cetuximab.

Conclusions

The PHWE technique has proven to be a sustainable and effective approach for the recovery of theobromine and caffeine from cocoa shells, allowing these agro-industrial by-products to be exploited as a possible source of bioactive molecules. in addition, the optimized extract showed promising activities in supporting chemotherapy against colon and breast cancer.

References

1. Panak Balentić, J., Ačkar, Đ., Jokić, S., Jozinović, A., Babić, J., Miličević, B., … Pavlović, N. (2018). *Molecules*, 23(6),1404.
2. Quelal-Vásconez, M. A., Lerma-García, M. J., Pérez-Esteve, É., Arnau-Bonachera, A., Barat, J. M., Talens, P. (2019). *Food Control*, *99*, 68-72.
3. Arlorio, M., Coïsson, J. D., Travaglia, F., Varsaldi, F., Miglio, G., Lombardi, G., Martelli, A. (2005). *Food Research International*, 38(8–9), 1009–1014.
4. Okiyama, D. C. G., Soares, I. D., Cuevas, M. S., Crevelin, E. J., Moraes, L. A. B., Melo, M. P., … Rodrigues, C. E. C. (2018). Food Research International, 114, 20–29.)
5. Ameer, K., Shahbaz, H. M., Kwon, J. (2017). *Comprehensive Reviews in Food Science and Food Safety*, 16(2), 295–315.
6. Mustafa, A., Turner, C. (2011). *Analytica Chimica Acta*, 703(1), 8–18.
7. Barbosa-Pereira, L., Guglielmetti, A., Zeppa, G. (2018). *Food and Bioprocess Technology*, 11(4), 818–835.

P23

Mineral content in plant-based drinks

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Summary: *Plant-based drink of different plant matrices were analyzed in this study. The objective was to determine the mineral content and the relative daily intake. The results showed that these matrices were not contaminated with toxic elements. Furthermore, it was possible to establish whether plant-based beverages are an excellent source of mineral elements.*

Keywords: *plant-based drinks, mineral elements, ICP-MS, DMA-80*

Introduction

Plant-based drinks are obtained from extracts of various plant matrices, including cereals, pseudo-cereals, legumes, nuts, and seeds. In the world, they are marketed as alternatives to cow's milk. In recent studies, the protein, energy content and selected mineral elements in some beverages have been evaluated [1-5].

Experimental

In this research, the Na, Mg, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, Cr, Al, B, Se, Mo, As, Ba, Cd, Pb and Hg content in 12 types of plant-based beverages (walnut, organic almond, organic soya, rice, organic oats, organic rice and coconut, organic buckwheat, organic rice and hazelnuts, sorghum, spelt, millet and coconut) by ICP-MS and DMA-80 was evaluated. Furthermore, the daily intake also was calculated.

Results

The highest amount of potassium and magnesium were found in organic buckwheat, while the highest concentration of sodium was found in "organic rice and hazelnuts" and, finally, the most important calcium content was determined in walnut. Iron, zinc, manganese, and copper were present in abundant amounts: organic buckwheat had the highest concentration of Fe and Zn; organic rice-coconut the highest content of Mn; organic soya the highest level of Cu.
Actually, there is no specific regulation for the toxic elements content in plant-based beverages. Considering the legal limits (Regulation N. 1881/2006 and Regulation N. 1323/2021) for Cd and Pb in cereal-based foods, none of the samples showed a content exceeding the maximum permitted values.

Conclusions

The results demonstrated the safety of such plant-based drinks, given the low contamination by toxic elements (As, Cd, Hg and Pb).

In addition, the one cap consumption (200 mL/day) of each plant-based drinks analyzed in this study resulted in an element intake below the recommended dose, except in the coconut and in “organic rice and coconut” drink for Ni contribution.

References

1. K. M. Collard and D. P. Mccormick, *Academic Pediatrics*., vol. 000, (2020), 1–3.
2. F. Takens, D. Rand and L.S. Young editors, *Dynamical Systems and Turbulence*, Warwick (1980), pp 36-38.
3. K. E. S. Ahrens, F. Ahrens and C. A. Barth, *European Journal of Nutrition*, 59, (2019), 19-34.
4. I. Fructuoso, B. Rom, H. Han, A. Raposo, A. Ariza-Montes, L. Araya-Castillo, and R. P. Zandonadi, *Nutrients*, 13 (2021), 2650.
5. S. K. Vanga and Vijaya Raghavan, *J. Food Sci. Technol.*, vol. 55, no. 1, (2018), 10–20.

P24

Determination of phytochemical compounds residues in raw cow milk

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Summary: *Milk is one of the most produced agricultural commodities in the world, and can be consumed as fresh milk or as processed products. We developed a streamlined analytical protocol involving a QuEChERS sample preparation step coupled with GC-MS/MS to analyze four phytochemicals, used to improve growth and animal health, in raw cow milk.*

Keywords: *phytogenics, raw cow milk, GC-MS/MS*

Milk is the third most produced agricultural commodity worldwide and cattle accounts for 83 % of the total milk production. Cow milk can be consumed fresh or after processing in a myriad of dairy products. Milk and dairy products are nutrient-dense foods supplying energy and significant amounts of protein and micronutrients [1]. Human consumption of cow milk continues to rise worldwide and this pushes the dairy industry for an increase of productivity, but without the use of antibiotic growth promoters which have been banned in EU since 2006 [2]. One of the most promising alternatives to antibiotics to improve growth and animal health, are a group of secondary plant metabolites that includes a wide range of naturally derived chemical compound classes like phenolic compounds, terpenes, alkaloids, lectins, aldehydes and ketones, called phytochemicals [3]. These substances may leave residues in the milk and meat from supplemented animals so it is important to determine these residues within the scope of consumer safety assessment.

We developed a streamlined analytical protocol involving a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) sample preparation step coupled with GC-MS/MS to analyze four phytochemical compounds (menthol, carvone, thymol and carvacrol) in raw cow milk.

QuEChERS is a valid alternative to the usual sample preparation techniques for food matrices, that still represent one of the most challenging ones due to their complexity [4]. The most effective match for the QuEChERS sample preparation method in terms of instrumental analytical technique is the GC-MS/MS operated in multiple reaction monitoring (MRM) mode, which reduces the matrix background, thus allowing to improve selectivity and sensitivity to achieve limits of quantitation (LOQ) in the low ppb range [5].

The method allowed to reach a LOQ of ≤ 10 ppb for all compounds with recoveries at the LOQ concentration ranging from 74 % to 115 % (RSD < 10 %; n = 6).

Real samples from the Italian market were analyzed and for all the aforementioned compounds the concentrations were below the LOQ.

References

1. *FAO The Global Dairy Sector: Facts,* (2016)
2. C. Yang et al.; *Pathogens*, 4 (2015), 137
3. R.G. Berger editor, *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*, 2007, 4
4. S.J. Lehotay et al.; *Journal of Chromatography A*, 1217 (2010), 2548
5. F. Hernández et al.; *Anal. Methods*, 5 (2013), 5875

P25

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

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Keywords: *LC-MS, milk, lipidomics, triacylglycerols, Q-Exactive Orbitrap*

Hay milk is a traditional dairy product recently launched on the market. It is protected as “tradi-tional speciality guaranteed” (TSG) and subjected to strict regulations. One of the most important restrictions is that the cow’s feed ration must be free from silages. There is the need for analytical methods that can discriminate milk obtained from a feeding regime including silages. This work has developed an optimized workflow for the targeted analysis of TAGs in milk by liquid chromatography coupled with a Q-Exactive Orbitrap mass spectrometer. First, the effects of resolution (17,500; 35,000; 70,000 and 140,000) and automatic gain control target (AGC, from 2x104, 2x105, 1x106 and 3x106) have been optimized with the goal to minimize the injection time, maximize the number of scans and minimize the mass error. Then, the flow rate of the liquid chromatography system was also optimized by maximizing the number of theoretical plates. The resulting optimized parameters consisted of a flow rate of 200 µL/min, mass resolution of 35,000 and AGC target of 2x105. Such optimal conditions were applied for targeted TAGs analysis of milk fat extracts. Up to 14 target triglycerides in milk fat were identified performing a data dependent HPLC-HRMS-MS2 experiment (t-SIM-ddMS2). Finally, with the use of these biomarkers and multivariate statistical analysis we were able to predict the use of maize and grass silages in the cow’s diet with 100% recognition percentage. Our findings suggest that the use of analytical approaches based on HRMS is a viable authentication method for haymilk.

P26

Traceability of pasture milk using alkaloid profile

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Summary: *Alkaloids are basic nitrogen-containing organic compounds widespread in nature. The study evaluated the possibility to use them for trace pasture milk.*

Keywords: *traceability, alkaloids, milk, Orbitrap*

Introduction

Alkaloids (alks), basic nitrogen-containing organic compounds, playing an important role in the interaction of plants with their environment. Some alks are responsible for the beneficial effects of plant extracts in humans in traditional medicine [1], nevertheless, other alks show highly toxic effects [2]. The alk profile of 62 different herbal plants, sampled in alpine pasture of north-eastern Italy were studied by Nardin et al. [1] bringing out an interesting bond with the plant families. The study evaluated the transfer of alks from herbs to milk and possibility to use these parameters as marker for milk traceability.

Experimental

12 milk samples, collected from cows grazing in two different north-eastern Italy pastures, were extracted with H2O/MeOH/FA (40:40:20 v/v/v) and fat was removed with hexane. 30 µL were injected in the UPLC with an online clean-up performed by loading the sample on a SolEx HRP SPE cartridge. All the chromatograms were recorded in profile mode through a full MS-data dependent MS/MS experiment employing a Q-Exactive™ hybrid quadrupole-orbitrap mass spectrometer (HQOMS) equipped with heated electrospray ionization (HESI-II) interface. A targeted and untargeted studies were performed using 41 analytical standard and a homemade database.

Results

In the milk samples, a significantly low number of alkaloids were detected showing that the transfer of these compounds does not always occur. Despite this, Figure 1 shows the samples perfectly divided in two groups corresponding to the pasture where the milk was produced.

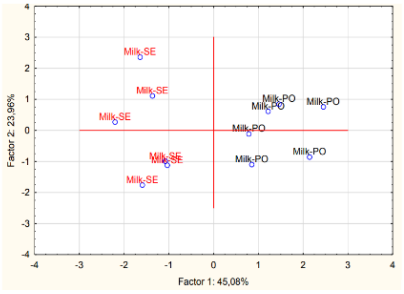

Figure 1. *Principal Component Analysis of mass milk samples according to the two alpine pastures (PO= Poion alpinae; SE= Seslerion caeruleae)*

Conclusions

Promising results have occurred in the use of alkaloids as markers for pasture milks.

References

1. Applequist, W.L., Moerman, D.E., Economic. Botany (2011), 65(2), 209-225.
2. Pabreiter, C.M., Phytochemistry (1992), 31(12), 4135-4137.
3. Nardin T, Larcher R, Barnaba C, Bertoldi D, Pasut D, Romanzin A, Piasentier E., Nat Prod Res. (2022), 12, 1-8.

P27

Occurrence of polyphenols and their metabolites in Pecorino cheese

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Summary: *Tyrosol and hydroxytyrosol are polyphenols widely studied in several plants, especially in olive products. Therefore, they could be present in food produced from herbivorous animals. In this study, the concentrations of these compounds and those of some of their phase II metabolites were measured in Pecorino cheese.*

Keywords: *cheese, polyphenols, LC-Q-Orbitrap*

Introduction

There is an extensive literature on the presence of tyrosol (Tyr), hydroxytyrosol (HTyr) and related compounds in various plants and, especially, in olive products (fruit, leaves and oil) as these molecules have demonstrated several beneficial health effects [1]. The olive oil production involves the generation of a large amount of solid residues and wastewater that can have a great impact on terrestrial and aquatic environments due to their high phytotoxicity. Therefore, many efforts have been made to reuse these wastes, including their use in animal nutrition [2]. In this context, some our recent papers have measured the concentrations of these polar polyphenols and some of their phase II metabolites in animal tissues, sheep milk and cheese produced by animals fed with diets containing waste by-products generated from olive oil extraction [3,4]. However, since HTyr and Tyr are contained in glycosylated form in several plants, these compounds could also be found in food produced by farm animals fed with conventional feed. In addition, Tyr and HTyr are synthesized endogenously in the human and animal organism as by-products of tyramine and dopamine metabolism, respectively [5]. The purpose of this work was the analysis of Tyr, HTyr and some of their phase II metabolites in commercial Pecorino cheeses in order to determine their "natural background" concentrations.

Experimental

Twelve Pecorino cheeses collected in local markets were analyzed to determine the following polyphenols and metabolites: tyrosol (Tyr), hydroxytyrosol (HTyr), tyrosol-sulphate (Tyr-S), hydroxytyrosol-4-O-sulphate (HTyr-4-S), hydroxy-tyrosol-3-O-sulphate (HTyr-3-S), tyrosol-glucuronide (Tyr-G) and hydroxy-tyrosol-3-O-glucuronide (HTyr-3-G). One gram of sample was weighted and extracted with 3 mL of a mixture formic acid 0.5% in MeOH/water 80/20 v/v and, after shaking and centrifugation, the supernatant transferred at -80 °C. Thirty minutes later, the supernatant was decanted in a 15 mL tube. The extraction was repeated for a second time and the reunited supernatants were evaporated. After dissolution, the sample was injected in an LC-Q-Orbitrap platform (LC-Q

Exactive Plus, Thermofisher Scientific, San Jose, CA, USA). The acquisition was carried out in ESI negative ionization mode performing full MS/dd-MS^2 experiments. Analytes were separated with an LC column Acquity BEH C18 (2.1 x 150 mm, 1.7 µm, Waters, Milford, MA, USA) using MeOH and water containing 2 mM ammonium acetate as mobile phases.

Results and discussion

HTyr was found only in four out of twelve analysed Pecorino cheeses in a range from 2 to 16 µg/kg dw (dry weight). Tyr, Tyr-S, HTyr-3-S and HTyr-4-S were always detected in the ranges 18-273 µg/kg dw, 7.1-33 µg/kg dw, 75-222 µg/kg dw and 91-278 µg/kg dw, respectively. Their mean concentrations were 91, 14, 152 and 192 µg/kg dw, respectively. Glucuronide metabolites were never found. In the Figure, the chromatograms and spectra of the two sulphate metabolites of HTyr detected in Pecorino cheeses are shown.

In our previous study performed administrating an enriched polyphenol diet to dairy sheep, Tyr-S, HTyr-3-S and HTyr-4-S in cheese were measured at mean values of 176, 549 and 773 µg/kg dw, respectively [4]. On the other hand, in cheese produced from milk of sheep belonging to control group, the concentrations of these compounds were significantly lower (from 14 to 70 µg/kg dw). The parent compounds, Tyr and HTyr, were found at 808 and 33 µg/kg dw, respectively, in cheese of treated animals. Similar levels were measured in cheese of sheep fed with conventional feed (613 and 29 µg/kg, respectively). These data highlight that the sulphate metabolites, which have still beneficial health effects [6] should be considered when Tyr and HTyr contents were measured in food produced from herbivorous animals, since their concentrations exceed those of parent compounds. Moreover, although the administration of polyphenol enriched diet to diary sheep significantly increases the levels of all sulphate metabolites in Pecorino cheese [4], their “natural” occurrence is not negligible.

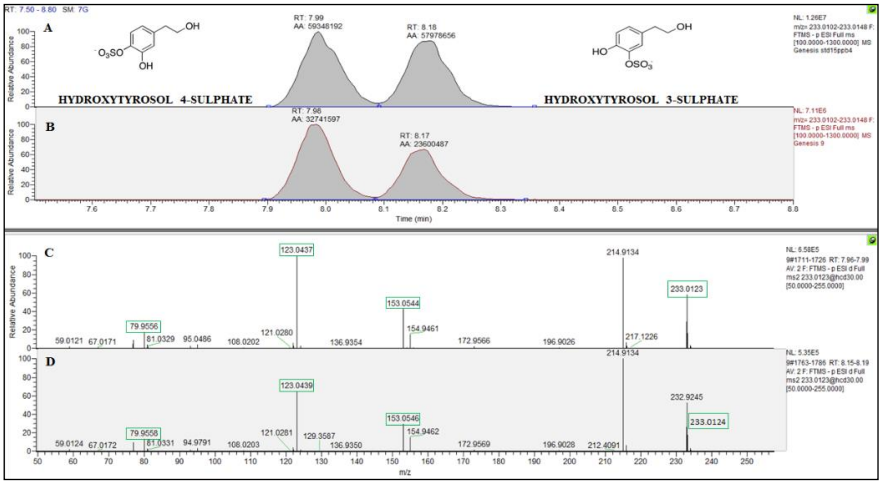

Figure 2. *LC-HRMS/MS chromatograms of the two isomers of hydroxytyrosol sulphate (HTyr-3-S and HTyr-4-S) in a standard solution at 150 ng/mL (A) and in a Pecorino cheese (B); MS^2 spectra of HTyr-4-S (C) and HTyr-3-S (D).*

Conclusions

For the first time, the levels of Tyr, HTyr and their sulphate metabolites were measured in cheese produced from milk of sheep fed with conventional feed. In some cases, the concentrations of Tyr, HTyr-3-S and HTyr-4-S reached also levels higher than 250 µg/kg dw. The origin of these compounds is most likely to be found in the vegetables included in dairy sheep feed such as corn, barley and soy.

Acknowledgments

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References

1. A. Karkovic Markovic, J. Toric, M. Barbaric, C.J. Jakobusic Brala; *Molecules,* 24, (2019), 2001
2. J. Berbel and A. Posadillo; *Sustainability*, 10, (2018), 237
3. R. Branciari, R. Galarini, D. Giusepponi, M. Trabalza-Marinucci, C. Forte, R. Roila, D. Miraglia, M. Servili, G. Acuti, A. Valiani; *Sustainibility,* 9 (2017), 1-13
4. R. Branciari, R. Galarini, D. Miraglia, D. Ranucci, A. Valiani, D. Giusepponi, M. Servili, G. Acuti, M. Pauselli, M. Trabalza-Marinucci; *Animals*, *10,* (2020), 1941
5. J. Rodríguez-Morató, A. Boronat, A. Kotronoulas, M. Pujadas, A. Pastor, E. Olesti, C. Pérez-Mañá, O. Khymenets, M. Fitó, Magí Farré, R. de la Torre; *Drug Metabolism Reviews,* 48(2), (2016), 218-236.
6. G. Serreli and M. Deiana; *Antioxidants*, 7 (2018), 170

P28

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

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Summary: *Polycyclic Aromatic Hydrocarbons (PAHs) are chemical compounds associated with risks to human health, especially carcinogenesis. PAHs in food originate from environmental deposits or arise from food processing. In this study a fast and effective method to quantify PAHs in difficult fatty food matrices, such as cheese, was proposed.*

Keywords: *PAHs, cheese, GC-MS/MS*

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are chemicals composed of hydrogen and carbon atoms arranged in two or more fused aromatic rings. More than a hundred different PAH compounds exist and almost always in mixtures. PAHs represents environmental carcinogenic compounds, and can arise as a result of human activity as well as during food processing. The major route of human exposure to PAHs for non-smokers is food. Because of their lipophilic nature, PAHs can accumulate easily in fatty food matrices such as milk, meat, edible oil, fish and cheese [1].
The aim of the presented study was to develop an effective and easy sample preparation procedure for the determination of PAHs in commercial cheese samples.

Experimental

For this work, 60 samples of non-smoked hard cheese were collected from the market. The optimised protocol for PAHs quantification was composed of three steps: 1) fast QuEChERS extraction using cyclohexane as solvent, 2) easily and fast mechanically clean-up by Silica Gel (70-200 mesh ASTM) and 3) concentration under gentle stream of nitrogen.
PAHs analysis was carried out on an Agilent Intuvo 9000 GC system coupled with an Agilent 7000 Series Triple Quadrupole MS using a HP-5MS Ultra Inert (30 m × 0.32 mm id × 0.25 µm film thickness) capillary column. Chromatograms were acquired in selected-ion monitoring (SIM) mode and target compounds were identified according to their ions and retention times.
For method development were considered 18 different PAHs: naphthalene (NaP), 2-methylnaphthalene (MeNaP2), 1-methylnaphthalene (MeNaP1), acenaphthene (Ace), acenaphthylene (Acp), fluorene (Flu), phenanthrene (Phen), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), benzo[a]anthracene (B[a]a), chrysene (Chr), benzo[b]fluoranthene (B[b]f), benzo[k]fluoranthene (B[k]f), benzo[a]pyrene (B[a]p), indeno[1,2,3-c,d]pyrene (I[cd]p), dibenzo[a,h]-anthracene (D[ah]a), benzo[g,h,i]perylene (B[ghi]P).

Results

The developed method was validated in terms of linearity, repeatability, reproducibility, recovery, limit of detection (LOD), and limit of quantification (LOQ). Linearity was evaluated between 0 and 500 µg/kg; the values of the R^2 were higher than 0.99 for all the considered compounds.

The repeatability and reproducibility were calculated from five spiking samples analysed on the same day and from three different days, respectively. The repeatability was lower than 10% for all analytes, whereas the reproducibility was in most cases better than 10%, going from 4 to 15%.

The recovery was calculated at two different concentration levels: 20 and 100 µg/kg. All results were found within acceptable limits and ranged from 75% to 110%. The LOD and LOQ were estimated at 0.3 and 0.9 µg/kg, respectively.

Once validated, the method was applied to analyze the entire set of collected samples. In only three cheese samples were identified the PAH compounds. Specifically, were quantified the three PAHs with the lower molecular weight: NaP, MeNaP2 and MeNaP1. However, in these samples the contamination degree was very low with a highest total concentration of 30 µg/kg.

Conclusions

The experiment conducted in this study demonstrated that the proposed method allows an effective clean-up of the samples and at the same time achieve acceptable recoveries, repeatability and reproducibility for PAHs quantification in cheese. For this reason, the method could be successfully applied at different food matrices with high fat content.

References

1. L.Duedahl-Olesen. 13 - Polycyclic aromatic hydrocarbons (PAHs) in foods - Persistent Organic Pollutants and Toxic Metals in Foods. Woodhead Publishing Series in Food Science, Technology and Nutrition 2013, pages 308-33.

P29

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

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Summary: *The VOCs profile of media fermented by lactic acid bacteria can be altered by changing the amino acid present. A better understanding of the complex metabolic pathways used by lactic acid bacteria will help to determine how to alter the substrate composition to maximise development of targeted flavour compounds. This knowledge could for example be used to more efficiently develop flavour compounds from plant-based substrates, that could be used to formulate meat or dairy flavours.*

Keywords: *meat or dairy flavours, plant-based substrates, and metabolic pathways*

Introduction

Reductions in meat and dairy consumption worldwide highlight consumers' shift toward plant-based foods[1,2]. However, many consumers still desire meals which are familiar in terms of their appearance, flavour, texture, and preparation methods[3]. This need has resulted in a large increase in the volume and diversity of analogues that resemble meat or dairy products[4]. The biggest challenge when producing these analogues is providing realistic meat or dairy-like flavours from plant components. Microbial biosynthesis/fermentation is a potential route for synthesizing meat or dairy-like flavours from plant substrates. A better fundamental understanding of microbial metabolic pathways during the fermentation of substrates present in plants will help in assessing the steps required more effectively produce targeted flavour components/chemicals required to combine to form realistic meat or dairy-like flavours. As a first step in the process, a defined nutrient medium was developed as a base for the growth of the lactic acid bacteria, *Lactobacillus brevis.* The defined media and defined media supplemented with different amino acids separately or in combination, was inoculated with *L. brevis* and the volatile organic compounds (VOCs) produced during fermentation measured.

Method and Materials

The defined medium (G) containing glucose, peptone, phosphate, acetate, mineral salts, and vitamins was supplemented with either leucine, isoleucine, phenylalanine, threonine, methionine, or glutamic acid (Table 1) separately or in combined was fermented by *L. brevis* - WLP672 for 16 days at 25 ^{o}C. Glutamic acid was added to all media except G. After 16 days, the fermented broth was centrifuged, 10 mL of the supernatant added to a headspace vial containing 3 g NaCl and 20 µL of 3-heptanone (0.01 mg/mL) was added, capped with a teflon faced-septa and stored at 4 °C until analysis by gas chromatography mass spectrometry (GC-MS). VOCs after fermentation were extracted using 50/30 µm,

DVB/CAR/PDMS fiber by head space - solid phase microextraction (HS-SPME) and analyzed using GC-MS.

Table 1. Medium composition

	Defined medium composition	Glutamic acid	Leucine	Isoleucine	Phenylalanine	Threonine	Methionine
A	✓	✓	✓				
B	✓	✓		✓			
C	✓	✓			✓		
D	✓	✓				✓	
E	✓	✓					✓
F	✓	✓					
G	✓						
H	✓	✓	✓	✓	✓	✓	✓

Results and discussion

The pH level affects the growth of the bacteria and can change during growth owing to compounds produced by the bacteria[5]. As expected, pH values decreased in all media over the 16 days of fermentation. Turbidity which is an indicator of cell numbers also increased over time.

VOCs formed by fermentation were strongly influenced by the addition of single or combined amino acids. The addition of amino acids resulted in the formation of VOCs that could be attributed to specific catabolic pathways by *L. brevis* which were specific to each amino acid.

Specifically, the addition of leucine to the medium resulted in high concentrations of 3-methyl-1-butanol (Isoamyl alcohol/isopentyl alcohol), and 3-methyl-1-butanoic acid (Isovaleric acid) (Leucine). Adding phenylalanine to the medium resulted in higher concentrations of benzaldehyde, 2-phenyl ethanol and 2-phenyl methanol (Benzyl alcohol) than the control with no added phenylalanine. Methionine addition to the medium led to higher concentrations of dimethyl trisulphide.

In the absence of the addition of glutamic acid, leucine, isoleucine, phenylalanine, threonine or methionine the amino acid catabolic derived VOCs presented above were still detected in the medium but at lower concentrations. This was attributed to the presence of the peptone in the defined medium. The addition of glutamic acid the medium increased the concentrations of amino acid catabolic derived VOCs compared to the medium containing only peptone. This is because glutamic acid is an important intermediate in all amino acids' catabolism[6].

Conclusion

Microorganisms utilize amino acids to synthesis cellular component's and for energy which results in the production of distinct VOCs through reaction including decarboxylation, deamination, or elimination by the action of enzymes.

In this study, *L. brevis* fermentation generated an array of flavour VOC distinct to each amino acid. This study will improve understanding on how to more efficiently target the production of VOCs that resemble meat or dairy-like flavours and help to explain their complex metabolic pathways. This knowledge will subsequently be able to be applied to plant-based substrates. Further, although flavour VOCs were measured in the HS-SPME-GC-MS, online tracking of VOCs using PTR-ToF-MS will better explain the changes in VOC production over time.

References

1. Sajeev E.P.M., Martin R., Waite C., M. Norman. 2020. Is the UK ready for plant-based diets? Report Produced for the Global Food Security Programme, December 2020. https://www.foodsecurity.ac.uk/publications/
2. Austgulen, M. H., Skuland, S. E., Schjøll, A., F. Alfnes. 2018. Consumer readiness to reduce meat consumption for the purpose of environmental sustainability: Insights from Norway. Sustainability. 10(9): 3058. https://doi.org/10.3390/su10093058
3. Szejda, K., Urbanovich, T., Wilks, M. 2020. Accelerating consumer adoption of plant-based meat: An evidence-based guide for effective practice. Five Working Papers. Washington, DC: The Good Food Institute
4. Euromonitor Internationals 2016. Accessed May 2022. https://www.euromonitor.com/article/tech-industry-driving-innovation-meat-dairy-analogues
5. Lee, L. W., Cheong, M. W., Curran, P., Yu, B., Liu, S. Q. (2015). Food Chemistry, 185, 182–191. https://doi.org/10.1016/j.foodchem.2015.03.124
6. Christensen J.E., Dudley E.G., Pederson J.A., Steele J.L. (1999) Peptidases and amino acid catabolism in lactic acid bacteria. Antonie van Leeuwenhoek, 76:217–246. doi: 10.1023/A:1002001919720.

P30

Functional compounds in experimental Provola Ragusan cheese

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Summary: *The aim of the work was to evaluate the effects of olive cake in the diet of dairy cows in terms of Provola cheese quality. Provolas in the experimental group are found to be better in both fatty acid profile and polyphenol content. For this reason, it could be well liked by consumers.*

Keywords: *Provola cheese, olive cake, polyphenols, fatty acids.*

Introduction

Provola cheese is one of the most famous and highly nutritional dairy products in southern Italy. It is also popular in the entire peninsula because it is a common ingredient in many gastronomic preparations. Olive cake, a by-product of olive mill waste, is a natural source of beneficial fatty acids, phenolic compounds and natural antioxidants [1]. The use of olive cake in the feeding of dairy cows is studied [2], but little information exists regarding the quality of the resulting cheese. The aim of this work was to evaluate the effects of olive cake supplementation in the diet of dairy cows in terms of the quality of obtained Provola cheese. In particular, the fatty acid profile and total polyphenol content of both olive cake and Provola cheese samples were evaluated in order to verify the real effect of olive cake supplementation in the finished product.

Experimental

The experiment was carried out during the period from March 2021 to July 2021 on 460 healthy multiparous dairy cows divided into two homogeneous groups named CTR and BIO. The cows were farmed in a commercial dairy farm located in Ragusa (Sicily (Italy)). The experimental group (BIO) received a concentrate supplemented with 8% pitted olive cake (OC) of the drattile matter dose; the control group (CTR) received a concentrate with no olive cake supplementation. Four representative Provolas samples (80 x 500 g) were analysed monthly for each group. Each representative sample was obtained from five Provolas randomly selected from 20 Provolas. Total lipid content, fatty acid profile and total polyphenols were determined in olive cake and Provolas cheese samples. Briefly, lipid extraction of olive cake was performed according to AOAC method 920.39, while lipid extraction of Provolas were performed according to the Folch method [3] with some modifications. Fatty acid methyl esters (FAME) were prepared by transmethylation of OC and Provola lipid extracts according to ISO 5509 2000 method and analyzed by gas chromatograph (GC). The total polyphenol contents of olive cake and Provolas cheese samples were determined spectrophotometrically using Folin-Ciocalteu reagent according to

the methods of Singleton, V. L et al. [4] and Shetty et. al. [5] respectively. Fifteen parameters of interest were modeled using an analysis of variance (ANOVA); a principal components analysis (PCA) was performed to find the parameters that best described the variability of the samples.

Results

The total lipid and polyphenol content of OC is respeatedly 180.80 g/kg and 10.18 g/kg, in agreement with Dal Bosco et. al. [6]. The OC fatty acid profile was characterized by a high amount of C18:1n-9 (66.63%), C18:2 (10.66%) and C16:0 (16.14%). Provolas total lipids content was 20% in average. Moreover, lipids content showed seasonal variability, but no correlation with diet was observed. The Provolas polyphenol content in the analyzed samples ranged from 92.07 ± 1.91 mg/kg to 165.84 ± 7.90 mg/kg for the BIO group, and from 72.11 ± 0.46 mg/kg to 112.26 ± 6.38 mg/kg for the CTR group. The lower polyphenol and lipids content recorded in May can be attributed to seasonal physiological changes in animal metabolism and variations in climatic conditions. In each case, the total polyphenol content of BIO samples was always higher than CTR samples. The fatty acid profile of BIO Provolas was characterized by of C16:0 (30.90%, average amount), C18:1 n-9 (19.90%), C18:0 (12.84%) and C14:0 (11.24%), while CTR Provola was characterized by of C16:0 (32.91%, average amount), C18:1 n-9 (19.60%), C18:0 (10.84%) and C14:0 (11.60%). According to PCA results BIO samples were separated from CTR samples on PC1. The first have highest values of C20:1, C18:0, and C22:0, whereas the last have highest values C16:0 and C14:0. On PC2 the months of March April and May at the top and June and July at the bottom can be distinguished.

Conclusions

A significant result of this research is the increased content of total polyphenols and fatty acid in BIO Provola compared to CTR. This provides added value to the final product. In conclusion, the nutritional and health composition of Provola cheese obtained from feeding dairy cows with olive cake supplementation is superior to that obtained from a conventional diet.

References

1. Benincasa, C., Pellegrino, M., Veltri, L., Claps, S., Fallara, C., Perri, E.; *Molecules*, 26(14), 2021, 4337.
2. Foti, P., Pino, A., Romeo, F. V., Vaccalluzzo, A., Caggia, C., Randazzo, C. L.; *Microorganisms*, 2022, 10(2), 237.
3. Folch, J.; Lees, M.; Stanley, G. S.; *J. Biol. Chem*. 1957, 226, 497-509.
4. Singleton, V. L.; Rossi, J. A.; *J. Enol. Vitic*. 1965 16, 144-158.
5. Shetty, K.; Curtis, O. F.; Levin, R. E.; Witkowsky, R.; Ang, W.; *J. Plant Physiol*. 1995, 147, 447-451.
6. Dal Bosco, A.; Mourvaki, E.; Cardinali, R.; Servili, M.; Sebastiani, B.; Ruggeri, S.; Mattioli, S.; Taticchi, A.; Esposito, S.; Castellini, C.; Meat Sci. 2012, 92, 783-788.

P31

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

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Summary: *The effect of milk collection, dairy factory, and seasonality on volatile organic compounds (VOCs) in hard seasoned cheese were studied to improve quality control analyzing via SPME/GC-MS the VOCs profiles of the wheels of Trentingrana cheese, a variety of the PDO Grana Padano over two years.*

Keywords: *Hard ripened Cheese, Volatile Organic Compounds, SPME/GC-MS, Quality control, Anova-simultaneous Component Analysis, Orthogonal Partial Least Squares Regression*

Introduction

Several Volatile Organic Compounds (VOCs) contribute to odors and flavors that play a priming role in sensory perception [1,2].
In cheese products, VOCs are both directly transferred from pasture to milk and produced by cow's metabolism, by catabolic activity of endogenous enzymes and microorganisms [3].
The VOC profile of cheese is a chemical feature that is related to the quality of the process and the sensory properties of cheese [4].
A large portion of the VOC profile in food products can be measured qualitatively and quantitatively using SPME/GC-MS analysis, obtaining a large amount of information related to different chemical properties of food.
Trentingrana cheese is produced in dairies located in the Trentino region (Italy) following the same production disciplinary of Grana Padano (EC Commission Regulation No. 1107, 1996) although minor variations in the processing condition and in the milk collection procedure are permitted. These factors, together with season variation in milk composition, are sources of variability in cheese production that need to be monitored to guarantee the quality of the final product.
In the present work, the overall variability of the VOC profile of Trentingrana cheese over two years of production was analyzed with SPME/GC-MS, sampling a representative batch of cheese wheels from the production quality control procedure. The work aims to estimate the overall VOC profile of Trentingrana cheese and the effect of the constant variables of the process (the dairy factory, the part of the year and the milk collection procedure adopted by the dairy) on it in the real-scale production process.

Experimental

A total of 317 cheese wheels (aged between 16 and 18 months) were sampled, between November 2015 and October 2017 on a two-months basis from 15 dairy factories belonging to the Trentingrana consortium. VOCs were sampled and analyzed by Head Space SPME/GC-MS [4]. VOC's data were analyzed using ANOVA simultaneous component analysis (ASCA) [5] to identify multivariate patterns associated with different factors related to the production process (dairy factory, part of the year, and their interaction) and to the analytical procedure (the effect of the distinct batches of SPME fiber).
An Orthogonal Partial Least Squares - Discriminant Analysis (O-PLS-DA) [6] classifier model was then trained to estimate the specific milk collection procedures adopted by the dairy factory.

Results

The SPME fibers and the dairy factories significantly affected the VOC content according to the ASCA model, while the time of the year was not significant. The results of ASCA multivariate decomposition of the dairy factory term are in Fig. 1. In the first component, which explains 28.8% of overall variance, the dairies were separated according to the content of ketones, medium-chain free fatty acids, and their esterified forms. The second component, explaining 22.5% of the overall variance, separated the dairy factory C-11 from the others. The separation was due to the content of limonene, alpha-thujene, 2,6-dimethyl pyrazine, and secondary alcohols. The disposition along the first component of the dairy factories is related to the different milk collection procedures adopted. To deeper investigate this effect, an O-PLS-DA model was trained and validated. The distribution in the second component, according to the model, can be related to the outlier behavior of the VOC profile of the cheese wheels produced in a singular dairy factory.

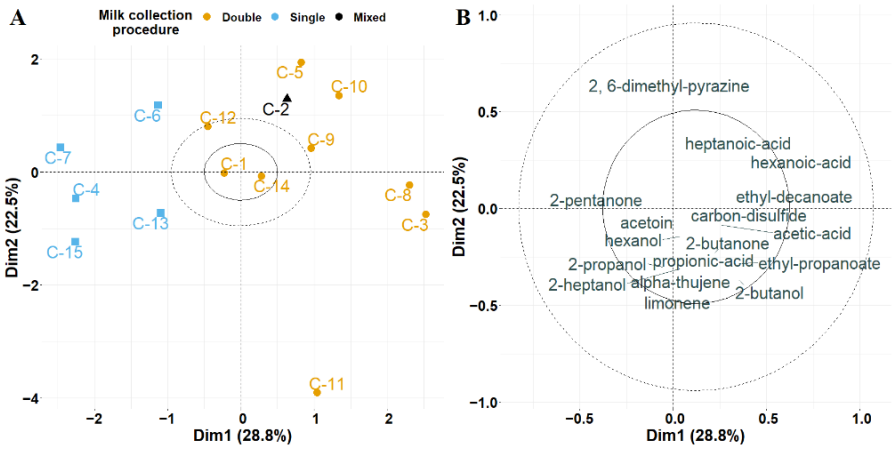

***Figure 1**. Score plot (A) and loading plot (B) of ASCA decomposition for the factor dairy factory: A) each dot represents a dairy factory, B) the loading values of each VOC are reported*

The classifier model detected 46 volatile compounds that are prominent according to the different milk collection procedures. In Fig. 2 the coefficients estimated for these molecules are reported, specifying in which milk collection procedure each compound is prominent.

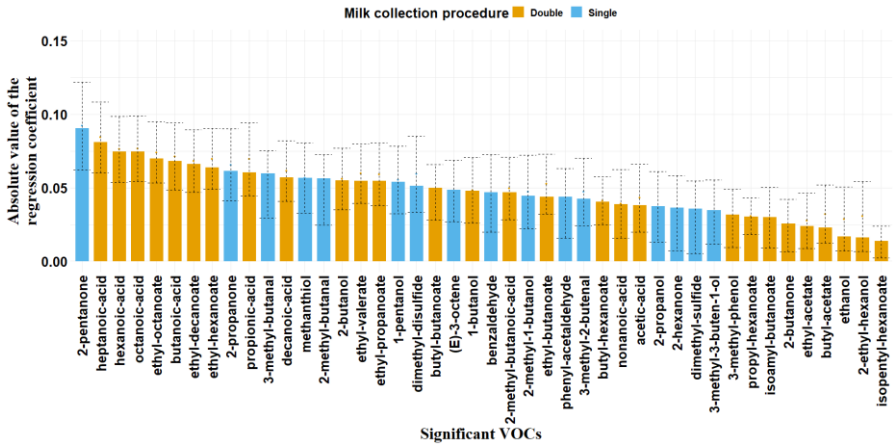

***Figure 2**. Absolute values of significant regression coefficients of the O-PLS-DA estimated using bootstrap, the VOCs are reported in axis x, the coefficients in axis y, the color indicates in which collection procedure the VOC is prominent*

Conclusions

The application of SPME/GC-MS on a large-scale sampling procedure directly from the quality control process detected the effect of the dairy factory on the VOC content in cheese. The differences between dairy factories could be due to process features, such as the milk collection procedure, the release of contaminants, and the effects of the Maillard reaction at low temperatures. Interestingly, cheese wheels produced using the double milk collection procedure had a higher level of VOCs related to the catabolism of fat: medium-chained free fatty acids, esters, and secondary alcohols. Those results demonstrated that the chemical profile of Trentingrana cheese is mainly affected by the first stages of the production process. The analysis of the VOC profile via SPME/GC-MS was a reliable instrument to estimate the effect of the production process on the quality of the product and to support a quality control procedure.

References

1. R. Khattab, H. A. Guirguis, S. M. Tawfik, M. A. Farag, *Trends in Food Sci. Technol.*, 2019 88, 343-360
2. P.M.G. Curioni, J.O. Bosset, *Int. Dairy J.*, 2002 12(12), 959-984
3. K. N. Kilcawley, H. Faulkner, H.J. Clarke, M.G. O'Sullivan, J.P. Kerry. *Foods*, 2018 7(3): 37
4. Endrizzi, A. Fabris, F. Biasioli, E. Aprea, E. Franciosi, E. Poznanski, A. Cavazza, F. Gasperi, *Int. Dairy J.*, 2012 23(2), 105-114
5. A.K. Smilde, M.E. Timmerman, M.M.W.B. Hendriks, J.J. Jansen, H.C.J. Hoefsloot, *Brief. Bioinformatics*, 2012 13(5), 524–535.
6. J Trygg, S. Wold. *J. Chemom.*, 2002 16, 119–128.

P32

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

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Summary: *The optimization of HS-SPME for the analysis of the X-ray irradiated mozzarella cheese volatile profile using a central composite design and a response surface methodology, was performed. The optimised conditions were applied to irradiated samples at different dose levels and the variations have been evaluated by a chemometric approach.*

Keywords: *HS-SPME/GC-MS, chemometric analysis, Food irradiation*

Introduction

Food irradiation is a process in which food products are exposed to ionising radiation, such as X-rays, to destroy and inactivate pathogenic and spoilage microorganisms [1]. In this study, X-ray irradiation was applied to mozzarella cheese produced with cow milk and the modifications in the composition of volatile organic compounds (VOCs) have been investigated. Headspace solid-phase microextraction (HS-SPME) technique coupled with gas chromatography mass (GC-MS) was used to extract, isolate and enrich the volatile fraction from the sample matrix [2]. Design of experiments (DOEs) and Response Surface Methodology (RSM) were used for the optimization of the HS-SPME process. Five parameters have been chosen: type of fibre, X-ray irradiation dose (kGy), extraction temperature (°C), extraction time (min) and sample amount (g). Type of fibre and X-ray irradiation dose level were evaluated through a first screening step. A central Composite Experimental Design (CCD) was used to optimise the remaining three factors, thus selected as independent variables. The influence of key parameters was evaluated on the total area and total number of VOCs. The optimised HS-SPME conditions were used to analyse a representative number of non-irradiated and X-ray irradiated mozzarella samples at three dose levels, i.e., 1.0, 2.0 and 3.0 kGy. The collected data were elaborated by Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA) and Partial Least Square-Discriminant Analysis (PLS-DA) to discriminate the variation of volatile profiles among non-irradiated and irradiated samples.

Experimental

The X-ray radiation treatment was performed using a low-energy X-ray irradiator (RS-2400, Radsource Inc., Texas, USA). To maximise chemical information of

irradiated mozzarella volatiles, the extraction performances of the four different SPME fibres (PDMS, DVB/CAR/PDMS CAR/PDMS and PDMS/DVB) were evaluated at the same analytical conditions. Then, a CCD of 17 runs was implemented, selecting three factors: sample amount, extraction time and temperature, assessing them at three different experimental levels. VOCs were analysed using a 6890N gas chromatograph (Little Falls, DE, USA) coupled with an Agilent 5975 mass selective detector, equipped with a Gerstel MPS auto-sampler (Gerstel, Baltimore, MD, USA). X-ray irradiated samples at three dose levels of 1.0, 2.0 and 3.0 kGy and the non-irradiated were analysed in the optimised HS-SPME conditions. Chemometric analyses including PCA, PLS-DA and LDA were performed using free software R version 4.1.1. These classifiers were chosen because they have been widely applied in similar contexts and they generally provided accurate results [3].

Results

The DVB/CAR/PDMS was the best fibre in terms of sensitivity for the analysis of X-ray irradiated samples and then it was used in the CCD analyses. The optimum HS-SPME conditions were estimated by means of the desirability function method, used for simultaneous optimization of the multiple responses. The surface responses, reported in Fig. 1, shows as the optimised combination of extraction temperature, extraction time and sample amount, corresponded to the following values: 75 °C, 75 min and 5 g. A total of 11 classes of compounds, including alcohols, aldehydes, alkanes, alkenes, aromatic compounds, carboxylic acids, esters, ketones, methyl esters and oxygen and sulphur-containing compounds were identified. A PCA preliminary data exploration was performed and showed how irradiated and non-irradiated samples were grouped in the PC subspace, also based on dose levels. The score dispersions at the three doses were influenced by the specific classes, i.e., from alcohols at 1.0 kGy, alkanes and alkenes at 2.0 kGy, ketones and aldehydes at 3.0 kGy. The Volcano plot and Variable Importance in Projection (VIP) were used for identifying and assessing the discriminant volatiles from the volatolomic dataset [4].

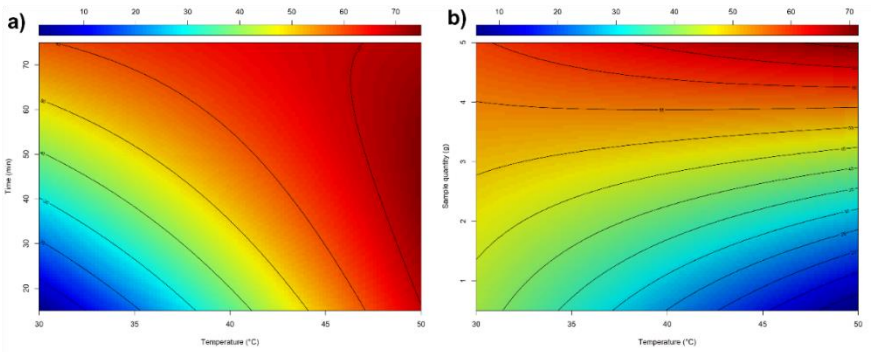

***Figure 1**. Responses surface plots for significant effect of extraction temperature (°C) and extraction time (min) on a) number of VOCs and of extraction temperature (°C) and sample amount (g) on b) total VOCs area*

The selected VOCs were used for PLS-DA and LDA. Both discriminant approaches were applied in double cross validation scheme [4] and the results highlighted the strong discriminating capacity of the PLS-DA and LDA algorithms in distinguishing irradiated samples from non-irradiated ones.

Conclusions

The effect of HS-SPME parameters in terms of fibre type, extraction temperature, extraction time and sample amount were optimised to obtain the maximum total area and number of VOCs of X-ray irradiated mozzarella samples. The best parameters of HS-SPME were used to investigate non-irradiated samples and the irradiated ones at three different dose levels. Some classes of compounds as hydrocarbons, oxygen-containing compounds, alcohols, aldehydes and ketones increased in irradiated samples, due to possible oxidation mechanisms induced by the irradiation treatment, as already reported for other matrices [5]. The classification by two discriminant approaches, LDA and PLS-DA, was effective in the indication of irradiation treatment and the dose levels too.

References

1. O. B. Odueke, K.W. Farag R.N. Baines, S.A. Chadd, *Food and Bioprocess Technology*, 9 (2016), 751-767.
2. A.S. Bertuzzi, P.L.H. McSweeney, M.C. Rea, K.N. Kilcawley, *Comprehensive Reviews in Food Science and Food Safety* 17 (2018), 371-390.
3. F. Di Donato, A. Biancolillo, D. Mazzulli, L. Rossi, A. A. D'Archivio, *Microchemical Journal* 165, (2021), 106133.
4. E. Szymańska, E.Saccenti, A. K. Smilde, J. A. Westerhuis, *Metabolomics*, 8 (2012), 3-16.
5. D.U. Ahn, C. Jo, D.G. Olson, *Meat Science*, 54 (2000), 209-215.

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P33

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

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Summary: *In this study, Folch procedure, Supercritical Fluid Extraction (SFE) and Microwave-Assisted Solvent Extraction (MASE) were compared for lipid characterization of mozzarella cheese. Extracts were analyzed by UHPLC-Q-Orbitrap-MS and the raw data were processed using LipidSearch™ software. The results confirm these techniques as complementary in lipidomics.*

Keywords: *Lipidomics, Lipid extraction, UHPLC-Q-Orbitrap-MS*

Introduction

Lipidomics is known as the large-scale and comprehensive study of all lipids in biological systems and widely used in the complex analysis of foods for different purposes [1]. Lipid extraction is the first step of lipidomics to isolate a subset of components and/or to remove some of those that would otherwise interfere with the detection process. It constitutes a fundamental step for high quality and exhaustive lipidomic approach [2]. Standard procedure for total lipid extraction from food samples, including milk and cheeses, was developed by Folch et al. [3], consisting in the use of a large quantity of solvents with different polarity for two-phase partition of both polar and non-polar lipids. Supercritical Fluid Extraction (SFE) is another efficient, environmental-friendly and selective alternative to conventional solvent techniques for lipid extraction from food matrices. This technology is based on the characteristic of supercritical fluids to exhibit liquid-like density and gas-like viscosity, allowing better penetration into the solid matrix. Finally, Microwave-Assisted Solvent Extraction (MASE) is an emerging and efficient extraction technique for various kinds of samples due to its low usage of extraction solvents and shorter extraction time [4] but, to our knowledge, at the moment, no study is reported that uses MASE in untargeted lipidomics. In this study, Folch procedure, SFE and MASE, were compared for lipid characterization of mozzarella cheese.

Experimental

In this study, a slightly modified Folch procedure was used for the total fat extraction, according to our previous work [5]. SFE was performed using a pilot unit SFT110XW System (Supercritical Fluid Technologies, Inc., USA) with an SFT10 CO_2 pump. For each SFE experiment, 2 g of mozzarella cheese + 150 µL of trinonanoin 1000 mg/L (IS) were loaded in the extraction vessel. Subsequently, 4 cycles were performed, including 15 min of maceration in static

conditions and 5 min of dynamic conditions during which the valves were opened and the extract was collected in a vial, keeping a CO_2 gas constant flow rate of 0.5 mL/min. MASE was performed using an ETHOS-ONE microwave system (Milestone s.r.l., Sorisole, Bergamo, Italy). EtOH/EtOAc (1:2, v/v) were used as solvents for the extraction and the temperature was increased to 65° C in 15 min, held for another 15 min and then reduced. The maximum extraction power was set to 500 W. The extracts were analysed by an Ultimate 3000 UHPLC system with a binary pump (Thermo Fisher Scientific, Waltham, MA, USA). A gradient elution was carried out employing as mobile phase A ACN/H_2O (60:40, v/v) and as mobile phase B IPA/ACN (90:10, v/v), both containing 10 mM NH_4HCO_2 and 0.1% HCO_2H. A Q-Exactive Focus Orbitrap Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was used for the detection of all lipid subclasses in positive and negative acquisition ion modes. Finally, the lipid identification from raw data was performed by Thermo Fisher LipidSearch™ 4.2.2.7 software. The MS and software parameters were set according to our previous work [5].

Results

By comparing the lipid profiles of mozzarella cheese, obtained using three different extraction procedures, it was evident that the composition of recoveries was different (Fig. 1). In particular, SFE did not allow the extraction of phospholipids while permitted the recovery of only lysophosphatidylserine (LPS) and of higher numbers of diacylglycerols (DG), bismethyl phosphatidic acids (BisMePA) and ceramides (Cer). On the other hand, MASE permitted the higher extraction of galactosylacylglycerols (GG) and lysophosphatidyl-ethanolamines (LPE). Finally, the results highlighted the best capability of the Folch to extract hexosyl ceramides (HexCer) and membrane lipids, such as phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidyl-inositols (PI), phosphatidylserines (PS) and sphingomyelins (SM). This enhanced extraction capacity of Folch was probably due to the use of methanol that allowed a more efficient breaking of the bonds between lipids and biopolymers [6]. Considering the complementary results obtained from the three types of extraction, it can be stated that the lipidome of mozzarella was characterized by a high number of triacylglycerols (TG) and a relatively low number of DG. In addition to TG and DG, which constitute the main energy storage resource, many types of phospholipids were found in the studied samples, mainly identified in negative acquisition mode.

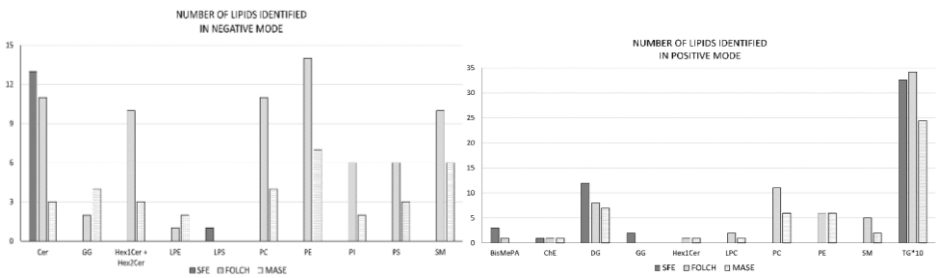

***Figure 1**. Subclasses of lipids identified in mozzarella cheese, using four different lipid extraction procedures*

Conclusions

In this study, an efficient characterization of the lipid profile of mozzarella cheese was carried out using Folch procedure, SFE and MASE, obtaining three different lipid profiles, both in the number of identified lipids and in the subclasses. These results suggest that the use of different extraction techniques, associated with high resolution mass spectrometry detection, allow a comprehensive investigation of the whole lipid profile in foodstuff. Moreover, the knowledge of the lipid-specific adeptness of extraction procedures, permits to choose the specific method based on the lipid subclass of interest. This aspect could be developed in the studies for the authenticity assessment of dairy products and for the evaluation of technological treatments, which are important issues that need to be further investigated.

References

1. T. Hyötyläinen, I. Bondia-Pons, and M. Orešič, *Mol. Nutr. Food Res.* 57 (2013), 1306.
2. Y. Xie, B. Wu, Z. Wu, X. Tu, S. Xu, X. Lv, H. Yin, J. Xiang, H. Chen, and F. Wei, *Food Chemistry* 319 (2020), 126547.
3. J. Folch, M. Lees, and G. H. Sloane Stanley, *J Biol Chem* 226 (1957), 497.
4. C. C. Teo, W. P. K. Chong, and Y. S. Ho, *Metabolomics* 9 (2013), 1109.
5. A. Mentana, R. Zianni, M. Campaniello, M. Tomaiuolo, A. Chiappinelli, M. Iammarino, V. Nardelli, *Food Chemistry* 394 (2022), 133542.
6. S. Pati, B. Nie, R. D. Arnold, B. S. Cummings, *Biomedical Chromatography* 30 (5) (2016), 695–709.

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P34

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

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Summary: *The study of the secondary metabolites in musts of three different grape varieties (Pinot grigio, Trebbiano, Garganega) was carried out by high-resolution mass spectrometry LC/QTOF. This approach made it possible to identify some suitable indices to discover the presence of white grape varieties blended with Pinot Grigio.*

Keywords: *Pinot grigio grape variety, high resolution mass spectrometry LC/QTOF, chemical markers*

Introduction

The area cultivated with Pinot Grigio variety in the world is 67,000 hectares. In Italy the number of hectares is 31,600 of which 27,000 are in the DOC "delle Venezie" area. The DOC includes Veneto and Friuli-Venezia Giulia regions and the province of Trento.
The disciplinary for the production of DOC wines specify that they must be obtained from a minimum of 85% of Pinot grigio grapes and up to a maximum of 15% of other white grape varieties belonging to the same "delle Venezie" area.
A study was developed to identify the chemical markers suitable to reveal the fraud implemented by addition of other not allowed grape varieties which can be potentially used due to lower commercial cost.

Experimental

Profiling of the secondary metabolites in musts of three different grape varieties (Pinot grigio, Trebbiano, Garganega) was performed by high-resolution mass spectrometry LC/QTOF. Two milliliters of must were diluted to 4 mL with deionized water and passed through a 360 mg Sep-Pak® C18 cartridge (Waters, Milford, MA, USA), the metabolites were collected with 2 mL of methanol, filtered with a Clarify-PTFE 0.22 µm filter (Phenomenex, Torrance, CA, USA) and directly analysed. 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 two homemade electronic databases of grape and wine compounds, made with molecular information from the literature and from other electronic databases.

Results

The profiling of the secondary metabolites of the three grape varieties by high-resolution mass spectrometry LC/QTOF allowed the identification of some indices suitable for discovering the presence of white grapes varieties in blend with Pinot grigio. A similar approach was previously performed to detect possible frauds committed in Amarone di Valpolicella wine and it was effective to detect the addition at 10% [1].

References

1. M. De Rosso, C. Marangon Mayr, G. Girardi, A. Dalla Vedova, R. Flamini; *Metabolomics* 14 (2018), 124-133.

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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Summary: *The article describes the impact of four autochthonous yeast strains on the volatile and chemical profiles of rosé and white sparkling wines produced on a pre-industrial scale. A combined analytical approach was adopted based on the correlation between data from the odorant* series, *GC-MS, and HPLC-HRMS.*

Keywords: *Apulian sparkling wines, metabolomics, chemometrics.*

Introduction

The production of sparkling wine (SW) through the *champenoise* method involves the "in bottle" secondary fermentation of the base wine commonly carried out using yeast strains belonging to the *Saccharomyces cerevisiae* species. Nevertheless, the use of few widely used strains can cause a flattening of the sensory profile. To the best of our knowledge, few studies [2, 3] have so far investigated the impact of native starter strains on the volatile and non-volatile profiles of SW. In addition, there are no studies based on the comparison of volatile and chemical profiles of sparkling wines produced from two different Italian indigenous cultivars, i.e. Bombino Bianco and Bombino Nero, using both native and commercial strains. The application of an untargeted metabolomic approach based on HPLC-HRMS and GC-MS techniques has shown that the yeast strain is able to affect the chemical profile of sparkling wines by modifying the quali- quantitative profile of several secondary products of microbial fermentation, including polysaccharides, organic acids, phenolic acids and lipids. Moreover, an HPLC-HRMS/GC-MS correlation analysis was performed, resulting in a map that is a useful tool for monitoring different aroma release patterns. This contribution provides information to modulate the quality of regional sparkling wines from minor indigenous grape varieties using selected microbial resources.

Experimental

Yeasts used and SWs production

The S. cerevisiae autochthonous starter strains ITEM 9351, ITEM 9520, ITEM

9518, and ITEM 17294 are deposited in the Agro-Food Microbial Culture Collection of ISPA (http://www.ispacnr.it/collezioni-microbiche) and have been previously described [1]. The commercial *S. cerevisiae* DV10 strain (Lallemand, Petaluma, USA) was used as a control. SWs from *Bombino Bianco* or *Nero* grapes, grown in Apulia region were manufactured according to *Méthode Champenoise*.

GC-MS Analysis: The analysis of volatile compounds was performed by a solid-phase micro-extraction coupled with a gas chromatography-mass spectrometry (SPME-GC/MS). The extraction and the instrumental analysis were carried out according to the literature [1].

HPLC-HRMS Analysis: HPLC/HRMS analysis was performed according to a modified protocol reported in the literature [2].

Chemometric evaluation: Principal Component Analysis (PCA) and Hierarchical Clustering Dendrogram (HCD), were performed on the collected data. The correlations between GC-MS and HPLC-HRMS data were studied by calculating the matrix of pairwise Pearson's linear correlation coefficients.

Results

Regarding GC-MS data, the interaction effect between the yeast strain and the *cultivar* is statistically significant for 26 molecules, while for the others, such as isoamyl acetate, ethyl hexanoate, and decanoate, α-terpineol, 6,7-dihydro-7-hydroxy linalool and 2,3-butanediol, the yeast plays an important role in determining a specific aromatic profile. The SWs produced with autochthonous yeast strains showed higher values of all molecules than two controls. Taking into account to odor activity value, 13 molecules with OAV>1 belonging to esters and terpenes classes, were detected. Fruity and floral notes seem to characterize especially the rosé and white SWs fermented from indigenous strains. Also, specific non-volatile metabolites were annotated by HPLC-HRMS metabolic analysis. Some of them were identified as biomarkers indicative of the adopted production procedure, in terms of yeast strain used for the refermentation step, i.e. commercial *vs* autochthonous. A semi-quantification of pivotal metabolites involved in the winemaking chain allowed the traceability of the production process. The information collected from the GC-MS and HPLC-HRMS analyses were combined and correlated (Fig. 1), allowing the identification of crucial bio-markers for discriminating between re-fermentation procedures adopting different yeast starters.

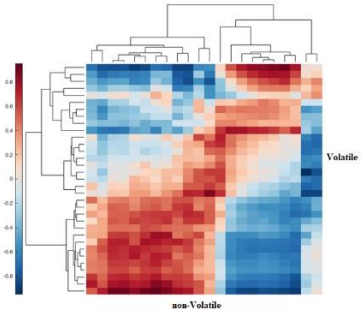

Figure 1. *Clustergram of the sample correlation coefficients between the volatile compounds detected by GC-MS and non-volatile compounds annotated by HPLC-MS*

Conclusions

The results indicated a significant strain-specific effect of the autochthonous yeast strains on the chemical composition of sparkling wines in terms of volatile and non-volatile compounds, which remarkably affect the sensory profile of the sparkling wines, differentiating them from the counterparts produced using a commercial strain.

Acknowledgement: This work was partially supported by the Apulia Region projects: “Innovazione nella tradizione: tecnologie innovative per esaltare le qualità dei vini autoctoni spumante della murgia barese—INVISPUBA”

References

1. M. Tufariello, G. Maiorano, P. Rampino, G. Spano, F. Grieco, C. Perrotta, V. Capozzi, F. Grieco; *LWT-Food Science and Technology*, 99 (2019), 188-196.
2. M. Tufariello, A. Rizzuti , L. Palombi, R. Ragone, V. Capozzi, V. Gallo, P. Mastrorilli, F. Grieco, *Food Control*, 126 (2021), 108099.

P36

Monitoring of a Sangiovese red wine volatile profile along one-year aging in different tank materials and glass bottle

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Summary: *The aim of this research was to study how different tank materials affected the volatile profile of a red wine during aging. For this purpose, a Sangiovese red wine was aged by using different tank materials including stainless steel, epoxy-coated and uncoated concrete, earthenware raw amphorae, new and used oak barrels.*

Keywords: *Sangiovese red wine aging, volatile profile, HS-SPME-GC-MS*

Introduction

During wine aging many physical−chemical reactions take place that change the wine chemical structure and sensory profile. Several factors, such as kind of tank, dissolved oxygen, and phenolic composition, are involved in the evolution and stabilization of wine. In particular, the choice of the aging tank affects the final wine characteristics, especially its volatile profile. A period of bottle aging is normally foreseen before the wine is ready to be commercialized and consumed. During this period, a series of reactions, depending on the aging treatments of wine before bottling and involving the polyphenol and volatile compounds, occur in wine [1,2]

Experimental

The red wine used for the one-year aging test was a Sangiovese from the 2018 harvest. After completing the malolactic fermentation, it was centrifuged at 0 NTU and sulfites were adjusted at 50 mg/ L of total SO_2 before the racking in the aging tanks. Stainless steel (SS), epoxy-coated concrete (CC), uncoated concrete (CR), earthenware raw amphorae (AM), new oak barrel (TN), used oak barrel (TO), and glass bottle (GB) were the materials used for the experimental. All the tanks were 5 hL of volume, and every treatment was set up in triplicate. The GB wine was used as references and bottled in 1 L glass bottles for the entire one-year aging, using a crown cap closure. At 6 months aging, 24 glass bottles (0.75 L Bordelaise, crown cap closures), equal to 3.6% of total tank volume (5 hL), were filled with wine from each different tank and let aged till the end of the experiment at one year aging. Wine samples were coded with the name of the tank material and the sampling time (_6: six months aging; _12: twelve months aging; _6+6: six months tank aging plus six months glass bottle aging).

Volatiles Profile by Headspace SPME GC−MS. This analysis was performed to evaluate the relation between the kind of tank material and the wines volatile

profiles. Thirty-five free volatile compounds were identified and quantitated, including six acetates, five esters, four terpenes, eight alcohols, three fatty acids, four ketones, and one aldehyde. Free volatile profile of wines was determined according to a method developed previously by Canuti *et al.,* [3]. The analytical system for the determination of the volatile compounds comprised an AutoSystem XL gas chromatograph (Perkin Elmer, Shelton, CT, USA) paired with a Turbomass Gold mass selective detector (Perkin Elmer). An HP-Innowax column (30 m × 0.25 mm o.d., 0.25 μm film thickness, Agilent Technology, Little Falls, DE, USA) was used for all analyses. The compounds were also verified using quantifier/qualifier ion ratios and published retention indices reported for a HP-Innowax column. The peak area of each standard (calculated as total ion), relative to the peak area of the octan-2-ol internal standard, were plotted against the standard concentration to create a standard curve. The linear regression equations obtained were used to calculate the concentration (mg/ L−μg/L) of each compound in the wine samples. Samples were prepared by transferring 8 mL aliquot of wine to a 20 mL amber glass headspace sample vial containing 3 g of NaCl and then adding 5 μL of the octan-2-ol internal standard solution (82 mg/L in ethanol solution) for a final concentration of 5.1×10^{-2} mg/L. The mixture was carefully shaken to dissolve the NaCl and then left for 1 h in the dark at room temperature (22 ± 1 °C) to equilibrate before analysis. The SPME fiber used for extraction was polydimethylsiloxane (PDMS), 100 μm thickness, 23 gauge. The prepared wine samples were warmed up to 40 °C for 10 min before exposing the SPME fiber to the sample headspace. Headspace extraction times of 30 min, at a temperature of 40 °C, were performed with continuous stirring (500 rpm).
Ethanal and Higher Alcohols Analysis by GC – FID. Ethanal and higher alcohols were determined with a method previously developed [4] and using an AutoSystem XL gas chromatograph equipped with flame ionization detector (FID) (Perkin Elmer).

Results

Wines appeared to be separated for the volatile composition in three distinctive groups according to the different times and modality of aging (tanks and bottles) (Fig. 1). On the left of the graph, there were the _6 wines; in the middle, the _12 wines; and on the right, the _6 + 6 wines. This last group resulted the most different from the _6 wines and characterized by the higher content of norisoprenoids (#16, #17, #18, #19) and terpenes (#14, #15), and to a lesser extent of acetates (#5) and esters (#30). On the other hand, _6 wines were richer in esters and acetates (#4, #6, #7, #8) and less in alcohols (#20, #21) and terpenes (#12). According to other authors [5], was evidenced an increase in eugenol (#14) in all wines both in tanks and in the bottle after 12 months aging. It could be observed that during wine aging, both in the tank and bottle, there was a decrease in esters, mainly acetates, except for the ethyl esters of diprotic acids such as ethyl lactate (#11) and diethyl succinate (#5) [6]. In particular, it was possible to observe an increase of diethyl succinate (#5) in the _6 + 6 wines in comparison with the same wines aged for 12 months in tanks.
All these findings allowed presuming that reductive condition of the 6 months bottle aging induced reactions that enabled the synthesis of varietal volatiles norisoprenoids by acidic hydrolysis of the precursors. These findings were in

accordance with other authors [2]. On the contrary, the more oxidative conditions of the tanks seemed to have reduced the speed of these kinds of reaction and, consequently, the 12 months wines resulted more like the 6 months wines.

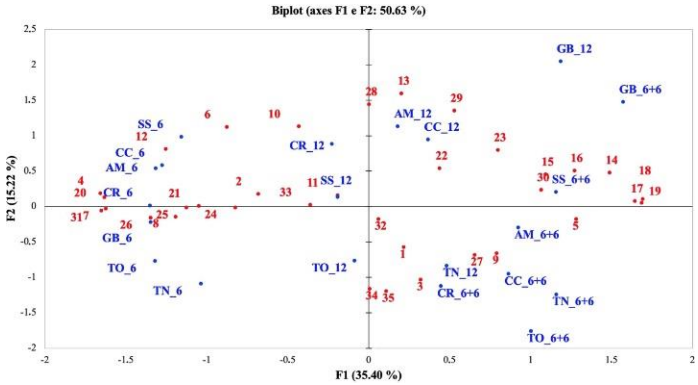

***Figure 1**. Principal component analysis (PCA): scores and loadings plot of the volatile compounds measured in Sangiovese red wine during aging (1.ethyl butanoate; 2. ethyl 3-methylbutanoate; 3. 3-methylbutyl acetate; 4. ethyl hexanoate; 5. hexyl acetate; 6. octan-2-one; 7. hexan-1-ol; 8. ethyl octanoate; 9. heptan-1-ol; 10. 4-terpineol; 11. vitispirane I; 12. vitispirane II; 13. ethyl nonanoate; 14. b-linalol; 15. riesling acetal; 16. diethyl succinate; 17. TDN; 18. β-citronellol; 19. β-phenethyl acetate; 20. n-pentadecanoic acid; 21. benzyl alcohol; 22. whiskey lactone; 23. β-phenylethanol; 24. cis-oak lactone; 25. octanoic acid; 26. eugenol; 27. nonanoic acid; 28. ethanal; 29. propan-1-ol; 30. ethyl acetate; 31. 2-methylpropan-1-ol; 32. 3-hydroxy-2-butanone; 33. 2-methylbutan-1-ol; 34. 3-methylbutan-1-ol; 35. ethyl lactate)*

Conclusions

These findings could be useful for winemakers since the tank material represents an important choice in the wine production process as a function of the oenological aim and definition of the wine style. The bottle aging, combined with different tank materials, enhanced the complexity of the wine volatile profile thanks to the reductive status inside the bottle that seemed to promote the varietal precursors hydrolysis.

References

1. Han, G.; Webb, M. R.; Waterhouse, A. L. *Food Chem*. 2019, 290, 208−215. (15)
2. Ugliano, M. J. Agric. *Food Chem*. 2013, 61, 6125−6136.
3. Canuti, V.; Conversano, M.; Li Calzi, M.; Heymann, H.; Matthews, M. A.; Ebeler, S. E. *J. Chromatogr*. 2009, 1216, 3012−3022.
4. Bertuccioli, M. Direct gas chromatographic determination of some volatile compounds in wine. Vini Ital. 1982, 138, 149-156.
5. del Alamo-Sanza, M.; Nevares, I.; Martínez-Gil, A.; RubioBretón, P.; Garde-Cerdán, T. *LWT* 2019, 101, 395−403.
6. Pérez-Prieto, L. J.; López-Roca, J. M.; Gómez-Plaza, E. J. *Food Compos. Anal*. 2003, 16, 697−705.

P37

Headspace solid-phase microextraction–gas chromatography–mass spectrometry for profiling free volatile compounds in Sangiovese grapes and wines obtained with different canopy vineyard treatment for organic production

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Summary: *HS-SPME coupled with GC-MS was applied for volatile profiling of Sangiovese grapes and wines obtained with different vineyard treatment. In particular, product were based on elicited molecules that can induce resistance in vine for fungine disease and affect the final composition of grapes and related wines.*

Keywords: *Solid-phase microextraction, volatiles, terpenes*

Introduction

Grape aroma is comprised of a large number of volatile compounds including alcohols, esters, acids, terpenes, norisoprenoids, thiols, and carbonyl compounds. During winemaking, the “free” volatile compounds are released as a result of physical crushing and subsequent chemical and enzymatic hydrolysis of the conjugated volatiles by grape, yeast, and/or industrial enzymes (glycosidases or peptidases) [1]. The volatile composition of grapes is one of the most important factors determining wine character and quality [2] and the management of vineyard can affect it. In agricultural practice, plant activators may represent an effective alternative to conventional agrochemicals [3]. They do not exert a direct antimicrobial activity against phytopathogens, but are able to boost the plant innate immune systems. They deserve particular attention because of their wide use against grey mould (*Botrytis cinerea*) and powdery mildew (*Erysiphe necator*) infections in vineyards.

Experimental

Three different Sangiovese grape from 2021 harvest were collected from a vineyard where three different treatments were applied. In particular, inside a same vineyard, a raw was the control (STD) with the conventional treatment, a second raw was treated with a product chitosan base (IBS), the third raw was instead treated with the same product chitosan-base and an antagonist fungus (IBP) with the aim of contrast grey mould (*Botrytis cinerea*) and powdery mildew (*Erysiphe necator*).

Grape and wine volatiles profile by Headspace SPME GC−MS. The grapes were analyzed for the free volatile profile and then vinified to produce three different wines that were also characterized after the alcoholic fermentation and after

malolactic fermentation. It was used a method previously developed by the same authors [4]. The analytical system for the determination of the volatile compounds comprised an AutoSystem XL gas chromatograph (Perkin Elmer, Shelton, CT, USA) paired with a Turbomass Gold mass selective detector (Perkin Elmer). An HP-Innowax column (30 m × 0.25 mm o.d., 0.25 μm film thickness, Agilent Technology, Little Falls, DE, USA) was used for all analyses. The compounds were also verified using quantifier/qualifier ion ratios and published retention indices reported for a HP-Innowax column. The peak area of each standard (calculated as total ion), relative to the peak area of the octan-2-ol internal standard, were plotted against the standard concentration to create a standard curve. The linear regression equations obtained were used to calculate the concentration (mg/L−μg/L) of each compound in the wine samples. Randomly selected and thawed grapes (∼30g; exact sample weight was determined prior to analysis) were manually peeled to separate the skin from the pulp. The skins were weighed and then centrifuged for 10 min at 4 °C and 5000 rpm in order to remove excess juice. The supernatant was discarded. The skins were again weighed and then diluted with 8 mL of buffer (2 g L^{-1} tartaric acid in water, pH 3.6 adjusted with NaOH). The grape skin mixture was homogenized with an ultraturrax and then centrifuged again for 10 min at 4 ◦C and 5000 rpm. An 8 mL aliquot of the supernatant was transferred to a 20 mL glass headspace sampling vial containing 3 g of NaCl and 5 ml of 2-octanol solution (82 mg L^{-1} in ethanol) as internal standard. The final 2-octanol concentration was 5.1 × 10−2 mg L^{-1}. Silicone septa from Supelco (Bellefonte, PA, USA) were used with 18 mm diameter screw caps to seal the 20 mL sample vials. The sealed vials were carefully shaken to dissolve NaCl and then left to equilibrate for 3 h in the dark at room temperature before the GC–MS analysis. All grape samples were prepared in triplicate. Wine samples were prepared by transferring 8 mL aliquot of wine to a 20 mL amber glass headspace sample vial containing 3 g of NaCl and then adding 5 μL of the octan-2-ol internal standard solution (82 mg/L in ethanol solution) for a final concentration of 5.1 × 10^{-2} mg/L. The mixture was carefully shaken to dissolve the NaCl and then left for 1 h in the dark at room temperature (22 ± 1 °C) to equilibrate before analysis. The SPME fiber used for grape and wine extraction was polydimethylsiloxane (PDMS), 100 μm thickness, 23 gauge. The prepared grape and wine samples were warmed up to 40 °C for 10 min before exposing the SPME fiber to the sample headspace. Headspace extraction times of 30 min, at a temperature of 40 °C, were performed with continuous stirring (500 rpm).

*Ethanal and Higher Alcohols Analysis by GC–FID***.** Ethanal and higher alcohols were determined in wines with a method previously developed [5] and using an AutoSystem XL gas chromatograph equipped with flame ionization detector (FID) (Perkin Elmer).

Results

Grapes free volatiles analysis. The analysis of variance evidenced that most of the volatile compounds found in grape skins did not showed significant differences among the different vineyard treatments. However, hexen-2-ol resulted significatively higher in IBS and IBP grapes, while b-linalol, octan-1-ol, b-citronellol and *trans*-geraniol higher in IBP grape. They are all considered

varietal compounds.

Wines free volatiles analysis. Wines after alcoholic fermentation resulted similar in volatile composition except for IBP wine significantly higher in a-terpineol, vitispirane I, b-linalol, phenethylacetate e b-phenylethanol compare to the IBS and STD wines.

The malolactic fermentation allows the wines to have a different evolution in volatile composition. In fact, the differences between IBP wine and the IBS and STD became higher with IBP the richest in hexylacetate, octan-2-one, vitispirane I and vitispirane II, ethylnonanoate, phenethylacetate, benzyl acohol, b-phenylethanol, and nerolidol.

Fig. 1 reported the distribution of the wines after alcoholic and malolactic fermentation according to the free volatile composition (Principal Component Analysis, 75% of total explained variance).

It is possible to evidence that the wines resulted divided into two groups according to the time of the fermentation process: on the right side the wines after alcoholic fermentation (coded with ALF) and on the left side the wines after malolactic fermentation (coded with MLF). The IBP wine resulted the one closer to the main groups of the volatile compounds in both the sampling times confirming that it was the most different among the wines.

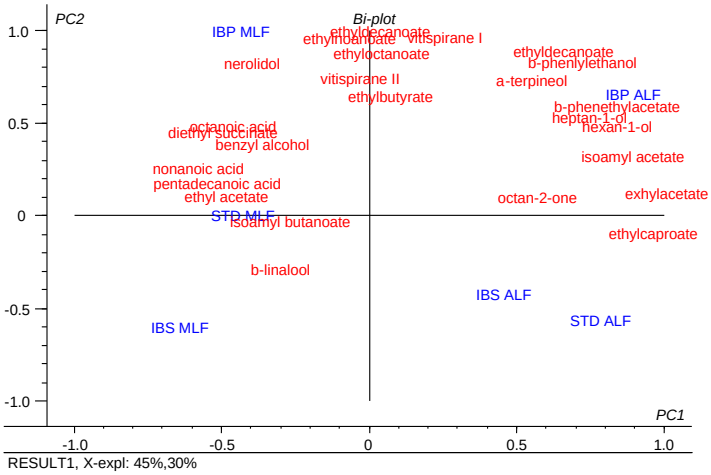

***Figure 1**. Distribution of the wines after alcoholic and malolactic fermentation according to the free volatile composition (Principal Component Analysis, 75% of total explained variance)*

Conclusions

The use in the vineyard of elicited products to treat the vine plants against grey mould (*Botrytis cinerea*) and powdery mildew (*Erysiphe necator*) significantly affected the volatile composition of the Sangiovese grapes and consequently of the related wines, thus adding useful information concerning the literature information for these grape varieties.

References

1. T. Lund, J. Bohlmann, Science 311 (2006) 804
2. S.E. Ebeler, A.C. Noble, Am. J. Enol. Vitic. 51 (2000) 205.
3. R. Buonaurio, M. Iriti, G. Romanazzi., 19 (2009), 130-148.
4. Canuti, V.; Conversano, M.; Li Calzi, M.; Heymann, H.; Matthews, M. A.; Ebeler, S. E. *J. Chromatogr*. 2009, 1216, 3012−3022
5. Bertuccioli, M. Vini Ital. 1982, 138, 149-156

P38

The contribution of varietal thiols in the diverse aroma of Italian monovarietal white wines

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Summary: *This research aims to map the volatile thiols (VTs) distribution over the Italian white wine scenario. An LC-MS/MS method, supported by sensory and sorting analyses, was used for the quantification of VTs. Müller-Thurgau, Lugana and Verdicchio showed a relevant amount of VTs whereas other cultivar had a lower content.*

Keywords: *Italian white wines, varietal thiols, Müller Thurgau*

Introduction

Volatile thiols (VTs) are organic molecules containing a -SH group and are known to be potent odorants, playing a key role in food aroma, due to their broad presence and low Odor Detection Threshold (ODT) [1]. Among the various volatile thiols present in wine, the so-called varietal thiols (VTs) are already present in grapes (usually in a bonded form) and are therefore considered as "varietal compounds" [2] even if the winemaking process in general, and the specific yeast strain activity in particular, can increase their content in wines [3]. The most powerful odor-active VTs in wine are 4-methyl-4-sulfanylpentan-2-one (4-MSP), 3-sulfanylhexan-1-ol (3-SH) and its ester 3-sulfanylhexyl acetate (3-SHA) [4]. Despite their important contribution to wine aroma, there are several issues that make the quantification of VTs a challenging task [5]. First, the VTs content in wine is usually detected in parts per trillion (ng/L), meaning that an enrichment technique and a sensitive instrumentation are required by most analytical protocols [6]. In addition, wine is a very complex and highly variable matrix, where the concentration of many compounds can heavily affect the measurement of trace analytes [7]. Finally, the thiols of interest are reactive molecules whose concentration can be affected by several reactions, impacting on their final concentration in wine [8]. A broad variety of protocols, based on many different instrumentations and sample preparation strategies, have been described in the last years, even if a reliable, efficient, robust and straightforward protocol is missing. The specific goal of this research was to quantify the varietal thiols 4-MSP, 3-SH and 3-SHA in the 18 monovarietal wines, and to test their olfactory impact by descriptive and sorting sensory analyses, for the first time. To do this, a simple, fast, and robust LC-MS method, based on that proposed by Román et al. [9], was optimized and validated.

Experimental

- *Sample set #1*. A first sampling consisted of 246 monovarietal white wines (vintage 2019) from 18 Italian grape cultivars collected in 9 Italian regions. For each variety, between 8 and 21 different commercial wines, all produced without wood refining, were collected from the main geographical areas of production.
- *Sample set #2*. An additional sample set of 50 Müller-Thurgau wines (2019 and 2020) was also analyzed.
- *Quantitation procedure*. 35 mL of wine sample, 35 µL of I.S. solution and 5 mL of acetonitrile were transferred into a falcon containing the salt mixture (12 g $MgSO_4$, 4 g NaCl, 1.5 g of Sodium citrate dibasic, 3 g of Sodium citrate tribasic), stirred and centrifugated. 2 mL of the organic phase were then spiked with 150 µL of Ebselen (600 mg/L) for the derivatization process. Quantitation was performed with an Exion LC system using an Acquity UPLC BEH C18 (1.7 µm, 2.1 mm x 50 mm) column for the separation, and an AB Sciex LLC QTRAP 6500+ operating in positive ion (MRM) for the ionization. 11 equal-to-real spiked samples (from 0.5 ng/L to 1000 ng/L) were prepared in matrix for the acquisition of calibration curves.
- *Sensory analyses*. 12 judges (22-50 years old; 5 males, 7 females) were selected and trained to perform the sensory assessment of wine samples as described by Pittari et. al. (10), with slight modifications.
- *Sorting analysis of Müller-Thurgau*. 12 enologists (28–62 years old; 6 females, 6 males) were requested to sort samples and to assign the most characteristic descriptors based on a pre-defined list reported in literature.

Results

Quantitative results on major VTs in the sample set #1 provided for the first time a comprehensive overview of the highly diversified Italian white wines scenario. From a varietal point of view, VTs were observed in at least one sample of each cultivar, implying that, in the context of Italian white wines, these compounds can be considered rather ubiquitous. Quantitative variations across cultivars, as well as within the same cultivar, were rather large, reflecting the complex array of factors that can determine the VTs content of wines, such as the pedoclimatic characteristics of the vineyard, the management of the winery pre-fermentative steps, the yeast strain used in fermentation, and the levels of oxygen exposure of the wines after fermentation and after bottling. It should be noted that Müller-Thurgau was in this survey the only cultivar that differs for its high 4-MSP content (Fig. 1).

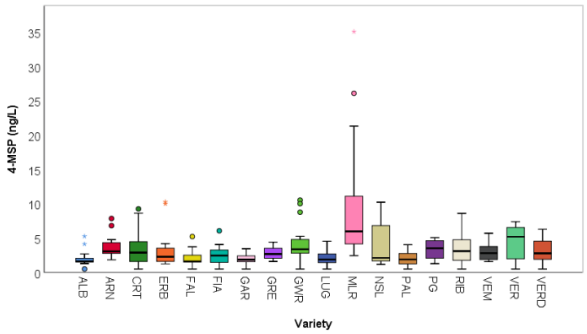

***Figure 1**. 4-MSP distribution in the Italian cultivars (ng/L)*

4-MSP is a highly identifying VT in Sauvignon blanc wines and is also present, at lower but detectable concentration, in other cultivars, while it was never detected before in Müller-Thurgau.
3-SH (Fig. 2a) was instead more ubiquitous, as it was present in almost all cultivars, even if the richest cultivar was Lugana followed by Müller-Thurgau and Verdicchio. About 3-SHA (Fig. 2b), its content was low and with no varietal dependency in any cultivar.

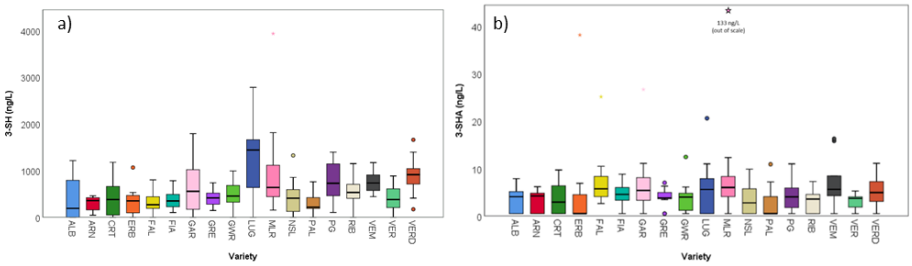

***Figure 2**. 3-SH (a) and 3-SHA (b) distribution in the Italian cultivars (ng/L)*

Descriptive sensory assessment showed that 4-MSP plays a key role in the aroma of Müller-Thurgau wines, which, unlike all the others, showed concentrations significantly higher than 10 ng/L, imparting a distinctive "Thiolic character". In agreement with descriptive analysis, sorting task performed by enologists showed that these descriptors were well represented in the set of analyzed wines.
In conclusion, even if VTs concentration appeared to be highly variable because of its correlation to many pedoclimatic and winemaking conditions, it can be stated that there is some cultivar, even in the Italian white wine's scenario, where these compounds can be labelled as varietal; these cultivars are Lugana and Verdicchio, because of their content of 3-SH, and in particular Müller-Thurgau for its unexpected richness in 4-MSP.

References

1. McGorrin, R. J. (2011). ACS Symposium Series, 1068, 3–31.
2. Villano, C., et. Al. (2017). In Food Control, 80, 1–10.
3. Swiegers, J. H., et. al. (2007). Applied Microbiology and Biotechnology, 74(5), 954–960.
4. Roland, A., et. al. (2011). Chemical Reviews, 111(11), 7355–7376.
5. Chen, L., et. al. (2019). Molecules, 24(13), 1–24.
6. Hart, R. S., et. al. (2019). Journal of Microbiological Methods, 165(August), 105699.
7. Lyu, J., et. al. (2021). Food Chemistry, 346(September 2020), 128957.
8. Liem-Nguyen, V., et. al. (2015). Analytical Chemistry, 87(2), 1089–1096.
9. Román, T., et. al. (2018). European Food Research and Technology, 244(3), 379–386.
10. Pittari, E., et. al. (2020). 1530 (9).

P39

Vine-wine industry by-products: Studies for extraction of polyphenols; chemical characterization by LC/MS

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Summary: *The objective of our work was to determine the concentrations of polyphenols from the by-products of the wine industry, through the use of LC/HR-MS with the final aim of enhancing waste and reusing it for many applications, in the field of cosmetics, in the production of food supplements or animal feed.*

Keywords: -*Polyphenols, HPLC/ MS, by-products of the wine*

Introduction

Wine contains a large amount of proteins and polyphenols which can lead to chemical and/or physical alterations.
One of the most used solutions to avoid this problem is the use of bentonites. Bentonites are clay-based compounds with a high surface area that have the function of cationic exchangers [1], thanks to their positive electrostatic charge they allow to interact with particles that are in suspension. In oenology, bentonites are used in the so-called clarification process which allows to prevent clouding of the wine and to stabilize it, removing proteins, heavy metals and polyphenols from it. In addition to white bentonite, black bentonite is widely used, characterized by the addition of activated carbon which greatly increases its adsorbing power.
Bentonites, together with grape skins, are considered by-products of the wine industry and an excessive production of these waste is not only an economic damage for companies, but also a threat to the environment [2], for this reason it is important to adopt recovery and enhancement strategies for wine waste [3].
The main objective is the enhancement of the by-products of grape processing, determining the amount of polyphenols, known for their antioxidant, anti-inflammatory and antiviral properties, through the use of HPLC / MS, after having carried out a very simple and fast extraction using "green" processes,
The final goal of the project is to enhance the waste and use it for many applications, in the field of cosmetics, in the production of food supplements or in the production of animal feeds with nutraceutical characteristics superior to those of feed currently on the market.
This research was developed with the "Smiling" project, funded by the Production Activities Department of the Sicilian Region as part of the FESR 2014-2020.

Experimental

The first step was to extract and isolate the mixture of polyphenols from the by-products of the wine industry such as white bentonite, black bentonite and the skins of white grapes and red grapes, with "low impact" techniques, traceable to green chemistry.

Four aliquots of 5 g matrix (20 g total) were weighed in 50 ml falcons and 50 ml MeOH added. They were immersed in an ultrasonic bath for 15 min at room temperature and centrifuged to easily separate the supernatant and the remaining solid. The latter was re-extracted with the same amount of fresh solvent. A total of 5 extractions were made for black bentonite and 4 for white bentonite, white grape skins and red grape skins. The volume of solvent was reduced by distillation of approximately ¼ as compared to the total volume used; the solvent recovered from distillation was used for subsequent extractions.

The quantifications for phenols were made by comparing the data obtained for individual species with the calibration curve for resveratrol, while the quantifications for anthocyanin and flavonoids were made by comparing the data obtained with the calibration curve for quercetin.

The analyses were carried out using HPLC (Waters 2695) interfaced with a mass spectrometer (waters QTOF premier). For further confirmation, the analyses were also carried out at the ATeN Center of the University of Palermo using the UHPLC-HESI-MS.

Results

The most representative analytes are caffeic acid, gallic acid and resveratrol between phenols and quercetin and campferol among flavonoids.

The results obtained from LC-MS analyses were compared with those obtained by the Folin-Ciocalteu method (F-C) [4]; the latter shows an overestimation of the total amount of polyphenols, since the reagent F-C reacts with a wide category of molecules and is not rigidly selective towards polyphenols.

The musts obtained from white grapes and red grapes were also analyzed, observing an absence of polyphenols, confirming that bentonites can retain these compounds.

Finally, the antimicrobial activity of polyphenols extracted from black bentonite against *Pseudomonas aeruginosa ATCC 15442, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Candida albicans ATCC 10231* was evaluated.

The substance has antibacterial activity against the four bacterial strains at a concentration of 2,5 mg/ml and against the fungal strain C. albicans at 1,25 mg/ml and bactericidal and fungicidal activity against P. aeruginosa and fungicidal activity against C. albicans at a concentration of 2,5 mg/ml.

Conclusion

The by-products of the wine industry, such as white bentonite, black bentonite and the skins of white and black grapes, can be enhanced to be reused in many applications. Through a simple "green" extraction we were able to extract the polyphenols present in the by-products of the wine industry and through an HPLC/MS analysis we determined their concentration by observing how they are rich in polyphenols, compounds known for their important health properties .

The valorisation of waste is an important aspect, both from an economic point of view for companies and from an environmental point of view. The next step is to use them in various fields of application, such as that of cosmetics, nutraceuticals and the production of animal feed characterized by higher nutritional values than those currently on the market.

References

1. N. Jaeckelsa, S. Tenzer, M. Meier, F. Will, H. Dietrich, H. Decker, P. Fronk; *Food Science and Technology 75 (2017) 335e343336*
2. V. Di Stefano, D. Bongiorno, C. Buzzanca ,S. Indelicato ,A. Santini, M. Lucarini ,A. Fabbrizio, M. Mauro, M. Vazzana, V. Arizza, A. Durazzo; *Sustainability 2021, 13(23);*
3. M. Sciortino, G. Avellone, A. Scurria, L. Bertoli, D. Carnaroglio, D. Bongiorno, M. Pagliaro, R. Ciriminna; *ACS Food Sci. Technol. 2021, 1, 937−942*
4. E. J. Lee, N. Nomura, B. S. Patil, K. S. Yoo; *International Journal of Food Sciences and Technology 2014, 49, 2364-2372*

P40

Volatolomic profile as tool to evaluate the influence of cereal-yeast-hop interactions on aromatic profile of craft beer

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Summary: *The volatolomic and chemical profiles of different craft beers were characterized, focusing on grain-yeast-hopper interactions. The aroma profiles were analysed by HS-SPME-GC-MS while the chemical profile by HPLC-DAD. The data obtained were subjected to multivariate analysis. The effect of ingredient composition for the production of aromatic beers will be discussed.*

Keywords: *craft beer, metabolomics profile, raw materials*

Introduction

In recent years, the growing demand for beers denoted by distinctive and complex aroma profiles has driven the study about novel ingredients to be introduced in both artisanal and industrial productions. Focusing on starchy adjuvants, of particular interest is the use of different grains including wheat, sorghum, quinoa, amaranth and rice in order to produce beers with improved organoleptic and nutritional quality. In the light of these new trends, our work aimed to study the influence of different cereals [soft wheat (Ri), durum wheat (Da) and spelt (Fa)], hops (*Humulus lupulus*) varieties, [Cascade (Ca) and Columbus (Co)], of *Saccharomyces cerevisiae* strains (two commercial) on the volatile and sensory composition of the beers produced. The ultimate goal of the work is to improve knowledge about the production of beers with high aromatic impact.

Experimental

Brewing materials. Barley malt cv. Fortuna was supplied by Agroalimentare Sud (Melfi, Potenza, Italy). The unmalted cereals, i.e. durum wheat cv. Dauno III, soft wheat cv. Risciola, and dehulled emmer cv. Padre Pio (Triticum dicoccum) came from the experimental fields of CREA-CI Research Centre for Cereal and Industrial Crops, Foggia, Italy. Two cultivars of dried hop cones were used: Cascade and Columbus (6.7 and 17.6% alpha-acid contents, respectively). Hops as well bitter orange peels and coriander were supplied by Birramia (Querceta, Lucca, Italy). For the wort fermentation trials, the following two Saccharomyces cerevisiae strains were used: M21 and M02 (Mangrove Jack's (Rosedale,

Auckland, NZ).

Beer production. The beers were manufactured using 60% of barley malt and 40% of unmalted cereals. Twelve different wit-inspired craft beers were produced by combining the 3 binary mixtures of barley malt and unmalted cereals (alternatively durum wheat cv. Dauno III, soft wheat cv. Risciola, or dehulled emmer), the 2 hop varieties (Cascade, Columbus), and the 2 yeast strains (M21 and M02).

All grains were milled with a roller mill (Albrigi Luigi, Stallavena, Verona, Italy). Brewing was performed in a 30 L Braumeister system (Speidel Tank-und Behälterbau GmbH, Ofterdingen, Germany). Mash-in temperature was 52°C, followed by a 20 min. stand at 55 °C, followed by a 30 min stand at 65 °C, a 30 min stand at 70°C, and a final mash-off at 78 ◦C for 10 min. The resultant wort was boiled for 90 min with addition, 30 min after the start of boiling, of bitter orange peels, coriander, and hop cones, the latter in the amounts required to obtain a final bitterness of around 15 IBU and a final original gravity of 1.043±0.005. The wort was cooled at room temperature, inoculated with 10 g dried yeast to 20 L wort and fermented at 20±2°C for 21 days (until it reached values of original gravity around 1.012±0.003), followed by maturation at 4±1°C for 4 days. Beers were packaged into 750 mL glass brown bottles with addition of 6g/L of sucrose) and bottles were conditioned at 20±1 °C for 1 months.

HS-SPME Procedure. The analysis of volatile compounds was performed by a solid phase micro-extraction in combination with a gas chromatography coupled to mass spectrometry (SPME-GC/MS) as reported by Tufariello et al. [1].

GC-MS Analysis: GC-MS analyses were performed on a GC 6890 (Agilent Technologies, Palo Alto, CA) coupled to an Agilent MSD 5973 Network detector using a HP-INNOWAX capillary column (60 m × 0.25 mm, 0.25 µm, J&W Scientific Inc., Folsom, CA, USA) as reported by Tufariello, et al.[1]. Concentration of each volatile compound was assessed by the internal standard method. The VOCs concentration was estimated, semi quantitatively, using the added amount of 4-methyl-2-pentanol (IS) according the following equation: VOCs concentration = (VOC GC peak area/IS GC peak area) × IS concentration. This semi quantification approach was already performed in previous scientific studies [2].

HPLC-DAD Analysis: Organic acids were identified onto an Agilent Hi-Plex H (300 × 7.7 mm) with internal particles of 8.0 µm (Agilent Technologies, Santa Clara, CA, USA). The temperature of the column compartment was maintained at 70 °C. The flow rate applied was 0.4 mL min^{-1} with a run time of 30 min. The phase was 4.0 mM L^{-1} H_2SO_4 in ultrapure water [3]. Standard solutions were injected to obtain the retention time for each compound. For the determination of tartaric, malic, lactic, citric, acetic, fumaric and succinic acids detection was conducted in the DAD at 210 nm. The maltodextrin, maltose, maltotriose, glycerol and ethanol concentration was quantified on to an Agilent Hi-Plex Ca column (300 mm x 7.7 mm) with internal particles of 8.0 µm (Agilent Technologies, Santa Clara, CA, USA). The mobile phase used was deionized water and a constant flow rate of 0.6 mL min^{-1} for a run time of 30 min. For sugars detection was carried out by refractive index detector (RID). Quantification of

individual organic acids and sugar were performed directly by ChemStation software (Agilent) using a five-point regression curve ($r^2 \geq 0,99$) on the basis of authentic standards.

Results

Our results highlight the key role of grain-yeast-liver interactions that significantly affect aromatic quality. The principal component analysis (Fig.1) and three-way anova indicated that three samples differed significantly. Beer brewed with durum wheat, cascade hops, and Belgian yeast (DaCaWi) differed in higher a-terpineol, trans-o-cymene, methyl decanoate, isoamyl acetate, ethyl dodecanoate, and 1-propanol content. The sample produced with the same grain and yeast but with columbus hops (DaCoWi) differs in high values of ethyl butanoate, phenylethanol, neral, camphora, and isoamyl alcohols. Finally, beer made with soft wheat, columbus, and cider yeast (RiCoCi) differs in higher concentrations of certain terpenes, such as geraniol, ethyl nonanoate, and ethyl heptanoate.

Conclusions

The obtained data have highlighted the key role of the volatolomics approach to study the contribution of different raw materials to improving the sensory profile of final beers. In addition, we propose some grain-yeast-hops combinations that can ensure the production of beers with high aromatic value.

Acknowledgments: This research was founded by the APULIA REGION, with the project "Birra: dal campo al boccale—BE^2R" (P.S.R. Puglia 2014/2020 - Misura 16.2).

References

1. Tufariello M., et al. LWT, 108 (2019) 268 - 276
2. Perestrelo R., et al., Molecules,23(2), (2018) 499
3. Coelho, E.M., et al., Journal of Food Composition and Analysis,66, (2018) 160-167.

P41

The wine is “naked”: flint glass bottles cause wine aroma identity degradation

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Keywords: *metabolomics, GC×GC-Tof-MS, lightstrike*

Transparent packaging is often used for food products, including wine, milk, beer and fruit juices. This choice is based on the marketing recommendation that consumers want to see the product before they buy it, although scientists point out that light can harm food quality and nutritional value.
Although the practice of bottling white wine in clear glass is known to cause a wine defect, the influence of light on the fruity and floral flavor profile of the wine is unknown. The aim of this work was to study the influence of light exposure on the white wine volatilome under the typical supermarket shelf conditions and to monitor the primary aroma compounds that characterize the sensorial identity and flavor of each cultivar using 1,052 bottles of 24 white wines [1]. The volatile profile was studied using a fingerprinting method able to maximize the number of volatiles detected, via comprehensive gas chromatography combined with time-of-flight mass spectrometry (GC×GC-ToF-MS) instrument.
After only 7 days of shelf life in flint glass bottles, a dramatic loss of terpenes (10 to 30%) and norisoprenoids (30 to 70%) was recorded, while colored glass bottles did not show such behavior. even after 50 days and the darkness has preserved the fruity and floral aromatic integrity of the wine. Flint glass bottles bring no benefit to the wines, while the multiples changes in the aroma composition can jeopardize the quality, depriving the wine of the identity of the variety and terroir. In other words, the wine is naked. In light of this understanding of the negative impact of flint glass on the aromatic identity and sensory character of white wine, this packaging should be strongly discouraged. The same results should apply to a wide range of different foods consumed daily in which clear packaging is used.

References

1. S. Carlin, F. Mattivi, V. Durantini, S. Dalledonne, and P. Arapitsas, *Proc Natl Acad Sci U S A*, vol. 119, no. 29, p. e2121940119, Jul. 2022, doi: 10.1073/pnas.2121940119

P42

Ultra-selective solid phase isolation of mercaptans using Cu(I) salts. Application to the selective isolation of five polyfunctional mercaptans from wine

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Summary: *A novel SPE procedure for the ultra-selective isolation of mercaptans has been developed and applied to the determination of wine polyfunctional mercaptans. Mercaptans are extracted in the SPE-bed, complexed with Cu(I), interferences removed, complexes cleaved, eluted with methanol, extracted with SBSE and determined by TD-GC-GC-MS reaching good detection limits.*

Keywords: *Polyfunctional mercaptans, Wine, Solid-phase extraction*

Introduction

Mercaptans are sulphur containing molecules that play important roles in food due to the characteristic aromatic impact they can give. Furthermore, most of them have very low threshold levels (ng/L), meaning that they can be smelled at tiny concentrations. From the sensory point of view, mercaptans are characterized by powerful and penetrating odours and, while some of them are known for their putrefaction-related unpleasant odours, others are responsible for positive flavour nuances, playing essential roles in products such as grapefruit [1], passion fruit [2], onions [3] or wine [4-5]. Some of them have been found amongst the most relevant odorants in wine 4-mercapto-4-methyl-2-pentanone (MP), furfurylthiol (FFT), benzyl mercaptan (BM), 3-mercaptohexanol (MOH) and 3-mercapto hexyl acetate (MHA) [4].

The analysis of these odor-active molecules is very challenging because of their low levels, high reactivity and poor spectrometric properties. Most current methods make use of their derivatization to form highly detectable derivatives, such as those obtained with PFBBr [6] or with Ebselen [7]. However, derivatives are odorless, which rends these methods useless for the qualitative identification of unknown mercaptans, and the yields of the derivatizations are extremely matrix-dependent, so that the use of isotopomers as internal standards becomes crucial. From both reasons, having at hand selective isolation procedures becomes essential.

In the present paper, we explore the known ability of Cu(I) salts to form strong complexes with mercaptans and take advantage of the strong retention properties of those complexes on SPE polymeric phases possibilities to remove all other compounds co-extracted with the mercaptans. Therefore, the main aims of this work are to undergo a thorough study of all the different aspects and variables affecting the selective SPE isolation of mercaptans and specifically

develop an optimal procedure for the selective isolation and subsequent SBSE extraction and TD-GC-GC-MS determination of the five main PFMs of wine.

Experimental

For the experiments, a 1 mL SPE polypropylene cartridge filled with 65 mg of the ISOLUTE ENV+ absorbent phase was used. Both real and model wines, spiked with 200 ppb of five PFMs, were used to optimize the procedure. Initial experiments investigated the best form of forming and extracting the Cu(I)-SR complexes. Once determined that they will be formed on the mercaptans already extracted in the SPE bed, the breakthrough volume for the five PMFs in wine was assessed to evaluate the amounts of sample that can be safely loaded in the cartridge (15 mL). Then the concentration, composition and volume of the CuCl aq. solution used to complex the mercaptans in the cartridge was investigated. Subsequently, cleaning, washing and elution steps using different solvents and solutions were studied and optimized. The washing step was found to be the most critical and many different combinations of solvents and antioxidants using different conditions were tested.

Finally, for the quantitative determination of the five underivatized main wine polyfunctional mercaptans, a procedure using methanol as elution solvent, further diluted with water and extracted with SPME or twister extraction was further developed. The most sensitive conditions were achieved by twister extraction (SBSE, PDMS 1 cm) and further TD-GC-GC-MS.

Results

Initial experiments showed that CuCl can be weakly retained in the cartridge, with breakthrough volumes as low as 7 mL, which precludes the use of CuCl-preloaded SPE beds for the preconcentration of mercaptans in hydroalcoholic solutions. Additionally, Cu(I)-SR complexes already formed in wine were just poorly retained in the cartridge, with non-ideal retention kinetics, suggesting that complexes cannot penetrate into the sorbent particles. Because of that, maximum advantage of the selectivity introduced by Cu(I) is achieved if complexes are formed once the mercaptans are extracted in the SPE cartridge.

Following, the BVs of five polyfunctional mercaptans in wine were determined and were found to be > 30 mL. Consequently, 15 mL of sample were chosen as optimal loading volume. Subsequently, CuCl solutions were evaluated at different concentrations (100 and 500 mg/L) and volumes (2 and 4 mL) for complexing PFMs already present in the cartridge. The best results were obtained by using 4 mL of an aqueous solution 500 mg/L in CuCl.

Different cleaning solvents were studied. Results revealed that Cu-mercaptan complexes are so strongly retained in the cartridge, that more than 6 mL of MeOH can be used for the washing step without eluting detectable amounts of mercaptans, however, 2 mL of MeOH were selected as optimal, since this volume is able to elute nearly 100% of the other volatile material retained in the sorbent.

Then, the cleavage of the Cu(I)-S complexes and further elution of the mercaptans was investigated. First essays with 4 mL of a cysteine aq. solution (50 g/L) followed by elution with DCM offered consistently poor recoveries. Acceptable results were obtained only for MP (73%) and AMH (89%). A thorough

research of the factors affecting this, revealed that the problem was not related to a lack of complex cleavage, solvent strength or elution volume, but to oxidation. Results much improved when antioxidants such as TCEP and, in particular, DTT (1,4-dithiothreitol) were incorporated. At the end, 100% recovery for the five PFMs was observed by using 4 mL cysteine 50 g/L spiked with DTT 5 g/L followed by a further percolation of a solution containing 10 mL DTT 3 g/L. Mercaptans can be further quantitatively eluted out of the cartridge by using 0.6 mL of DCM or 1.3 mL MeOH both containing also DTT (2 g/L). The isolation procedure was highly selective, quantitative and repetitive.
In order to get enough sensitivity in the analytical determination of underivatized PFMs extracted from wine, the elution with MeOH was selected followed by dilution with water and further extraction with solid phases. SPME was found to be unable to provide enough concentration factors. SBSE, however, followed by TD-GC-GC-MS made it possible to reach detection limits in the low-ppt range.

Conclusions

Cu(I) can be satisfactorily used to complex and stabilize mercaptans previously retained in a polymeric SPE cartridge. The level of fixation is strong enough to achieve a complete separation of fixed mercaptans from any other volatile retained in the SPE bed. By using strong reducing agents during complex cleavage and elution, quantitative recoveries of completely isolated mercaptans is achieved. For the automated analysis of underivatized mercaptans, best sensitivities were obtained by using MeOH elution, and further SBSE extraction followed by TD-GC-GC-MS.

References

1. A.S.P. Buettner; *Journal of Agricultural and Food Chemistry*, 47 (1999), 5189-5193.
2. P. Werkhoff, M. Güntert, G. Krammer, H. Sommer and J. Kaulen; *Journal of Agricultural and Food Chemistry*, 46 (1998), 1076-1093.
3. M. Granvogl, M. Christlbauer, P. Schieberle; *Journal of Agricultural and Food Chemistry*, 52 (2004), 2797-2801.
4. L, Mateo-Vivaracho, J. Zapata, J. Cacho, V. Ferreira; *Journal of Agricultural and Food Chemistry*, 58 (2010), 10184-10194.
5. L. Chen, D.L. Capone, D.W. Jeffery; *Molecules*, 24 (2019), 2472.
6. L. Mateo-Vivaracho, J. Cacho, V. Ferreira; *Journal of Chromatography A*, 1185 (2008), 9-18.
7. D. L. Capone, R. Ristic, K. H. Pardon, D. W. Jeffery; *Analytical Chemistry*, 87 (2015), 1226-1231.

P43

Volatile metabolome analyzed through HS-SPME/GC-MS. The case of garlic

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Summary: *The volatile metabolome of garlic powder, assessed through HS-SPME/GC-MS, revealed a crucial dependency on both the employed SPME-fiber and the temperature set for the of solid⇆headspace equilibration and sampling steps. Beside the expected sulfur-containing-compounds, fatty acid esters and alcohols were found as representative classes of compounds.*

Keywords: *HS-SPME/GC-MS, volatile metabolome, garlic powder*

Introduction

Garlic (*Allium Sativum* L.) is a member of Amaryllidaceae family, representing one of the most widely produced plants all over the world. It has been used since the ancient times as food flavoring as well as in folk medicine, for prevention of strokes and cardiovascular pathologies. Aroma components of garlic mainly consist of several thiosulfinates and other sulphur-containing-compounds (SCC) that are enzymatically formed from odorless precursors after crushing fresh leaves or cloves [1]. In this work [2], the volatile metabolome of garlic powder (provided by Grappolo S.r.l., Soliera, Modena (Italy)) has been determined by combining GC-MS analysis with headspace solid-phase microextraction (HS-SPME), wherein several extraction parameters have been tuned to assess the most proper analytical conditions for targeting specific classes of compounds.

Experimental

Four SPME-fibers, namely, PDMS-DVB, CAR-PDMS, CAR-DVB-PDMS, and PA, have been compared to determine the greater extraction efficiency based on the number of extracted compounds. The following three parameters were tuned to optimize the extraction conditions during the equilibration (eq) and sampling (sa) steps (Table 1): thermostat bath temperature (adjusted at 50, 70, 80, 90 °C) during both the equilibration and sampling steps, equilibration time (t_{eq}: 20, 40 min) and sampling time (t_{sa}: 20, 35 min). After sampling, fiber was exposed into the GC inlet at 260 °C for 0.5 min. Furthermore, the powder shelf-life was evaluated by analyzing the sample after four-month storage at room temperature.

Table 1. *HS-SPME tuned parameters.*

Temperature (°C)	**50**	**70**	**80**	**90**
Equilibration time (min)		20 40		
Sampling time (min)		15 35		

Results

The best efficiency, in parallel to the higher number of detected compounds, was obtained with the DVB-CAR-PDMS fiber ($T_{eq/sa}$ = 80 °C, t_{eq} = 20 min and t_{sa} = 15 min) which extracted three prevailing classes of compounds, namely alcohols, SCC and fatty acid esters (FAE) whose relative abundance crucially depends on the HS-SPME conditions. Using this set of parameters, diallyl trisulfide was found the most abundant component (22.0%) followed by ethyl linoleate (17.8%), ethyl palmitate (9.68%), diallyl tetrasulfide (6.80%), and diallyl disulfide (6.18%). Diallyl trisulfide is also the major analyte extracted by the PDMS-DVB fiber in the same analytical conditions (37.52%). The fiber selectivity presents remarkable differences: the DVB-CAR-PDMS cartridge has a similar selectivity for FAE as well as for SCC (39.4% and 40.3%, respectively) and a very low affinity for alcohols (3.6%). This pattern is reversed when CAR-PDMS results are considered: indeed, alcohols definitely represent the principal class of compounds absorbed by this phase (61.2%). The selectivity of PDMS-DVB cartridge is pronouncedly oriented toward SCC (52.1%) and significantly less toward FAE (30.4%). When t_{sa} was increased to 35 min, PDMS-DVB and CAR-PDMS fibers show a similar pattern to the result obtained for t_{sa} = 15 min; on the contrary, the DVB-CAR-PDMS one extracted very efficiently FAE (69.1%). It is noteworthy that the VOC distribution was not altered by the four-months room temperature storage.

Conclusions

HS-SPME/GC-MS technique was applied to the study of the volatile metabolome of garlic powder, by tuning several extraction parameters. The most efficient extraction, evaluation based on the number of extracted compounds, was achieved using the DVB-CAR-PDMS fiber ($T_{eq/sa}$ = 80 °C, t_{eq} = 20 min and t_{sa} = 15 min). Three classes of compounds were found to be the most representative, namely alcohols, SCC and FAE, whose relative abundance strongly depends on the set of extraction parameters. The SPME-fiber and sampling/extraction temperature proved to be the most critical parameters.

References

1. S. N. Lee, N.S. Kim, D. S. lee; *Analytical and Bioanalytical Chemistry*, 377 (2003), 749-756.
2. L. Recinella, Chiavaroli, F. Masciulli, C. Fraschetti, A. Filippi, S. Cesa, F. Cairone, E. Gorica, M. De leo, A. Braca, A. Martelli, V. Calderone, G. Orlando, C. Ferrante, L. Menghini, S. C. Di Simone, S. Veschi, A. Cama, L. Brunetti, S. Leone; *Nutrients*, 13 (2021), 2332-2353.

P44

Ion mobility spectroscopy and chemometrics for the analysis and discrimination of tomato sauce

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Summary: *Sixteen tomato sauce bottles, purchased from the market, were analyzed by gas-chromatography coupled to ion mobility spectroscopy (GC-IMS). The data were subjected to chemometric analysis (principal components analysis, PCA) with the aim of discriminating different brands and finding the most discriminating volatile molecules.*

Keywords: *tomato sauce; GC-IMS; PCA*

Introduction

Tomato products are used all over the world and it is estimated that 30% of global production of tomato is destined for industrial processing. The main producers are United States (Italy), and China, covering together 56% of world tomato production.

The volatile profile of tomato contains a great number of different molecules. However, not every one of them contributes equally to its aroma. Volatile molecules are also partly responsible for the taste of tomato and tomato products.

In this work, a faster, cheaper, and greener analytical procedure was developed for the analysis tomato sauce volatile organic fraction by gas-chromatography coupled to ion mobility spectroscopy (GC-IMS) [1].

Experimental

Sixteen tomato sauce samples were purchased from the market and analyzed by GC-IMS. Samples were analyzed without any chemical pre-treatment: an aliquot (2g) of each sample was put in a vial, sealed, and directly put in the GC-IMS auto-sampler. The GC system aspires an aliquot of the vial headspace, and injects it into the instrument.

GC-IMS result is a 2D map in which the vertical axis represents GC retention times, while the horizontal axis represents the IMS drift times. An example is shown in Fig. 1, where the red dots are the 2D peaks. Such map is converted into a one-dimension vector by the instrument software, and the samples' vectors can be chemometrically processed by principal components analysis (PCA) [2].

Results

Tomato sauce samples were divided into six brands. The PCA carried out on the GC-IMS data showed some interesting behavior for each brand.

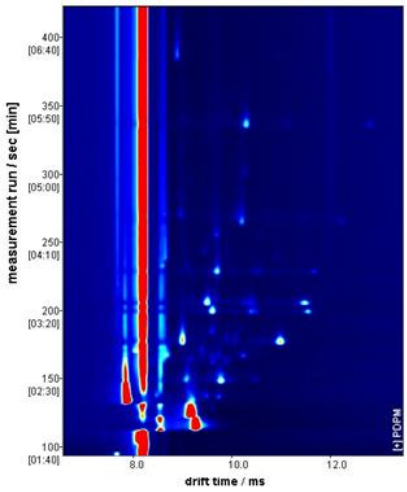

Figure 1. *GC-IMS 2D plot*

The scores plot is reported in Fig. 2. It can be seen that, while brands 1, 2, 5, and 6 are well discriminated, with their samples well grouped between them, both brand 3 and brand 4 show two distinct groups. By looking at the loadings plot, it was possible to find the molecules that mostly concurred to such samples division. Moreover, it was found that samples at positive values of PC1 have, in general, higher concentrations of volatile compounds.

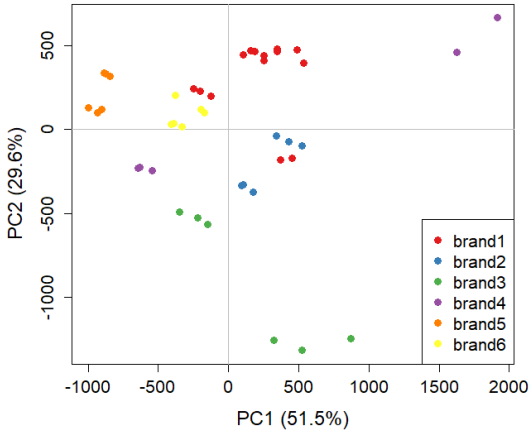

Figure 2. *PCA scores plot*

References

1. Arvanitoyannis, I., Vaitsi, O.; *Critical reviews in food science and nutrition*. 47 (2007), 675-699
2. Melucci D., Bendini A., Tesini F., Barbieri S., Zappi A., Vichi S., Conte L., Toschi T.G.; *Food Chemistry* 204 (2016) 263-273

P45

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 Figure 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. 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].

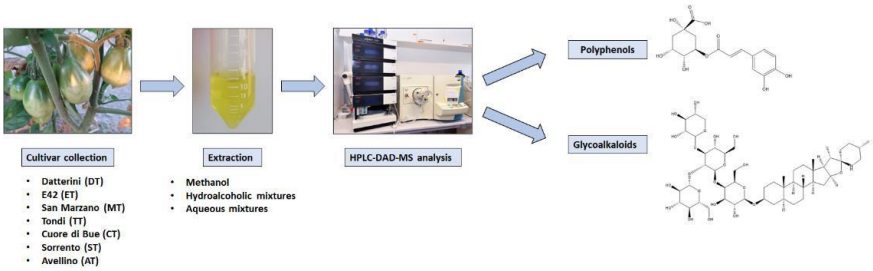

Figure 1. *Workflow of the analysis of glycoalkaloids and polyphenols.*

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 (R^2 > 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 Figure 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*-pentosyl-rutinoside.

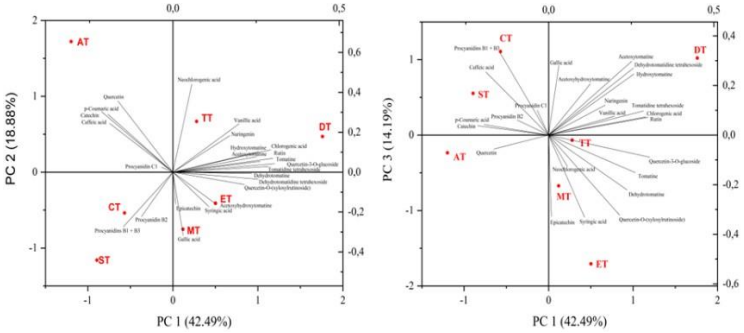

Figure 2. *Biplot of the principal component analysis (PCA) of 7 unripe tomato cultivars and 24 quantified compounds.*

Conclusions

During the processing of the tomato industry, a large amount of unripe tomatoes are 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 HPLC-DAD-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.

References

1. Matei, E.; Râpă, M.; Predescu, A.M.; Țurcanu, A.A.; Vidu, R.; Predescu, C.; Bobirica, C.; Bobirica, L.; Orbeci, C.; *Materials (Basel)*, 14 (2021), 221-232.
2. Rick, C.M.; Uhlig, J.W.; Jones, A.D. *Proc. Natl. Acad. Sci. U. S. A.*, 91 (1994), 12877-12881.
3. Agency, E.M. ICH Topic Q 2 (R1), 2 (2006), 1-15.

P46

In depth characterization of fresh leaves from basil (*Ocimum Basilicum* L.) by HPLC-DAD-MS, HS-SPME-GC×GC/TOF and non-destructive fluorescence-based method

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Summary: *Basil is widely used as food ingredient all over the world. It is characterized by a typical fragrance capable to enrich many dishes. In this work basil fresh leaves with different intensity color were characterized for phenolic and volatile fractions with innovative techniques and a non-destructive fluorescence-based method.*

Keywords: *Volatile compounds, biomolecules, food quality*

Introduction

Sweet basil (*Ocimum Basilicum* L.) is a vegetable widely used as food ingredient all over the world. Fresh basil is characterized by a typical fragrance and it is used as an ingredient in various dishes and food preparations, especially in the Mediterranean cuisine. Carovic-Stanko et al. [1] identified six *O. basilicum* morphotypes on the basis of morphological traits. *O. basilicum* cultivars are also distinguished by their varying chemical compositions. Basil essential oil contains many aromatic compounds that contribute to the herb antioxidant properties [2], and accessions have been grouped into chemotypes based on their highest concentration volatile component such as methyl chavicol (estragole), linalool, or methyl eugenol [3]. Chemotyping based on non-volatile leaf compounds is less common, yet variations in foliar phenolic concentrations have been reported among basil cultivars [4]. The biomolecules found in basil leaves – including rosmarinic acid, caftaric acid, chicoric acid and procyanidins – are important for both their strong antioxidant capacities as well as their chemopreventive potentials [5]. In this work, an innovative method HS-SPME-GC×GC-TOF was developed to characterize the volatile fraction of 5 italian basil accessions. Comprehensive GC-MS (GCxGC-MS) is a powerful technique that provides two-dimensional chromatography data acquisition capability and it's suitable for a variety of applications, including profiling of complex matrices such as natural products, and grouping analysis based on 2D chromatograph patterns. Basil leaves were also analyzed for their polyphenolic content by HPLC-DAD-MS.
Moreover, a preliminary evaluation of basil metabolites was performed to assess a non-destructive fluorescence-based method with Multiplex portable sensor [6]. This technique provides a series of numerical indices, some of them sensitive in particular to the concentration of nitrogen and chlorophyll, others sensitive to the presence of flavonoids or anthocyanosides within the sample. Further investigations will therefore concern the possibility of optimizing a rapid non-destructive measurement method, complementary to the HPLC-DAD-MS and

GCxGC-MS/TOF analysis, for in-field monitoring of metabolites in basil plants.

Experimental

Five *O. basilicum* samples were purchased from LANDLAB Srl (Quinto Vicentino, Vicenza (Italy)). There were more intense green leaves and yellow leaves for each of the 5 accessions. Volatile organic compounds (VOCs) were analyzed by HS-SPME-GCxGC-MS-TOF analyses. VOCs were absorbed from the headspace of 20-ml screw cap vial by a 2-cm fiber (DVB/CAR/PDMS), for 15 min under orbital shaking at 60°C. Compounds were tentatively identified comparing mass spectra with those reported in mass spectral databases; identification was confirmed by their retention index. *HS-SPME-GCxGC-MS-TOF*: a SRA-Agilent 7890B GC (Agilent Technologies, Palo Alto, CA, USA), with flow modulator device for 2D separation, coupled with a time-of-flight mass spectrometer (TOF-DS Markes International Ltd., Llantrisant, UK) was used. Chromatographic separation was performed using a (1D) HP-5 column (0.18x0.18mm, 20 m) and a (2D) column (0.23x0.32 mm, 5 m). *HPLC-DAD-TOF*: a HP1100 liquid chromatograph equipped with a DAD detector and a Agilent TOF MS with an ESI source was used (Agilent Corp, Santa Clara, CA, USA). Compounds were separated by using a 250x4.6 mm i.d, 5 µm LUNA C18 column (Phenomenex, USA). The TOF analysis worked using full-scan mode and the mass range was set at m/z 100–1500 in both positive and negative modes. The conditions of ESI source were as follow: drying gas, high purity nitrogen (N_2); drying gas temperature, 350°C; drying gas flow-rate, 6 L/min; nebulizer, 20 psi; capillary voltage, 4000 V (negative) 4000 V (positive); fragmentation, 80-150 V, and skimmer, 60 V. The portable Multiplex fluorescence sensor (FORCE-A, Orsay, France) was previously described in detail [6]. It is equipped of 4 LED excitation sources and 2 detection channels to detect the fluorescence of chlorophyll at different spectral bands.

Results and conclusions

GCxGC-MS/TOF analysis showed that the main compound in the basil accessions under study is methyleugenol. The other main compounds present are bergamotene and eugenol, whereas estragole, on the other hand, was not detected in these samples. Fig. 1 shows the contour plot obtained by GCxGC-MS/TOF analysis of sample 1 and 5. Comprehensive two-dimensional GC fingerprint analysis allows a direct comparison of the VOCs: in particular, the analyzed samples show differences in terms not only of intensity of the VOCs present, but also in the absence of some compounds. In the 2D images, the regions corresponding to the class of monoterpenes and sesquiterpenes are highlighted. Sample 5 is the one with the highest number of VOCs and of greater intensity, furthermore there are aldehydes and esters not present in sample 1 compared.

The HPLC-DAD-MS characterization showed a higher average content of total polyphenols for green leaves with respect to the yellow ones (10.49 mg/g of green leaves *vs* 8.72 mg/g yellow leaves); in all samples rosmarinic acid, hydroxycynnamic derivatives and procyanidins were detected and quantified, with a prevalence of the first one (average content of rosmarinic acid with respect to total polyphenols: 85% p/p yellow leaves; 72% green leaves). No flavonoids

were detected in any of the samples. Preliminary data collected with Multiplex portable fluorescence sensor confirmed the scarcity of flavonoids and, consequently, the most reliable indices turned out to be the ones sensitive to chlorophyll. For example, the trends of the NBI (Nitrogen Balance Index) and SFR (Simple Fluorescence Ratio) indices reflected the different abundance of chlorophylls in the green and yellow samples.

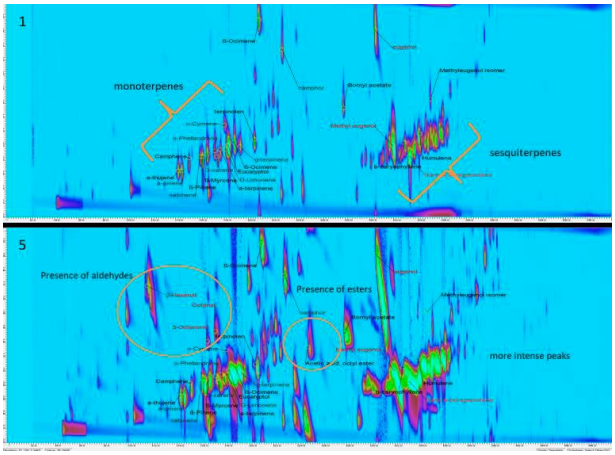

***Figure 1**. Contour plot from GCxGC/TOF analysis of sample 1 and 5*

Acknowledgement
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References

1. Carovic-Stanko et al., *Plant Biosyst.* 145: (2011), 298–305.
2. Purushothaman et al. *J. Nat. Rem.* 18, (2018), 71–85.
3. Valtcho et al., *J. Agric. Food Chem*. 56, (2008), 1.
4. Kwee, et al., *Food Chem.* 128, (2011), 1044–1050.
5. Sestili et al., *Expert Opin. Drug Metab. Toxicol.* 14, (2018), 679–692.
6. Agati et al., *J. Agric. Food Chem. 61, (*2013), 12211- 12218

P47

Statistical differentiation of pesto products using SPME-GCxGC-TOFMS and ChromaTOF Tile Software ® enhanced aroma characterization

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Summary: *The high number of chemicals critical to taste and aroma in Pesto are present in varying concentrations. Several low-level species, with high aromatic potency, often co-elute with those at high concentrations. The use of GCxGC and in combination with the Software TILE allows having a remarkable separation capacity.*

Keywords: *Non-Target, GCxGC-TOFMS, Class Differentiation*

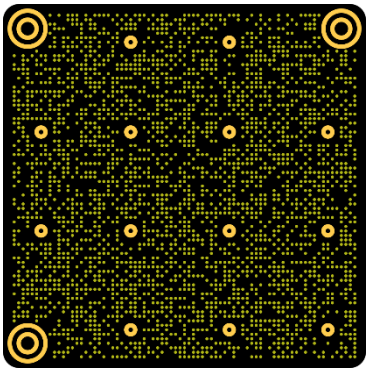
Introduction

Pesto and its origins date back to ancient times. Although the main ingredients are well known (typically based on olive oil, basil, and pine nuts), the recipes of numerous commercially available pesto products can strongly differ. The aroma profile of the pesto is highly influential on consumers' perceptions and preferences. Thus, its examination plays an important role in definition of the "right" recipe, product stability, and product quality. The ability to comprehensively analyze and characterize pesto recipe variations allows more optimal product development and therefore higher brand awareness and customer satisfaction. The complexity of GCxGC data can quickly become overwhelming due to a necessity of maintaining peak-tables of hundreds or thousands of analytes for each sample. To find statistically significant differences between data sets often requires time-consuming and intensive data-mining on an expert-level. Now, Tile, a data processing software platform developed using Prof. Synovec's Tile-based Fisher ratio ChromaTOF approach, uses a revolutionary algorithm for the comparison of multiple GCxGC-TOFMS data in a fast and user-friendly way, while using nominalized mass traces for area calculation. Statistically significant differences can be then easily highlighted, reducing the time required to locate the differences between the sample sets. The easy and user-friendly approach to the differentiative analysis is demonstrated on a data set of 11 pesto samples from three different producers. Samples were acquired by SPME-GCxGC-TOFMS.

Results and Discussion

The aim of this case study was to find the differences in products of three producers utilizing an easy and fast differentiation software tool. The degree of complexity in differential analysis depends on the methodology employed. A basic approach is to locate differences based on visual comparisons of contour

plots using TIC and/or selected masses (e.g. 93 for terpenoids). A more advanced strategy is to utilize a statistical comparison software. The latter workflow—ideally fully automated— allows discovery of differences that aren't easily visible or would be time consuming to identify, due to low intensity and high analyte complexity across numerous samples. LECO Tile locates the important spots by tessellating the ChromaTOF GCxGC plots by tiles (1D, 2D RT windows). The application of Fisher-ratio (F-ratio) analysis to the supervised comparison of sample classes algorithmically reduces complex GCxGC-TOFMS data sets to find class distinguishing chemical features. The area of each tile is calculated on each individual nominal mass, and the integrals are used for Fisher ratio (F-ratio) computation to discover statistically meaningful differences, as described by Synovec.1,2 Although multiple ways of displaying the results of differentiative analysis can be employed, one of the most used is the Principal Component Analysis.



Conclusions

The capability of Tile software to facilitate a fast class differentiation in non-targeted approaches for multiple ChromaTOF samples was demonstrated in this application note. The features that are responsible for statistically meaningful samples' clustering were easily found and identified, thus highlighting key ingredient differences which could then, for example, aid product development processes in conjunction with sensory and consumer test data. In general, the utilization of this differential analysis tool allows enriched information and trends and patterns to be leveraged quickly in complex sample sets obtained by GCxGC-TOFMS. Therefore more time can be spent focusing on meaningful results, rather than laborious data mining.

References

1. L.C. Marney, W.C. Siegler, B. A.Parsons, J. C.Hoggard, B.W. Wright, R.E. Synovec, 115 (2013) 887-895, Talanta, http://dx.doi.org/10.1016/j.talanta.2013.06.038
2. B. A. Parsons,L.C. Marney, W.C. Siegler, J.C. Hoggard, B.W. Wright, R.E. Synovec, 87 (2015) 3812-3819, DOI: Anal. Chem 10.1021/ac504472s

P48

Metabolite profiling for typization of "Rucola della Piana del Sele" (PGI), *Eruca sativa*, through UHPLC-Q Exactive-Orbitrap-MS/MS analysis

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Summary: *Eruca sativa from different geographical origins was characterized by UHPLC-MS analysis. Various types of extraction were developed. Metabolite profiling of E. sativa extracts revealed that some metabolites were specific to geographical origins, while others were common in all extracts.*

Keywords: *metabolomic analysis, geographical origin, and glucosinolates.*

Introduction

Eruca sativa (Brassicaceae) originated in the Mediterranean region is also known as Rocket, Rocket Salad or White Pepper. Young plants can be used as a salad, vegetable, or green fodder. The properties of this herb are well recognized in traditional medicine as astringents, depurative, diuretics, digestives, and emollients [1].
"Rucola della Piana del Sele" (PGI) received the mark of quality in 2020. There are no methods for typing and characterization of the geographical area of origin. The main metabolites contained in *Eruca* belong to the class of glucosinolates and phenolic compounds. In particularly glucosinolates present in Brassicaceae were found to have anticarcinogenic, antifungal, antibacterial, and antioxidant activities [2].
As *E. sativa* has a very wide cultivation, this study sought to compare metabolic profiles between Rocket grown in Eboli, Bergamo, Brescia, as well as in Switzerland, making a comparison also with the metabolic profile of wild *E. sativa* grown spontaneously.

Experimental

Different types of extraction were carried out. Firstly, the plant was extracted both fresh and fresh-dried, previously frozen at -80 °C. Two extractions were set up with both types of matrices, one with a methanol solution and the other with ethanol/water solution (70/30), both assisted by ultrasonic bath.
The extracts thus obtained from different types of *E. sativa* were examined through a Metabolite Profiling approach by using liquid chromatography coupled with mass spectrometry equipped with an electrospray source (UHPLC-Q Exactive-Orbitrap-MS/MS) in negative mode. Xcalibur software was used for instrument control, data acquisition and data analysis.

Results

The comparison of metabolic profiles did not reveal any important differences between the methanolic and hydroalcoholic extracts obtained from the dry

matrix. These extracts were found to be rich in polyphenolic compounds such as flavonoid glycosides, particularly quercetin and kaempferol derivates like quercetin-3, 3', 4'-triglucoside, quercetin-3, 4'-diglucoside-3'-(6-sinapoyl-glucoside, and kaempferol-3,4-diglucoside.
Glucosinolates were detected especially in the extracts obtained from fresh plant, suggesting that lyophilisation can reduce the amount of these metabolites in the samples. Glucoraphanin, glucosativin and glucoiberverin are among the most frequently detected glucosinolates in *E. sativa* extracts.
The analysis of rocket extracts of different origins showed a similarity of metabolites in the rocket from Brescia, Bergamo, and Switzerland unlike that from Eboli, especially with regards of glucosinolates and flavonoids. The peaks with the highest intensity in *E. sativa* PGI profile are represented by oxylipins, which could represent useful markers for the recognition of the botanical origin of the plant species.

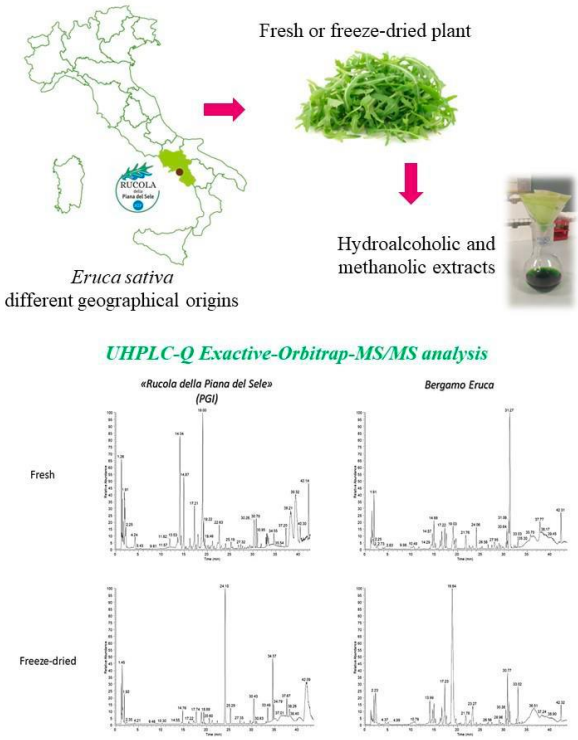

***Figure 1**. Research workflow*

Conclusions

This study sought to develop a suitable extraction method for *E. sativa*, highlighting that extraction from a fresh matrix allows to obtain mainly glucosinolates, while polyphenolic compounds are extracted from a freeze-dried matrix, to perform a method to discriminate "Rucola della Piana del Sele" (PGI) from others *Eruca* plants.

Metabolite profiling of *E. sativa* from different geographical origins using UHPLC-MS analysis revealed that all extracts contain common metabolites, as well as some metabolites that were specific to geographic sources.

References

1. G. Garg, V. Sharma; *Journal of Herbs, Spices & Medicinal Plants*, 2014, 20:2, 171-182
2. L. Bell, H. N. Yahya, O. O. Oloyede, L. Methven, C. Wagstaff. *Food Chemistry*, 2017, 221, 521-534.

P49

Rapid authentication of oregano: the combination of TD-DART-HRMS and LASSO method

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Summary: *This contribution describes the first application of TD-DART-HRMS to spices authenticity. The combination of this ambient mass spectrometry approach, characterized by no sample preparation, and the LASSO method allowed an accurate and rapid authentication of the oregano samples.*

Keywords: *fraud, classification model, ambient mass spectrometry*

Introduction

Spices are very precious commodities that can be used as preservative and flavour enhancer. Oregano (*Origanum vulgare*) is one of the most used and prized spice. A recent survey carried out in 2021 by the European Joint Research Centre revealed that 48% of the oregano sampled from the European market are at risk of contamination (1). On the basis of these results the European Commission has called on the operators for a rapid and effective action plan to rectify this critical issue and protect the consumers. The set up of new accurate methods able to verify the authenticity of a large number of spices, before the introduction in the EU market, is thus mandatory. The use of DART-HRMS in spices authentication has also been recently reported in literature (2). This contribution describes the development of a fingerprinting method for the authentication of oregano by thermal desorption direct analysis in real time high resolution mass spectrometry (TD-DART-HRMS) coupled to least absolute shrinkage and selection operator (LASSO). In this study, a homemade TD-DART-HRMS system was set up. The LASSO method was applied to create a classifier able to predict the authenticity of an oregano sample based on its TD-DART-HRMS fingerprint.

Experimental

In our study, a total of 24 samples were analysed by DART-HRMS in positive ion mode. The 12 authentic samples were provided by a recognized spices supplier and originated from Italy, Turkey, Albany and Bangladesh. We then spiked authentic samples with olive leaves, myrtle, strawberry tree leaves, sumac and rock rose, obtaining 12 adulterated samples. The concentration of adulterants in the spiked samples ranged between 20 and 45%. A DIY heating device was located between the DART gun and the inlet of the mass spectrometer. The heating device generated a temperature ramp between 25°C and 150°C in 40

seconds. The warm plume of volatile compounds was directly ionized and analyzed by DART-HRMS from about 50 mg of sample. To this aim, an orbitrap mass spectrometer (Exactive plus from Thermo Fisher Scientific) was coupled to a DART SVP 100 ion source (IonSense, Saugus, MA, USA). The resolution was set to 70,000 FMHW and the spectra were acquired between 75–750 Da. Three aliquots of each sample were analysed in order to catch the heterogeneity of the sample analysed. The triplicate spectral data were statistically analyzed using MetaboAnalyst 5.0 web portal (www.metaboanalyst.ca) and Rstudio 3.6.1 software with the *caret* package. Initially, a PLS-DA was performed to explore the capability of this ambient ionization approach to discriminate authentic (typical) and adulterated samples (atypical). The data were split into training (18 samples) and test sets (6 samples). A LASSO classifier was generated on the training set, and its performances established by 5 time repeated 5 fold cross-validation, The performances of the classifier were then established on the test set. Accuracy, sensitivity and specificity rates of the classifier were calculated

Results

Fig. 1A shows representative TD-DART-HRMS spectra of the authentic (typical) and adulterated (atypical) oregano. As shown in Fig. 1B a good discrimination of the typical and atypical samples was achieved by PLS-DA. These results encouraged the generation of a LASSO classifier for the automatic authentication of oregano from the TD-DART-HRMS spectra.

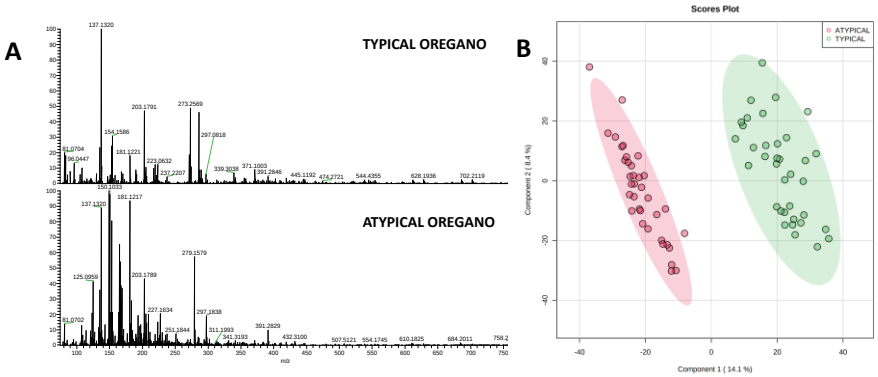

Figure 1. *A) TD-(+)DART-HRMS spectra of authentic (typical) and adulterated (atypical) oregano samples with 30% olive leaves. B) PLS-DA score plot showing a good clustering of the two groups of study*

Initially, LASSO selected 19 molecular features (ions) that were then used to create a predicting model. The model was cross-validated on the training set achieving excellent performances (Table 1). The performance of LASSO classifier was then evaluated on the withheld test set, achieving excellent results in terms of overall accuracy, sensitivity, and specificity (Table 1).

Table 1. *Statistical figures of merit (sensitivity, specificity and accuracy) for LASSO classifier obtained in cross-validation on the training set and in validation on the test set.*

TD-DART-HRMS dataset	Sensitivity	Specificity	Accuracy
Training set	100%	100%	100%
Test set	100%	100%	100%

Conclusions

This study demonstrated that TD-DART-HRMS, coupled with LASSO method, can be a powerful analytical strategy for oregano authentication.
We confirmed that this approach can identify the existent variability between authentic and adulterated oregano by rapidity and accuracy.

References

1. A. Maquet, A. Lievens, V. Paracchini, G. Kaklamanos, M.B. De La Calle Guntinas, L. Garlant, et al. 2021. Contract No.: EUR 30877 EN.
2. A. Massaro, A. Negro, M. Bragolusi, B. Miano, A. Tata, M. Suman, R. Piro, Food Control , 126 (2021), 108058.

P50

Valorization of Rice *(Oryza sativa L.)* Husk as a source of *in vitro* antiglycative agents

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Summary: *Our research investigates rice husk as a source of antiglycative compounds. Microwave-assisted extractions* were *performed and optimized to recover polyphenols using statistical models. The richest extract obtained (RHE) was* characterized *and its antiglycative effect was evaluated using in vitro models. Results shows RHE as a promising ingredient with antiglycative capacities.*

Keywords: *rice husk valorization, antiglycative activity, HPLC-MS*

Introduction

Currently, the minimization and reutilization of agro-food wastes are extremely relevant since they represent a good source of bioactive compounds with a potential positive impact on health [1]. For a sustainable development, the European Commission itself defined long-term strategies to give a second life to wastes and support a circular economy strategy [2]. We focused our attention on the valorization of rice husk (Oryza sativa L.), an agro-food by-product of the milling process, promising as source of chemically different bioactives [3,4,5]. Several epidemiological studies have shown the correlation between advanced glycation end products (AG Es) and the development of different chronical disorders [6]. Due to the current increasing interest in the antiglycative agents from natural origin [7], the object of our work was to evaluate the antiglycative capacity of rice husk extract obtained with innovative microwave assisted extraction coupled with hydroalcoholic mixture.

Experimental

A microwave-assisted extraction (MAE) by using hydro-alcoholic mixtures was set-up to extract polyphenols from rice husk. Extraction parameters (temperature, time, solid to solvent ration and percentage of ethanol in extraction mixtures) were optimized according to design of experiments approaches. Afterwards, the extracts were filtered through a filter paper and lyophilized to obtain a dry extract, after removing the organic solvent.
The extract with the highest recovery of phenolic compounds (RHE) was selected for further studies.
Phytochemicals in RHE were identify using a Thermo Finnigan Surveyor Plus HPLC apparatus equipped with a quaternary pump, a Surveyor UV-Vis photodiode-array detector, a Surveyor Plus autosampler and a vacuum degasser connected to a LCQ Advantage Max ion trap spectrometer.

Then, different *in vitro* BSA-based systems were set-up to evaluate the antiglycative capacity of RHE at different stages of the glycation reaction.
The inhibition of Amadori products and advanced glycation end products (AGEs) formation was evaluated by NBT assay and BSA-methylglyoxal (MGO), BSA-glucose (GLU) system, respectively [8,9]. The trapping capacity of the extract against MGO, well known AGE precursor, was evaluated using the RP-HPLC-DAD method described by Mesias et al. (2013), with slight modifications. Moreover, DPPH and ABTS assays were performed to determine RHE antioxidant ability [10].

Results

In this study, the identification of the optimal MAE extraction conditions was the results of two steps of work: a screening phase where a Full Factoria Design 2^4 was used to identify the significant factors that affect the quality process, and an optimization phase where a Box-Behnken design (BBD) was employed to endorse the setting of the process and find out the optimal conditions, then validated verifying that the experimental phenolic compounds yield was in accordance with the predicted one. The extract with the highest polyphenolic recovery was obtained at 90°C, 1:35 g/mL solid to solvent ration, 80% of ethanol and 5 min of extraction.
For the identification of the phytochemicals in RHE ion trap mass spectrometry was used. More than 1 0 compounds were tentatively identified: they belong to different chemical classes, mainly hydroxycinnamic acids, flavonoids and ferulic acids derivatives.
Results concerning the antiglycative activity indicated that RHE was able to inhibit 70-90% of AGEs generated in the used *in vitro* systems at different stages of the glycation reaction, in a dose-dependent matter. In addition, a good capacity to directly trap MGO was registered. In parallel, RHE showed a good antioxidant activity, as demonstrate by scavenger capacity against ABTS cation and DPPH stable radicals.

Conclusions

MAE showed to be an effective method for the isolation of rice husk polypbenols. The thus obtained RHE was active as anti-glycative agent in the incubation system tested, consisting of a protein and a sugar or MGO; moreover, it could directly trap MGO, avoiding AGEs formation.
The research is going on by performing extract bioaccessibility and bioavailability studies and stability investigation to obtain a rice-husk based ingredient for food supplement.

References

1. K. Kumar, A. N. Yadav, V. Kumar, P. Vyas, H. Singh Dhaliwal; *Bioresources and Bioprocessing,* 4 (2017), 18
2. Grean Deal Europeo 2015, Available on line at: https://ec.europa.eu/info/strategy/priorities-2019-2024/europeangreen-deal_it
3. A. B. Das, V. V. Goud; *Food Measure,* 12 (2018), 332-345.
4. P. Jha, A. J. Das, S.C. J. Deka; *Food Science and Technology,* 54 (2017),

3847-3858.
5. S. Butstat, S. Siriamornpun; *Food Chemistry,* 119 (2010), 603-613.
6. Q. Wei, T. Liu, D.W. Sun; *Trends in Food Science & Technology,* 82 (2018), 32-45.
7. S. Velinchkova, K. Foubert, L. Pieters; *Planta Medica,* 87 (2021), 780-802.
8. M. Mesias, M. Navarro, V. Gokmen, F.J. Morales; *Journal of Food and Agriculture,* 93 (2013), 2037-2044.
9. M. Maietta, R. Colombo, R. Lavecchia, M. Sorrenti, A. Zuorro, A. Papetti; *Food Research International,* 100 (2017), 780-790.
10. S. Dudonne, X. Yitrac, P. Coutiere, M. Woillez, J.M. Merillon; *Journal of Food and Agriculture,* 57 (2009), 1768-1774.

P51

Profiling by HPLC-DAD-MS of maior and minor ellagitannins from chestnut bark (*Castanea sativa* Mill) aqueous extracts.

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Summary. *The work aims to investigate the composition of chestnut bark water extracts obtained by autoclave at 90°C. A total of 52 compounds, including ellagitannins and gallotannins, were identified by HPLC-DAD-MS. The total tannin content ranged 126.5-349.0 mg/g on dry extracts in cortex alone and inner part chips, respectively*

Keywords: *HPLC-DAD-MS, ellagitannins, gallotannins.*

Introduction

Sweet chestnut (*Castanea sativa* Mill) belongs to the family Fagaceae, and it is one of the most common chestnut species in European countries, mainly in Italy. Chestnut bark extracts are rich in hydrolyzable tannins, which include gallotannins and ellagitannins. The main ellagitannins found in chestnut bark are castalin, vescalin, castalagin and vescalagin [1, 2]. Traditionally, chestnut bark is used in tanning and textile industries, but also to clarify wine and stabilize its organoleptic properties. The chestnut extract and/or its fractions alone or in combination with other polyphenols was reported as antioxidant, antibacterial, and for lowering nitrosamines and mycotoxins in raw food materials and food products for both people and animals [3]. Recently the potential use of cortex extracts as component of dietary supplements was authorized [4]. Despite the widespread use of chestnut bark extracts in industry, at the best of our knowledge, there is very little data on the characterization of minor polyphenols. The aim of this research was to characterize the tannin content in different parts of chestnut trees (basal chips, cortex, chips without cortex, and whole chips), maintained in suitable environmental conditions for a shorter or longer time for seasoning.

Experimental

Samples were collected over the years 2021-2022: i) whole fresh chips, ii) whole chips 2 years seasoning, iii) inner part 2 years seasoning, iv) cortex 2 years seasoning. The samples were produced by the Italian factory Pizzicannella S.r.L. (Latina (Italy)), by applying water/chestnut chips ratios 23kg/112L, at 90° C in an autoclave (Panini S.r.L.); extraction time 90 min. The extracts were analyzed by an HP 1260L liquid chromatography with a DAD detector (Agilent Technologies, Palo Alto, CA, USA); the column was a C18 Luna 250×4.6 mm, 5µm from Phenomenex; the eluent was a binary mixture of acidic water (pH 3.2 by HCOOH) as the solvent A and CH_3CN as the solvent B. The MS experiments were performed by the same chromatographic system and an HP 1260 MSD

(G6125B) mass spectrometer. The analyses were in negative ionization mode. Ellagitannins were determined using a calibration curve of gallic acid (280 nm) as external standard.

Results

A total of 52 compounds, including ellagitannins and gallotannins, were identified by combining data of MS fragmentation, UV-Vis spectra, retention time and earlier MS literature. We detected ellagitannins such as roburin, vescalin, castalin, vescalagin and castalagin, and gallotannins such as mono-, di- and trigalloyl-β-D-glucose, trigalloyl-HHDP-glucose, and di-HHDP-galloyl-glucose (Table 1). The identific-ation of these minor polyphenols in the chestnut wood extracts was possible thanks to the co-presence in several cases of three very diagnostic ions: $[M-H]^-$, $[2M-H]^-$ e $[M-2H]^{2-}$. Some further diagnostic fragments of the MS spectra allowed us to tentatively identify the compounds reported in Table 1.

Table 1: *Phenolic compounds of* chestnut bark *in aqueous extract tentatively identified by MS spectra in negative ion mode; in bracket the number of the isobaric compounds.*

Compound	**$[M-H]^-$**	**$[2M-H]^-$**	**$[M-2H]^{2-}$**	**ions**	**Ref**
Vescalin (2)	631	1263		481, 331, 301	[2]
Monogalloyl-glucose (2)	331	663		313, 271, 169	[1]
Castalin and isobars (2)	631	1263		481, 331, 301	[2]
HHDP-glucose (3)	481			331,169	
Galloyl-HHDP-DHHDP-hexoside (2)	951	-		783, 633,613, 481, 301,483	
Roburin E (4)	1065[a]		532	924, 915, 301	[1,2]
Gallic acid	169			125	
Roburin A/D (2)	1849		924	924, 915, 331, 169	[2]
Vescalagin	933	1867	466	915, 615, 301	
Vescavaloninic acid (2)	1101		550	550, 528	[1,2]
Digalloyl glucose (3)	483		967	331,169	
Castalagin	933	1867	466	301	[1,2]
β-1-*O*-ethylvescalagin (2)	961		480	633, 331, 301, 169	
1-*O*-galloyl-castalagin isomer	1085			542,483, 301	[1]
Valoneic acid	505			483, 301	
Tellimagrandin (3)	785		483	301, 169	[1,2]
Trigalloyl glucose (8)	635			277,453,169	[1,2]
Di-HHDP-galloyl-glucose (casuarictin/potentillin) (2)	935		527	633,301	[1]
Tetragalloylglucose (5)	787		317	317,393	
Trigalloyl-HHDP-glucose	937			633, 467,169	[1]
Pentagalloyl glucose	939			469, 169	[2]
Ellagic acid	301				
Ellagic acid-deoxyhexose	447	301			

The maximum % yields of dry extract was very different ranging from 2.8 % for WCF to 5.6 % for $IP2_y$. The total tannins were mainly located in the inner part and not in the cortex (348.8 mg/g on DE). The total tannins in the dried aqueous extracts of the different samples (with exception of cortex) were in similar amounts (Table 2).

Table 2. *Yields expressed as % dry extracts on dried chips and tannin content in dry extracts; the data are a mean of triplicate; dry matter (DMs), dry extracts (DE).*

Sample	Description	% Yields	Tot tannins mg/g DMs	Tot tannins mg/g DE
WFC	Whole fresh chips	2.8 ± 0.01	8.86 ± 1.8	320.2 ± 61
$WC1.5_y$	Whole chips1.5 years	3,5±0,18	9,47±0,24	268,8±11,1
$WC2_y$	Whole chips 2 years	4.9 ± 0.16	15.67 ± 0.8	318.9 ± 4.1
$IP2_y$	Inner part 2 years	5.6 ± 0.60	19.7 ± 1.2	348.8 ± 15.6
$C2_y$	Cortex 2 years	2.9 ± 0.2	3.6 ± 0.3	126.5 ± 2.1

Conclusions

A total of 52 compounds, including ellagitannins and gallotannins, were identified by HPLC-DAD-MS. The highest tannin content was in Whole chips, with a 18.1 % on DMs, and 35.5% on DE. And it is very important to define the phytochemical profile of chestnut bark to study their possible application in the food sector.

References

1. Comandini P, Lerma-García, M. J, Simó-Alfonso, E. F; *Food Chemistry* 157 (2014): 290-295.
2. Sanz M, et al; Journal *of Agricultural and Food Chemistry* 58.17 (2010): 9631-9640.
3. Bargiacchi E, Bellotti P, Pinelli P., Miele, S., Romani, A; *U.S. Patent Application* No. 14, (2015). 615,615.
4. Ufficiale, G. Gazzetta Ufficiale. Allegato 1, Serie Generale n.224 del 26-09-2018

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Tannins in chestnut by-products: characterization and biological activity in intestinal epithelial cells

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Summary: *Polyphenolic profile of Castanea sativa L. pruning by-product (bud and wood) was carried out through LC/MS-MS. The potential antioxidant and anti-inflammatory activity of chestnut by-product extracts in intestinal epithelial cells was evaluated.*

Keywords: *chestnut; by-products; tannins*

Introduction

Castanea sativa L. tree plays a significant role in the economic and environmental context of mountain areas. The fruit can be used as a source of dietary energy due to their starch, carbohydrates, and low-fat content. Chestnut processing generates a large amount of by-products, namely leaves, wood, shells, and spiny burs. Among these by-product, woody parts obtained by pruning are an interesting source of polyphenolic compounds, mostly phenolic acids and tannins. Indeed, chestnut wood barrels are nowadays commercialised in the field of wine brandies' aging due to its remarkable number of hydrolysable tannins [1]. Ellagitannins, one of the main classes of hydrolysable tannins, exerts various biological activities such as antioxidant, anti-diabetic, cardioprotective, antifungal, antimicrobial and anti-inflammatory [2]. Among inflammatory processes, several studied explored the role of tannins in both in vivo and in vitro models of intestinal disease (IBD, Chron disease) [3] [4]. A sustainable use of pruning by-product could be the extraction of its bioactive molecules, which may be applied as functional ingredients in several industrial sectors. The main purpose of this study involves a metabolomic characterization of chestnut woody by-product extracts, along with the exploitation of its anti-inflammatory properties.

Experimental

Quiescent buds and wood (fresh and dried) were manually ground under liquid nitrogen and hydroalcoholic extractions were conducted. All extracts were sonicated, centrifuged, filtered, and evaporated to dryness to be redissolved in DMSO. DMSO extracts were diluted in MeOH prior to LC-MS/MS detection.
Polyphenolic profile of chestnut by-product extract was acquired by an analytical approach based on HPLC coupled with LTQ ion trap. HPLC was performed through a Surveyor MS PUMP PLUS System (Thermo Fisher Scientific) The MS/MS analysis was carried out with a LTQ ion trap mass spectrometer (Thermo Fisher Scientific) coupled with an ESI source operating in negative mode. The analytes were separated on a Waters XSelect HSS T3 XP (100 Å, 2.5 µm, 2.1 ×

100 mm) column with a mobile phase composed of 0.1% formic acid in water (A) and acetonitrile (B) at a rate flow of 0.150 mL/min. LC-MS/MS quantification of single molecules in wood and bud extracts was performed through an Exion LCTM AC System (AB Sciex) and a Triple QuadTM 3500 system (AB Sciex) in ESI negative ionization. The analytes were separated on a Phenomenex Synergi Hydro-RP (80 Å, 4 µm, 150 × 4.6 mm) column with a mobile phase composed of 0.1% formic acid in water (A) and methanol (B) at a rate flow of 0.800 mL/min.
Total polyphenol index of extracts was assessed by Folin-Ciocalteu assay.
Anti-inflammatory activity of the extracts was assayed in an in vitro model of undifferentiated human intestinal cells (Caco-2) stimulated with IL-1β-IFNγ.
Antioxidant activity of extracts was assessed by DPPH and ORAC assays.
Cytotoxicity of extracts in Caco-2 cells was assessed by MTT and Neutral Red assays.

Results

The HPLC/LTQ ion trap/MS/MS- analysis of hydroalcoholic extracts of *C. sativa* wood and quiescent bud allowed to identify more than thirty compounds belonging to several classes of metabolites, mostly tannins both hydrolysable (galloyl glucose derivatives and ellagitannins) and condensed (proantho-cyanidins), with a minor number of other molecules, such as flavonoids, ellagic acid and phenolic derivatives.
Vescalagin and castalagin amount in the extracts was therefore quantified by an HPLC/ TripleQuad TM/MS/MS-.
Total polyphenol index in bud, fresh and dry wood hydroalcoholic extracts was about 428.3 ± 3.3, 391.2 ± 1.2 and 365.6 ± 6.5 mg gallic acid equivalent/g (mean ± s.e.), respectively.
Neutral Red and MTT assays did not report any cytotoxic effect of *C. sativa* by-product extracts in Caco-2 cells. *C. sativa* wood and bud extracts inhibited the NF-kB driven transcription and the release of CXCL-10, both induced by IL-1β-IFNγ, in a concentration-dependent fashion.
Antioxidant activity in bud, fresh and dry wood hydroalcoholic extracts was about 51 ± 1.8, 46 ± 1.7 and 47.1 ± 1 mM Trolox equivalent/ g (mean ± s.e.), respectively.

Conclusions

This work represents an interesting preliminary contribution to the exploitation of the polyphenolic profile of *Castanea sativa* by-product obtained by pruning practices and promotes a sustainable reuse in several fields as a source of bioactive compounds. Further analysis will be carried out to clarify the biological activity of these by-product in vitro models of human intestinal cells.

References

1. M. Aliaño-González, J. Gabaston, V. Ortiz-Somovilla, E. Cantos-Villar; *Biomolecules*, 12.2 (2022), 238.
2. M. Fraga-Corral, P. Otero, J. Echave, P. Garcia-Oliveira, M. Carpena, A. Jarboui, B. Nuñez-Estevez, J. Simal-Gandara, M.A. Prieto; *Foods*, 10.1 (2021), 137.
3. S. Hollebeeck, J. Winand, M.-F. Hérent, A During, J. Leclercq, Y.

Larondelle, Y.-J. Schneider; *Food & function,* 3.8 (2012), 875-885.
4. M. Larrosa, A. González-Sarrías, M. J. Yáñez-Gascón, M. V. Selma, M. Azorín-Ortuño, S. Toti, F. Tomás-Barberán, P. Dolara, J. C. Espín; *The Journal of nutritional biochemistry,* 21.8 (2010), 717-725.

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Capturing the human gut volatilome: high throughput VOCs detection during colonic in-vitro fermentation

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Summary: *The human gut encloses a large community of bacteria producing a wide range of volatile organic compounds (VOCs) when fermenting undigestible substrates. This study aims to provide a high throughput method to unveil the gut volatilome when different fecal donors process undigestible dietary ingredients (e.g., complex carbohydrates, polyphenols, proteins)*

Keywords: *Gut Microbiota, VOCs, Fermentation*

Current human gut metabolomic research focuses on microbial non-volatile metabolites (conventional metabolome), leaving VOCs as a missing component [1]. Recent research has focused on the volatile component of gut microbial metabolites [2]. This research effort intends to give more in-depth information on how gut microorganisms metabolize food components during fermentation by merging cutting-edge analytical approaches with in-vitro gut simulators. The large intestine ferments chemicals not absorbed by the host's endogenous machinery, creating an odorous gas mixture known as "volatilome" [3]. Microbes generate and consume volatile organic and inorganic compounds for metabolic, signalling, and ecosystem-to-global interactions [2]. Certain classes of VOCs are tough to be correlated with the gut homeostasis, even if there is still limited evidence on their potential as non-invasive biomarkers in gastrointestinal health due to the big interpersonal variability of the gut ecosystem [4]. Colonic batch fermentations are used to evaluate the impact of dietary components on gut microbiota in a short period of time (24-48 h) [5]. These models are useful for initial screening of various food components and faecal donors compared to dynamic fermentation systems, which need long incubation (weeks), considerable sampling over time, and restrictions in assessing multiple donors. The sample capacity of current approaches to track VOCs during batch fermentation is limited. Sampling and analysis are normally done manually at discrete time periods, and only a specific amount of liquid can be extracted to not alter the fermentation outcome. This impacts the ability to define VOCs during microbial growth. A quick method to screen the volatilome produced by different faecal donors would assist obtain insight on the metabolism that characterizes the gut ecology of an individual, especially when exposed to complex substrates comprising polysaccharides, phenolics and/or proteins. This experiment will examine if healthy donors produce different volatile organic compounds (VOCs) when digesting the same substrates. Due to their complementary analytical capacities, two techniques are presented for efficient separation and identification of analytes and rapid quantitative analysis without sample preparation. The first detection approach in this investigation is head-

space solid phase micro extraction coupled with gas chromatography and mass spectrometry (HS-SPME-GC-MS). The technology can capture the VOCs profile throughout fermentation without touching the liquid phase and is fully automated. SPME is a non-exhaustive extraction method, using a small amount of extraction phase to get a high analyte concentration [7]. This approach does not impact analyte concentration in the sample, unlike exhaustive extraction, which depletes analyte concentration in the environment [7] and can affects bacterial metabolism. Also, SPME sampling uses free concentrations rather than total concentrations, which is a superior estimate of bioavailability [8]. By measuring free concentrations using SPME, biological system dynamics may be monitored [8]. The fermentation is performed in eutrophic medium, in presence of non-digestible dietary ingredients and of faecal inoculum. To shorten desorption time and increase screening throughput, in-vitro batch fermentation is performed directly in glass vials with magnetic screw tops and silicone/PTFE septa. Autoclaved vials are filled with sterile media and flushed with nitrogen before inoculation. Incubation with faecal inoculum is performed at 37 °C and vials are exposed to a multi-polar fiber composed of 50/30 μm Divinylbenzene/ Carboxen/Polydimethylsiloxan (DVB–CAR–PDMS) (Bellefonte, PA, USA) every 4 hours over the entire incubation time (24h) in a completely automated manner by an autosampler. In our preliminary results, the arrangement was able to concentrate microbiota-related VOCs and track their relative abundance over time. Microbial end-products of interest [3,4], such as indole, butanoic acid, and propionic acid, were captured by the multi-polar DVB–CAR–PDMS fiber and their peaks were tracked over time by matching the acquired mass spectrum with spectral libraries. Proton Transfer Reaction Time of Flight Mass Spectrometry (PTR-ToF-MS) is also used in this study, because of its superior sensitivity and the feasibility of continuous, real-time monitoring without VOCs pre concentration and chromatographic separation. PTR-MS is well adapted to the investigation of live organisms [9,10]. The goal is to link GC-MS profiling with PTR-ToF-MS untargeted detection and track a subset of masses of interest to reveal concentration change during gut fermentation. By combining these two complementary techniques, various research questions will be addressed. Healthy donors create unique VOCs from similar substrates? Do microbial VOC emission patterns convey biological information, such as microbe identification and differentiation? Are gut-associated VOCs always emitted or do their amounts vary throughout fermentation? Our findings may assist create a routine examination of the volatile fraction of the gut microbiota metabolome. The temporal information collected by PTR-ToF-MS can be used to observe and analyse the dynamics of bacterial foraging on complex undigestible food substrates to gain new mechanistic insights on the gut bacterial ecosystem.

References

1. Laura K Meredith and Malak M Tfaily. Trends in microbiology (2022).
2. Laure Weisskopf, Stefan Schulz, and Paolina Garbeva.. Nature Reviews Microbiology 19.6 (2021) 391–404.
3. Nidhi M Sagar et al. Gastroenterology research and practice 2015 (2015).
4. Jin-Hyung Lee, Thomas K Wood, and Jintae Lee. Trends in microbiology 23.11 (2015), 707–718.

5. Pérez-Burillo, S., Molino, S., Navajas-Porras, B., Valverde-Moya, Á. J., Hinojosa-Nogueira, D., López-Maldonado, A., ... Rufián-Henares, J. Á. (2021). Nature Protocols, 16(7), 3186-3209.
6. Tomasz Majchrzak et al. Analytica chimica acta 1035 (2018) 1–13.
7. Nathaly Reyes-Garces et al. Analytical chemistry 90.1 (2017) 302–360.
8. Yu, M., Roszkowska, A., Pawliszyn, J. (2021). ACS Environmental Au, 2(1), 30-41.
9. Franco Biasioli et al. TrAC Trends in Analytical Chemistry 30.7 (2011), 968– 977.
10. Michael Bunge et al. Applied and environmental microbiology 74.7 (2008), 2179–2186.

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HPLC-HRMS untargeted metabolomic characterization of Honeys produced in the Venice Lagoon

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Summary: *Untargeted metabolomic analysis of 4 honey samples of different floral origin, collected in the Venice lagoon area was performed, to study the relation between chemical composition and botanical origin. The analytical methodology was optimized, and floral biomarkers of each honey typologies were identified using HPLC-ESI-LTQ-ORBITRAP technology.*

Keywords: *honey, untargeted metabolomic, HPLC-HRMS*

Introduction

Honey is a food that honeybees produce starting from the nectar of blossoms. The main components of honey are sugars, particularly glucose (38%) and fructose (31%), and water (17%). The minor components of honey, as amino acids, organic acids and polyphenols, despite being present in low amount, are very important to define the quality and the organoleptic properties of honey [1]. Moreover, since the nectar composition directly influences honey features, each honey presents different physicochemical, organoleptic, and nutraceutical properties depending by its floral origin [2]. In this context, the development of analytical methods for the assessment of honey quality, in relation to its nutraceutical properties, is an emerging field, due to the increasing awareness of consumers. The variability of floral sources, depending by seasons and geographical and climatic conditions, determine a high differentiation in the composition of honey, which need to be investigated to valorize the peculiarities of each product. The metabolomic approach, being able to qualitatively analyze a high number of known and unknown compounds, demonstrated to be advantageous in the characterization of complex matrices, and especially in the nutraceutical field [3].

In this study, HPLC-HRMS technology has been applied for the untargeted characterization of 4 kinds of honeys of different floral origins (salt marsh, spring multifloral, autumn multifloral and acacia). These honeys are produced in the S. Erasmo island by "Miele del doge", in the northern lagoon of Venice. Sample treatment procedure has been developed in order to:

-remove sugars, which prevent the determination of minor honey components;
-assure the extraction of flavonoids, considered as target bioactive compounds of honey, evaluating the recovery of four compounds (apigenin, kaempferol, quercetin, naringenin).

The analysis was conducted by a metabolomic approach and permitted to identify marker compounds for each honey typology and to find a relation between honey composition and botanical origin of honey.

Experimental

The sample treatment procedure was optimized by selecting the most appropriate stationary phase for solid phase extraction and removal of matrix effect. Tests were conducted on commercial glucose-fructose syrup to which known amount of four flavonoids were added. Three kinds of phases were tested: Styrene-divinylbenzene (Strata X, 6 mL, 33 µm Phenomenex, California USA), N-vinylpyrrolidone-divinylbenzene (Oasis HLB, 6 mL, 30 µm) and Sep-Pak C18 (3 mL, 55-105 µm) both of Waters Corporation, Massachusetts USA. Moreover, different sample amounts (300 and 400 mg) and different elution volumes (3 and 5 mL) were tested to select the method with the highest recovery for the target flavonoids. The final preanalytical protocol included the solubilization of 400 mg of honey in 1.5 mL of ultrapure water acidified with 0.1 of formic acid (AF). The solution was homogenized for 30 minutes in ultrasonic bath and then centrifugated; the supernatant was extracted by C18-SPE, washed with acidified water and eluted with 5mL of MeOH. The samples were then concentrated in thermostated bath until the final volume of 500 µL. The instrumental analysis has been conducted using an UltiMate 3000 (Dionex) coupled to an ESI-LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA), as described by Scalabrin et al., 2015. The column employed was a C_{18} SB-Aq Narrow Bore RR 2.1 x 150 mm, 3.5 µm (Agilent Technologies, Wilmington, USA). The column was eluted by AF 0.01% and ACN acidified with AF 0.01%. The ion source was operated in both negative and positive polarity at 300°C. The MS analysis were performed in full scan mode, in a mass range of 90-1500 Da, at a high mass accuracy (<5 ppm). The resolving power was 60000. To trace a complete profile of fragmentation of the analyzed ions, data dependent analysis was carried out. The identification of compounds was based on the most probable molecular formula and by comparison of fragmentation spectra with literature and available online libraries. The identification level assigned was in accordance with the protocol of Sumner et al., 2007 [5].

Results

The method was selected based on the best performance obtained by glucose-fructose syrup, spiked with flavonoid standards. StrataX SPE were excluded because the Felhing assay highlighted the presence of sugars, therefore they were not effective in removing sample matrix. C18-SPE proved to be efficient in the extraction of kaempferol, which was not retained by HLB-SPE. The results highlighted that the best method was represented by C18 extraction, combined with 400 mg of samples weighted and the elution volume of 5 mL. The selected method permitted the analysis of flavonoids in honey samples. Particularly, naringenin, quercetin and rutin showed the highest intensities in “salt marsh” honey while apigenin demonstrated to be particularly high in acacia honey.

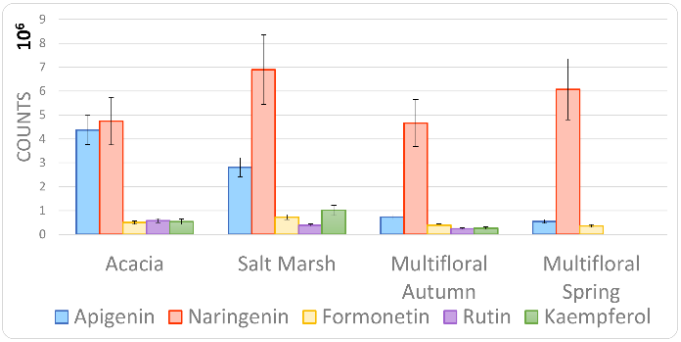

***Figure 1**. Intensities of flavonoids*

In addition, the method permitted the analysis of other 80 compounds of nutraceutical interest, related to the botanical origin of honey, especially: flavonoids, organic acids, phenolic acids, terpenes, iridoids. Among all the compounds, some biomarkers for each honey typology were identified. Acacia honey presented high levels of abscisic acid and of oleandrose, a sugar related to *Nerium oleander*; this plant, start flowering together with *Robinia pseudoacacia*, in May, continuing until September.

The spring multifloral honey was characterized by dihydroconiferin attributable to *Taraxacum officinale*, and isosuspensolide, an iridoid probably related to the flowering of *Viburnum.* The autumn multifloral showed high levels of the iridoid genipin gentibioside, found in the plants of Gardenia *jasminoides*. The salt marsh honey showed the presence of compounds related to the particular vegetation of this area (Fig. 2): 7,2'-Dihydroxy-6-methoxyisoflavone, which is found in *Salicornia europaea*, and the flavone chrysin, which is present in many plants and could be related to *Artemisia campestris*.

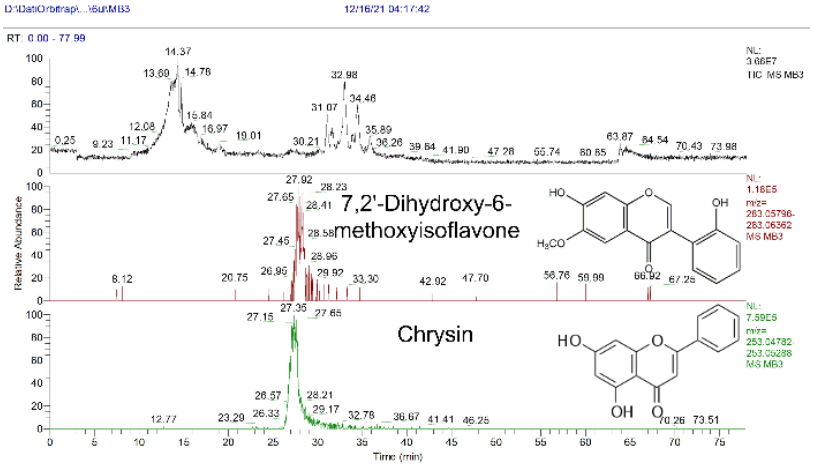

***Figure 2**. Chromatograms of the two compounds characterizing salt marsh honey*

Chrysin is known also to characterize the acacia honey, indeed its presence in this kind of honey was higher than in multiflorals. Luteolin glucosides are present in all the typologies of honey in similar levels and are probably related to the plants of *Cynara scolymus*, which are typical cultivations of this island.

References

1. Schievano E, Morelato E, Facchin C, Mammi S (2013) *J Agric Food Chem* 61:1747–1755.
2. Di Marco G, Manfredini A, Leonardi D, Canuti L, Impei S, Gismondi A, Canini A (2017) *Plant Biosyst* 151:450–463.
3. Montoro P, D'urso G, Kowalczyk A, Tuberoso CIG (2021) *Molecules* 26:1–14.
4. Scalabrin E, Radaelli M, Rizzato G, Bogani P, Buiatti M, Gambaro A, Capodaglio G (2015) *Anal Bioanal Chem* 407:6357–68.
5. Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin C a., Fan TW-M, Fiehn O, Goodacre R, Griffin JL, Hankemeier T, Hardy N, Harnly J, Higashi R, Kopka J, Lane AN, Lindon JC, Marriott P, Nicholls AW, Reily MD, Thaden JJ, Viant MR (2007) *Metabolomics* 3:211–221.

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Preliminary data of Moroccan honey traceability

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Summary: *Moroccan honeys of different botanical and geographical origins were analyzed in this study. The objective was to use chemical data together with chemometric instruments to verify the botanical/geographical origin of honey. The results showed that it is possible to discriminate honey based on its botanical origin using PCA.*

Keywords: *Moroccan honeys; traceability; chemometric analysis.*

Introduction

Honey has always been a natural and healthy food and the standards that guarantee its quality allow consumers to utilize it safely. Morocco is a valuable territory for honey production due to its honey varietal sources [1]. Currently, the studies about the food traceability are oriented at defining their botanical and geographical origins in relationship to physicochemical analyses of foods.
To achieve this goal quali/quantitative methods are developed and coupled with chemometric analysis [2,3]. Aim of the present work was applied these tools with samples belonging to four honey types (*Ziziphus lotus*, *Citrus sinensis*, *Euphorbia resinifera* and *Globularia Alypum*) from three different provinces (Khénifra, Béni-Mellal and Azilal) in Beni Mellal-Khènifra region of Marocco, and to carry out a correlation between data results of honey and their botanical and/or geographical origin with the purpose to use these correlations for traceability.

Experimental

The analyses were carried out on 12 honey samples obtained from beekeepers in 2021 including *Ziziphus lotus* (ZL1, ZL2 and ZL3) from Khénifra, *Citrus sinensis* (CS1, CS2 and CS3) and *Globularia Alypum* (GA1, GA2 and GA3) from Béni-Mellal, and *Euphorbia resinifera* (ER1, ER2 and ER3) from Azilal. The honeys (about 125 g in weight) were collected in glass jar and were stored in the dark at 4 °C until analysis. Physicochemical parameters (pH, free acidity, combined acidity and total acidity, moisture, brix grade, and electrical conductivity), pesticide, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), phthalate esters (PAEs), non-phthalate plasticizers (NPPs), bisphenols (BPs) and mineral elements were evaluated. Physicochemical analyses were carried out according to the methods reported in the Official Gazette of the Italian Republic (2003/185, August 11, 2003). Residue levels of 83 pesticides, 18 PCBs, 13 PAHs, 10 PAEs and 8 NPPs were determined by gas chromatography coupled mass spectrometry (GG-MS);

residue levels of 9 BPs were determined by liquid chromatography and tandem mass spectrometry (HPLC-MS/MS); 16 mineral elements were determined by inductively coupled plasma mass spectrometry (ICP-MS).
Statistical methods have been conducted on starting multivariate matrix where the cases (12) were the analyzed honey samples, and the variables (54) were the concentrations of detected analytes. The data set was subdivided into four groups according to the botanical origin. Initially the non-parametric Kruskal Wallis test was applied on data loge-transformed to highlight the differences among four groups. Then, the data set was normalized, and lastly a Factor Analysis with Principal Components extraction was performed.

Results

The values of physicochemical parameters investigated were within the limits set by Legislative Decree 2004/179 of May 21, 2004. No samples were found to be free of pesticides residues. According to the pesticide current legislation (Regulation (EC) No. 396/2005 and subsequent amendments), 75% of the samples exceeded the MRLs for carbaryl and cyromazine; 50% of the samples exceeded the MRLs for acephate, dimethoate and diazinon; and 25% of the samples exceeded the MRLs for alachlor, carbofuran and fenthion sulfoxide. Quinalphos, methalaxyl-M and fenthion sulfone residues, additionally, were determined but within their limits.
Only samples of *Ziziphus lotus* and *Citrus sinensis* contained residues of PAH (fluorene and chrysene in all samples; acenaphthylene, phenanthrene, and anthracene only in *Ziziphus lotus* samples; and benzo[a]anthracene only in *Citrus sinensis* samples). Furthermore, also two PCB residues were determined: PCB118 in *Ziziphus lotus* samples and PCB180 in all other samples. The Regulation (EC) No 1881/2006 does not set limits for PCBs and PAHs in honey. Five PAEs (DEHP, DEP, DPrP, DiBP, and DBP) and five NPPs (DEHT, DEA, DiBA, DBA, and DEHA) plasticizers were determined at concentration higher than their LOQ in all analyzed samples. Furthermore, also three BP residues were determined: BPA only in *Citrus sinensis* and *Euphorbia resinifera* samples; BPB in all samples except in *Globularia Alypum;* and BPAF in all samples.
About element analysis, only Cd was lower than its LOQ in all analyzed samples. The most abundant elements decreased in the following order: K, Ca, Na, Mg and Fe for *Ziziphus lotus* and *Globularia Alypum;* K, Ca, Mg, Na, and Fe for *Citrus sinensis* and *Euphorbia resinifera.* Only for Pb exists a limit value in honey of 0.1 mg/kg, as established by EU Reg. 2015/1005. In all samples of *Citrus sinensis*, *Euphorbia resinifera* and *Globularia Alypum*, Pb was higher than established limit. The results of Kruskal-Walli's test (with significant p-level below 0.05) showed that all variables were significantly different among four groups, except pH, DEHT, DEHA, BPAF and Pb. In the Principal Component Analysis (PCA), four principal components with eigenvalues exceeding one (325.627, 18.195, 7.575 and 1.257) were extracted, according to Kaiser Criterion. The extracted components explained up to 97.507% of total variance (47.457%, 33.695%, 14.029% and 2.327%, respectively). In the scores plot for the first two principal components, retained 81.152% of the variability of the system, four clusters are clearly distinguished according to botanical origin of honey samples. Some degree of separation was also achieved by geographical origin.

Conclusions

The discrimination among honey types was achieved by Principal Component Analysis (PCA), adding knowledge to the determination studies of the honey botanical/geographical origin.

In relation to the analysis performed and the results obtained, the study is certainly very promising. This was a preliminary study; in the future a complete characterization analysis of these honeys will be carried out in order to ensure complete traceability.

References

1. Sabbahi, R.; *International Journal of Tropical Insect Science*, 2022, 42(2), 1275-1284.
2. Fanelli, V., Mascio, I., Miazzi, M. M., Savoia, M. A., De Giovanni, C., Montemurro, C.; *Foods*, 2021, 10(07), 1644.
3. Liu, S., Lang, D., Meng, G., Hu, J., Tang, M., Zhou, X.; *Food Chemistry*, 2022, 371, 131066.

P56

Occurrence of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in Italian honey

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Summary: *PFASs are hazardous and persistent environmental contaminants. An internally developed method for the determination of 40 PFASs by LC-Q-Orbitrap was applied to the analysis of 50 Italian honey samples. The concentrations found were always below 40 ng kg^{-1}; PFPeA and PFHxA were the most frequently detected compounds.*

Keywords: *PFASs, honey, LC-Q-Orbitrap*

Introduction

Per- and polyfluoroalkylated substances (PFASs) are a class of synthetic fluorinated compounds with unique physicochemical properties (e.g. heat stability and water/grease repellence) useful in the production of textiles, food packaging, electronics, firefighting foams and household products [1]. The use and abuse of PFASs in recent decades has led to their accumulation in the environment with severe consequences for human health [2]. Diet exposure to PFASs was detailed by an EFSA Scientific Report in 2012 [3] including 27 perfluoroalkylated substances analyzed in various food; four of them (PFHxA, PFOA, PFDA and PFOS) were found in honey. In addition, it is well known that bees may be exposed to PFAS through contaminated soils, waters, plants and nectar and, therefore, honey is not only a valued food, but it can also serve as an indicator of environmental pollution. Nevertheless, at present, few data are available about the presence of PFAS in this food [3,4]. The aim of this work was to apply a recently developed method [5] to the determination of 40 PFASs in fifty honey commercial samples produced in Italy.

Experimental

Fifty honey samples of different botanical varieties were purchased in local markets (8 acacia, 4 citrus, 19 chestnut, 3 honeydew and 16 multi-floral). The collected samples were from twelve Italian regions. Forty PFASs were determined via isotopic dilution by means of an LC-Q-Orbitrap platform (LC-Q Exactive, Thermofisher Scientific, San Jose, CA, USA). The acquisition was performed in ESI negative ionization mode operating SIM, full MS/dd-MS^2 and t-SIM/dd-MS^2 experiments [6]. Analytes were separated with a LC column Kinetex XB-C18 (100 mm × 3 mm, 2.6 µm, Phenomenex, Torrance, CA, USA) using water and MeOH both containing 5 mM ammonium acetate as mobile phases. Sample extraction and clean-up have been previously detailed [5,6]. The method

was fully validated for the main performance characteristics following the "Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed" [7].

Results

Although the analyzed honey samples showed a high prevalence (88%) of contamination with at least one analyte above the relevant limit of quantification, the measured concentrations were always lower than 40 ng kg^{-1}. The most found PFASs were C_5-C_{10} carboxylic acids (PFPeA, PFHxA, PFHpA, PFOA and PFDA). In contrast to other monitoring data [3,4], PFOS was never detected. Surprisingly, chestnut and honeydew varieties seem to be the most contaminated ones (Table 1). Since these honey types are also the richest of interfering substances, a deep evaluation of analyte identity was carried out, assessing retention time tolerance (≤ 1%) and the presence of at least two ions within the required mass accuracy (≤ 5 ppm) [7]. Co-chromatography experiments were performed in order to further support analyte identification. With regards to PFAS levels, the concentrations measured in this study were in good accordance with the data included in the EFSA Scientific Report published in 2012 [3]. In contrast, analyzing 26 honey samples collected in various European countries, Surma et al. reported PFAS concentrations up to 10 times higher than those we measured [4].

Table 1. *Concentrations of PFASs measured in fifty Italian honey samples.*

Honey type	**Frequency of detection (%)**	**Concentration range (ng kg^{-1})**				
		PFPeA	**PFHxA**	**PFHpA**	**PFOA**	**PFDA**
Acacia	75	< LOQ-6.4	< LOQ-4.0	< LOQ	< LOQ	< LOQ
Citrus	50	2.2-4.1	< LOQ	< LOQ	< LOQ	< LOQ
Chestnut	100	8.7-20	12-25	< LOQ-4.1	< LOQ-9.7	< LOQ
Honeydew	100	9.7-33	8.7-39	< LOQ-12	< LOQ-20	< LOQ-3.4
Multi-floral	88	< LOQ-6.4	< LOQ-11	< LOQ-2.5	< LOQ-25	< LOQ

Conclusions

Based on our results, PFAS exposure through honey consumption does not appear to pose tangible risks to Italian consumers. No correlation was observed between contamination and geographical origin; conversely, some types of honey (chestnut and honeydew) seem to be more polluted than other botanical varieties. Further studies should be carried out both to confirm the here measured levels and the possible relationship between PFAS concentrations and botanical origin of honey.

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References

1. E.M. Sunderland, X. Hu, C. Dassuncao, A. K. Tokranov, C.C. Wagner, J.A. Allen. *Journal of Exposure Analysis and Environmental Epidemiology* 29 (2019), 131–147
2. G. Pitter, F. Da Re, C Canova, G Barbieri, M.Z. Jeddi, F Daprà, F. Manea, R. Zolin, A.M. Bettega, G. Stopazzolo, S. Vittorii, L. Zambelli, M. Martuzzi, D. Mantoan, F. Russo. *Environmental Health Perspectives* 128-2 (2020), 27007
3. European Food Safety Authority (EFSA). Perfluoroalkylated substances in food: occurrence and dietary exposure. EFSA Journal. 10-6 (2012), 2743
4. M Surma, H Zieliński M. Piskuła. *Bulletin of Environmental Contamination and Toxicology* 97-1 (2016), 112–118
5. C. Barola, S. Moretti, A. Piersanti, T. Tavoloni, A. Stramenga, D. Giusepponi, F. Paoletti, S. Sdogati, R. Galarini. Massa 2022. 20-22 giugno Carlentini (SR). OR (23), p. 65. (https://www.spettrometriadimassa.it/Congressi/MASSA2022_4MSWineDay/programMASSA2022-4MSWineDay.html)
6. C. Barola, S. Moretti, D. Giusepponi, F. Paoletti, G. Saluti, G. Cruciani, G. Brambilla, R. Galarini; *Journal of Chromatography A*, 1628 (2020), 461442
7. EURL “Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed.” (2022) (https://eurl-pops.eu › news › guidance-document-pfas)

P57

Chloramphenicol residues in honey and muscle tissue: LC-MS/MS method and validation according to new decision level

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Summary: *An easy and rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed to determine chloramphenicol in honey and muscles tissue of farm animals.*

Keywords: *chloramphenicol, validation*

Introduction

Chloramphenicol (CAP) is a broad spectrum antibiotic exhibiting activity against both gram-positive and gram-negative bacteria as well as other groups of micro-organisms. This property, together with its low cost and ready availability, makes it extensively used in the treatment of farm animals all over the world, including food producing animals. However, treatment of food producing animals with CAP is banned in many countries, including the European Union (EU), because it is associated with serious toxic effects in humans, such as bone marrow depression and the particularly severe fatal aplastic anemia. For this reason, in the past, CAP was classified in Annex IV under Commission Regulation (EU) 37/2010 with a Minimum Required Performance Limit (MRPL) of 0.3 µg/kg [1]. In 2019, a new EU regulation [2] established a new reference point for action as 0,15 µg/kg for CAP in food of animal origin.

To meet this new requirement, a sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed and validated to determine CAP in honey and in livestock muscle tissue (cattle, poultry).

Results and conclusions

The procedure starts with a liquid-liquid extraction of the sample, followed by SPE purification (honey) or lipid extraction (muscle tissue). The extracts are directly injected onto the LC-MS/MS system. Prior of extraction, all test samples are spiked with an isotopically labelled internal standard CAP-D(5) to compensate for analyte loss and potential ion suppression during the MS stage. The method was validated by InterVal Plus Software according to the latest EU criteria for the analyses of veterinary drug residues in food of animal origin [3]. The procedure provides a sensitive and reliable method for the determination of residues of chloramphenicol in food, like honey and muscle of livestock.

References

1. Reg. (EU) 37/2010 of 22 December 2009on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Official Journal of the European Union,* L 15, 20/01/2010.

2. Reg. (EU) 2019/1871, of 7 November 2019 on reference points for action for non-allowed pharmacologically active substances present in food of animal origin and repealing Decision 2005/34/EC. *Official Journal of the European Union,* L 289, 8.11.2019.
3. Reg. (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC. *Official Journal of the European Union,* L 180, 21/05/2021.

P58

Direct injection and chromatography, mass spectrometry and ion mobility: a synergic approach for strawberry volatilome analysis

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Summary: *The aroma profiles of 15 strawberry cultivars were evaluated by PTR-ToF-MS, GC-IMS and GC-MS. The obtained results showed the complementarity of these techniques*

Keywords: *PTR-ToF-MS, GC-MS, GC-IMS*

Introduction

Strawberry quality and aroma

Flavour is one of main factors impacting strawberry quality and consumer appreciation. Since flavour involves the perception of a plethora of volatile organic compounds (VOCs), their assessment is crucial to guarantee the selection and marketability of high-quality fruits. Thus, the great impact of VOCs on fruit marketability stimulates the need to step forward in the understanding of this quality trait. High priority should be given to replace poor flavour cultivars with favourable ones, exploiting the variability already available in nature. However, the analysis of the aroma trait in many samples, necessary to overcome the usually massive biological and genetic variability among samples, may be laborious and time consuming.

VOC phenotyping is currently a limiting step in breeding programs, due to high costs and complex analytical techniques. Another limitation also raised by the elevated, and difficult to be controlled, interaction between fruit genetics and environmental effects. While there is substantial flavor variation within fruit species, most plant breeding programs have historically neglected it, given its intrinsic complexity and costs to phenotype. As a consequence, the drop-off in flavor quality has become one of the major causes of consumer dissatisfaction. To correct this inconsistency and incorporate flavor into breeding program routines, it is necessary to identify the sources of flavor variability, understand the role of genetic and environmental factors, and define cost-effective methods of selection.

Analytical techniques for VOC analysis

The most used instrumental analytical techniques for identifying volatile aroma compounds in food is gas chromatography–mass spectrometry (GC–MS). However, due to the complexity of food matrices, complicated pre-treatments generally required before analysis and long detection times this technique may not meet the rapid detection requirements for many analytes. Some complementary techniques, based on direct injection mass spectrometry (DI-

MS), have been developed to investigate VOC emissions for fruit flavor analysis, such as proton transfer reaction mass spectrometry (PTR-MS) and selected ion flow tube mass spectrometry (SIFT-MS). According to recent publications both techniques can be considered as powerful high-throughput phenotyping tool for both genetic and quality related studies [1]. The rapidity and the moderate cost of DI-MS analysis may allow to perform a detailed aroma characterization of strawberry with a peculiar attention to the VOC fold changes caused by *ad hoc* storage and transformation experiments, tailored to simulate the "from farm to fork" chain. However, a weak aspect of DI-MS methodologies for VOC assessment is still the compound identification. Fragmentation, complex peak structure and/or the presence of isomeric compounds may still make this challenge unpractical, especially in complex matrices.
Another alternative to GC-MS analysis, recently applied in food studies [2], is the application of an ion mobility spectrometry. Gas chromatography–ion mobility spectrometry (GC–IMS) combines the high separation capacity of GC and the fast response of ion mobility spectrometry (IMS). These features make GC-IMS a powerful technique for the separation and sensitive detection of VOCs in fruit and vegetables.

Experimental
In this study, the application of GC-IMS (FlavourSpec, G.A.S., Dortmund, Germany), PTR-ToF-MS (PTR-ToF-MS 8000, Ionicon, Austria) was evaluated for a comprehensive volatilome analysis on 15 strawberry cultivars. Results were compared and validated with SPME-GC-MS (AutoSystem XL gas chromatograph coupled with a TurboMass Gold mass spectrometer, Perkin-Elmer, Norwalk, CT) analysis. 9 fruits of each cultivar were firstly analysed by PTR-ToF-MS in non-destructive way (1 fruit in 250 mL jar). Then each fruit was frozen, grinned in liquid nitrogen and sampled in 20 mL vials for analysis with all three techniques.

Results
The variability of aroma profile of different cultivars was observed for all analysis. Moreover, the differences of VOC release analysed by PTR-ToF-MS between the same fruits measured as intact and grinned were also observed. The integration of four data sets were performed in the novel mixOmics framework DIABLO [3].

Conclusions
Outcomes of this study demonstrated the complementarity of three analytic methodologies and the prospect to apply them both for broad volatilome screenings and for fruit quality assessment. In particular, a proper application of these methodologies would enable, in a close future, for a more precise selection of the most favourable new accessions distinguished by superior fruit flavour, suitable for different market segments.

References
1. M. Bordiga, L.M.L. Nollet, Food Aroma Evolution: during food processing, cooking, and aging, CRC Press 2020, pp 217-240.

2. S. Wang, H. Chen, B. Sun, Food Chemistry, 315 (2020), 1-7
3. A. Singh, C.P. Shannon, B. Gautier, F. Rohart, M. Vacher, S.J. Tebbutt, K.A. Lê Cao, Bioinformatics, 35/17 (2019), 3055–3062.

P59

Ginseng root extract: ginsenosides quantitation by HPLC-MS and biological studies to counteract obesity

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Summary: *Obesity is a worldwide health challenge characterized by an increased inflammatory and oxidation process. This work aimed to prepare a ginseng root extract rich in ginsenosides and to evaluate the antioxidant and anti-inflammatory activities of the extract using THP-1 LPS-stimulated cells. The ginsenosides content has been studied by HPLC-MS.*

Keywords: *ginseng root extract, HPLC-MS, anti-obesity properties*

Introduction

Obesity is a worldwide health concern especially in the developed countries and in 2016, more than 1.9 billion adults were overweight, among them over 650 million were obese [1]. The trouble lies in the fact that obesity condition is associated with an increased risk of developing several diseases such as type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease, asthma, various types of cancer, cardiovascular and neurodegenerative diseases [2]. This status is characterized by low-grade chronic inflammation and an elevated oxidative stress in young and old subjects [2-4]. Several papers demonstrated that ginseng and ginsenosides exert a potential anti-obesity effect [5], however, just a limited number of studies deeply focused on the potential antioxidant and anti-inflammatory activities of ginsenosides and ginseng root extract. Therefore, the aim of the present paper was to evaluate the potential role of ginseng in hampering an inflammatory and oxidative environment. For this purpose, three diverse extraction methods using various solvents have been evaluated with the aim to prepare a ginseng extract rich in ginsenosides. The content of ginsenosides, i.e., ginsenoside Rb1, Rb2, Rg1 and Rg2, was studied by HPLC-MS system and the best performing procedure in term of total ginsenoside content and recovery levels was selected and applied to prepare a dried extract from *Panax ginseng* roots. Individual ginsenosides as well as the extract were tested for their capacity to counteract inflammation and oxidation in THP-1 LPS-stimulated cells.

Experimental
Different extraction methods, i.e., solid-liquid extraction (SLE), solid-liquid extraction assisted by ultrasound (ultrasound-assisted extraction, UAE) and solid-liquid extraction assisted by Naviglio extractor, using various solvents, have been evaluated. The content of ginsenosides, i.e., ginsenoside Rb1, Rb2, Rg1 and Rg2, was studied by HPLC-MS system in SIM mode and the best performing procedure in term of total ginsenoside content and recovery levels was selected and applied to prepare a dried extract from *P. ginseng* roots. Individual ginsenosides as well as the extract were tested for their capacity to counteract inflammation in THP-1 LPS-stimulated cells by studying their effect on a) cytokines gene expression, b) mtDNAcn modulation, c) DNA methylation at the gene promoter of downregulated cytokines, and d) gene expression patterns of enzymes involved in the regulation of the epigenetic homeostasis. In addition, the capacity to hamper oxidative stress influencing reactive oxygen species/reactive nitrogen species (ROS/RNS) levels, amounts of markers for protein oxidation, the expression of nitric oxide synthase (NOS) and NADPH oxidase (NOX), was also evaluated.

Results
The best extraction solvents were ethanol:water 50:50 and methanol:water 70:30 in both SLE (total ginsenosides concentrations: 6499.65 ± 82.10 µg/g and 6318.93 ± 84.32 µg/g, respectively) and UAE (6062.10 ± 271.42 µg/g and 5055.93 ± 102.65 µg/g, respectively) and satisfactory recovery levels were found for all these procedures since the recovery values ranged from 91.8 to 118.4% for all analytes. The ethanol:water 50:50 (*v/v*) mixture has been selected to prepare the dried extract of ginseng roots and it was tested for anti-inflammatory and antioxidant activities. The analytical procedure has been validated by studying the linearity, limit of detection (LOD), limit of quantification (LOQ) and repeatability. All the analytes showed good linearity since R^2 were 0.9984−0.9998 while LOQ was 15 µg/L for all ginsenosides which implied a good method sensitivity. Repeatability was also studied by injecting five injections of standard concentrations in the same day and in three consecutive days. The intra-day repeatability ranged from 2.9 to 6.3% while the inter-day repeatability was 6.3−13.9%. Once validated, the method has been applied to quantify the four ginsenosides, i.e., Rb1, Rb2, Rg1 and Rg2 in the dried extract as well. The dried extract contained a total ginsenoside concentration of 25.39 ± 0.32 mg/g. Ginsenoside Rg1 (13.07 ± 0.29 mg/g) was the most abundant, followed by ginsenoside Rb1 (7.64 ± 0.03 mg/g), Rb2 (3.28 ± 0.01 mg/g) and Rg2 (1.39 ± 0.07 mg/g). Biological studies demonstrated that ginsenosides and the extract strongly reduced the expression of interferon-γ and the extract was able to down-regulate the mtDNAcn at levels similar to control. In addition, the extract and the tested ginsenosides exerted antioxidant effects in macrophages decreasing the amount of ROS/RNS, markers for protein oxidation and the activity and expression of enzymes involved in oxidative processes such as NOS and NOX.

Conclusions
In the present work, different ginsenosides extraction procedures have been evaluated and compared among them, obtaining a valid analytical approach able

to produce a ginseng extract rich in ginsenosides. Interestingly, the extract and individual ginsenosides showed anti-inflammatory (reduction of IFN-γ, NOS activity and iNOS expression, control of the mtDNAcn increase) and antioxidant properties (decreasing the amount of ROS/RNS, the content of markers for protein oxidation and the expression of NOX).
Considering that chronic over-nutrition and obesity are characterized by increasing inflammatory and oxidative processes, ginseng extract and ginsenosides could assist the treatment of this altered state by hampering oxidation and inflammation, together with the other reported anti-obesity actions.

References

1. World Health Organization online site, “Obesity and overweight,” available at https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight, 2022.
2. J. Karczewski, E. Śledzińska, A. Baturo, I. Jończyk, A. Maleszko, P. Samborski, B. Begier-Krasińska, A. Dobrowolska, *European Cytokine Network* (2018), 83–94.
3. P. Mathieu, I. Lemieux, J. P. Després, *Clinical Pharmacology & Therapeutics* (2010), 407–416.
4. H. K. Vincent, K. E. Innes, K. R. Vincent, *Diabetes Obesity and Metabolism* (2007), 813–839.
5. Z. Li, G. E. Ji, *Journal of Ginseng Research* (2018), 1–8.

P60

HPLC-MS characterization of polyphenols in sweet cherries from Apulian region

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Summary: *This work deals with an extensive analytical approach, which uses two complementary tandem mass spectrometry techniques, to characterize the flavonoids and chlorogenic acids present in three sweet cherry varieties (Ferrovia, Sweet Heart, and Lapins) grown in Apulian region. A different quantitative pattern of anthocyanins, flavonols, and chlorogenic acids was revealed.*

Keywords: *HPLC-MS^n, HPLC-MS/MS, regio-isomers, geometrical-isomers.*

Introduction

Nowadays, there is a general trend in food science to link food and health. The first step to discover the role of food in preventing future diseases is undoubtably the characterization of its chemical composition, particularly regarding bioactive and nutraceutical compounds, such as polyphenols [1, 2]. Polyphenols present in fruit, such as sweet cherry, show a considerable structural diversity and can belong to several different classes of compounds, including flavonoids and cinnamates. Considering that only few commercial standards are available, the accurate qualitative-quantitative analysis of these compounds in complex fruit matrix is very difficult. Therefore, in this study, an extensive analytical approach was carried out to identify flavonoids and hydroxycinnamate derivatives in hydro-alcoholic sweet cherry extracts, by means of HPLC-DAD-ESI-MS^n analyses, with emphasis on the structural isomers present. Moreover, an UHPLC-MS/MS approach was also employed to quantify the detected compounds.

Experimental

Deseeded, lyophilized, and homogenate cherry pulp samples (0.28 g) were put into 2 mL Eppendorf tubes in presence of water/ethanol 1:1 (1 mL). Then, the obtained solution was sonicated at 100W and 37 kHz for 3 min at 25 °C. Afterwards, the extracts were centrifuged at 4000 g for 15 min at 5 °C, filtered through a 0.20 µm syringe cellulose filter, and analyzed by HPLC-MS. The adopted HPLC-MS systems along with the chromatographic and spectrometric conditions were the same as described in previous reports [3, 4].
Means and standard deviations of the raw data, derived from six biological replicates of sweet cherries from each variety, were compared through one-way ANOVA analysis coupled to Tuckey's post-hoc test.

Results

Five anthocyanins, 4 flavan-3-ols, 9 flavonols (among which quercetin-3-O-rutinoside-7-O-glucoside, kaempferol-3-O-rutinoside-7-O-glucoside, quercetin-3-O-galactosyl-rhamnoside, and quercetin-3-O-coumaroyl glucoside), and 16 hydroxycinnamates (among which 4 methyl coumaroyl quinate and 3 methyl caffeoyl quinate isomers) were detected by means of HPLC-MS^n analyses on the basis of their molecular ion and fragmentation patterns (MS^n up to MS^4) and UV/Vis absorption maxima (I_{max}). Then, the main identified compounds were quantified by multiple reaction monitoring (MRM) experiments through UHPLC-MS/MS as listed in Table 1.

Table 1. *Identified and quantified polyphenols in sweet cherries pulp.*

Compound	I_{max} (nm)	M+ (*m/z*)	$[M-H]^-$ (*m/z*)	MRM transitions
Cyanidin-3-O-sophoroside	516	611		611 >> 287
Cyanidin-3-O-glucoside	516	449		449 >> 287
Cyanidin-3-O-rutinoside	516	595		595 >> 287
Pelargonidin-3-O-rutinoside	520	579		579 >> 271
Peonidin-3-O-rutinoside	520	609		609 >> 301
Quercetin-3-O-rutinoside-7-O-glucoside	344		771	771 >> 609
Kaempferol-3-O-rutinoside-7-O-glucoside	350		755	755 >> 593
Quercetin-3-O-galactosyl-rhamnoside	355		609	609 >> 301
Quercetin-3-O-rutinoside	354		609	609 >> 301
Quercetin-3-O-glucoside	354		463	463 >> 301
Kaempferol-3-O-rutinoside	350		593	593 >> 285
Kaempferol-3-O-glucoside	350		447	447 >> 285
Quercetin-4'-O-glucoside	354		463	463 >> 301
5-O-(4'-O-caffeoyl glycosyl)-quinic acid	298sh, 315		515	515 >> 341
trans-3-O-caffeoylquinic acid	303sh, 324		353	353 >> 191
Caffeic acid hexose I	290, 304sh		341	341 >> 281
cis-3-O-coumaroylquinic acid	306		337	337 >> 163
trans-3-O-coumaroylquinic acid	302sh, 311		337	337 >> 163
Caffeic acid hexose II	292, 306sh		341	341 >> 281
trans-5-O-caffeoylquinic acid	302sh, 326		353	353 >> 191
trans-4-O-caffeoylquinic acid	292, 324		353	353 >> 173
cis-4-O-coumaroylquinic acid	295sh, 313		337	337 >> 173
cis-5-O-caffeoylquinic acid	295sh, 313		353	353 >> 191

As depicted by Fig. 1, different contents of polyphenols were determined in the 3 analyzed sweet cherries. In particular Sweet Heart and Lapins showed higher concentration of hydroxycinnamic acids and anthocyanins and flavonols, respectively.

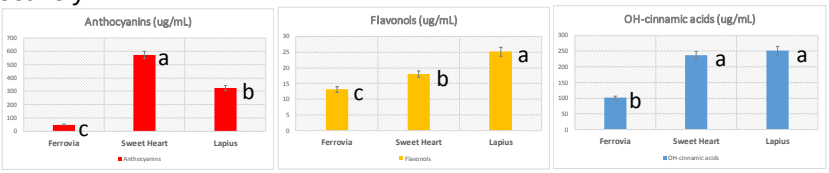

Figure 1*. Polyphenols content in the hydroalcoholic extracts of the 3 sweet cherry varieties*

Conclusions

A comprehensive analytical methodology, based on ‘in-time’ and ‘in-space’ tandem mass spectrometry (MS) techniques, proved to be effective for the complete characterization of polyphenols present in 3 sweet cherries grown in Apulian region.

References

1. V. García-Caňas, C. Simó, M. Herrero, E. Ibáñez, A. Cifuentes; *Analytical Chemistry*, 84 (2012), 10150–10159.
2. F. Corbo, G. Brunetti, P. Crupi, S. Bortolotti, G. Storlino, L. Piacente, A. Carocci, A. Catalano, G. Milani, G. Colaianni, S. Colucci, M. Grano, C. Franchini, M.L. Clodoveo, G. D’Amato, M.F. Faienza; *Frontiers in Immunology*, 10 (2019), 1001.
3. P. Crupi, R. Genghi, D. Antonacci; *Journal of Mass Spectrometry*, 49 (2014), 1025 – 1034.
4. P. Crupi, G. Bleve, M. Tufariello, F. Corbo, M.L. Clodoveo, L. Tarricone; Journal of Food Composition and Analysis, 73 (2018), 103–111.

P61

Effect of the postharvest drying process on the major phenolic compounds of Italian walnuts, using HPLC-MS/MS

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Summary: *Walnuts (Juglans regia L.), known for their healthy properties attributed to different bioactive compounds, are usually consumed after a drying process. In this work was evaluated the influence of drying on the major phenolic compounds by HPLC-MS/MS. A total of 45 compounds were identified based on mass spectra and literature.*

Keywords *walnuts, drying process, phenolic compounds*

Introduction

Walnuts (*Juglans regia* L.) are a nutrient-rich food of the Mediterranean diet that provide macronutrients and micronutrients, as well as other bioactive constituents [1]. Many studies have shown as walnuts can provide many health-promoting properties, such as reduce the risk of arteriosclerosis, hypercholesterolemia, cardiovascular disease, hypertriglyceridemia, diabetes mellitus, and cancer [2,3,4].

These benefits are due to the multiple phytonutrients (essential unsaturated fatty acids, tocopherols and phytosterols) they contain among which phenolics are considered as one of the most important groups of bioactive compounds. Walnuts have the most diverse phenolic profile and the highest phenolics content among the tree nuts [5].

Walnut kernels can be consumed as freshly picked fruits or after dehydration, even if most of the nuts are subjected to a drying process before consumption or processing. Drying stabilizes the product mass, lowering moisture content and water activity, and prevents the microbial spoilage and the deterioration in kernel quality, extending the fruit shelf life.

Until now limited published data are available on the comparison between fresh and dry walnuts about their composition and quality. Therefore, the aim of the presented study was to determine the phenolics content in Chandler walnuts, with particular focus on the effect of nitrogen fertilisation and post-harvest drying on the content of the individual phenols and phenolic groups in fresh and dry fruits.

Experimental

Orchard growing conditions and sampling

Walnuts were collected from an experimental walnut (*Juglans regia*) orchard of the cv. Chandler (Pedro x UC-56-224) grafted onto seedling rootstock grown in

the southern Italian Po Valley. At commercial harvest, fresh fruits were selected from the whole tree yield and stored until analysis. Additional subsamples were air dried at 30 °C one month, then processed as described for other samples.

Phenol extraction and determination

The phenolic compounds were extracted using the optimised protocol described by [6] The final polyphenol extracts were analysed by RP-HPLC-QqQ-ESI-MS as the method established by [7] was used. Quantification analysis was performed on a 6420 Triple Quadrupole (QqQ) mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) with an electrospray ionization (ESI) source in negative mode. Phenolic separation was performed on a 100 mm × 3.0 mm Zorbax Poroshell C18 column (Agilent Technologies, Millford, MA, USA) at 25 ºC. All compounds were monitored in the multiple reaction monitoring mode (MRM). The compound specific parameters of MRM (fragmentor voltage and collision energy) were automatically established with MassHunter Optimizer for each target compound.

Results

Walnut phenols were determined by HPLC-QqQ-ESI-MS and a total of 45 phenolic compounds were identified and quantified. As expected, gallic derivatives were the most abundant and numerous compounds in all samples with a percentage of about the 50% of the phenolic total content. Ellagic derivatives were the second class of compounds, representing more than 30%, mainly in the dried walnuts, whereas the flavan-3-ols and the flavonoids were present in low quantities, ranging from about 2 to 9 % of the total content. According to literature, the main phenolic compounds in all samples was Glansreginin A that is an important bioactive component of walnut because of its ability to exert neuroprotective effect via anti-inflammation in the brain [8],

About the total phenolic amount, data showed a significant decrease (19-44%) from fresh to dried walnut samples, mainly for the flavan-3-ols and gallic acid derivatives.

Conclusions

The use of LC coupled with a Triple Quadrupole (QqQ) allowed to carry out a comprehensive identification of phenolic compounds present in walnuts from *J. regia L* cv. Chandler, Besides, despite the complex and rich content in phenolic compounds of walnuts, the results suggested how the dry process causes a profound loss of most of them. Nevertheless, this technological process is needful to increase the shelf-life and to ameliorate the taste of this nut.

References

1. M. Kornsteiner, K.H. Wagner and I. Elmadfa; *Food Chemistry*, 98 (2006), 381–387.
2. D.K. Banel, and F.B. Hu; *The American Journal of Clinical Nutrition*, 90 (2009), 56-63.
3. A. Pan, Q. Sun, J.E. Manson, W.C. Willett and F.B. Hu; *The Journal of Nutrition*, 143 (2013), 512-518.
4. P.A. Davis, V. T. Vasu, K. Gohil,H. Kim, I.H. Khan, C.E. Cross et al.; *The British Journal of Nutrition*, 108 (2012), 1764–1772.

5. B.W. Bolling, C.Y.O. Chen, D.L. McKay, J.B. Blumberg; *Nutrition Research Reviews*, 24 (2011), 244–275.
6. V. Verardo, A. Bendini, L. Cerretani, D. Malaguti, E. Cozzolino, M.F. Caboni; *Journal of Food Quality*, 32 (2009), 262–281.
7. E. Díaz-de-Cerio, U. Tylewicz, V. Verardo, A. Fernández-Gutiérrez, A. Segura-Carretero, S. Romani; *Food Analytical Methods* 10 (2017), 2781–2791.
8. R. Haramiishi, S. Okuyama, M. Yoshimura, M. Nakajima, Y. Furukawa, H. Itoc and Y. Amakura; *Bioscience, Biotechnology, and Biochemistry*, 84 (2020), 187–197.

P62

Profile of phenolic compounds of antioxidant dietary fiber obtained from avocado peel, mango peel, and mango testa

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Summary: *Phenolic compounds present in antioxidant dietary fibers obtained from agro-industrial by-products were identified by means of HPLC-MS. These fibers have potential use as natural antioxidants for meat products.*

Keywords: *polyphenols, antioxidant activity, antioxidant dietary fiber.*

Introduction

Polyphenols are an important class of bioactive compounds, and they are one of the main constituents in human diet. Their chemical structures are characterized by the presence of at least one aromatic ring with one or more hydroxyl functional groups attached [1]. The extraction and use of bioactive constituent, as phenolic compounds (PCs) from agro-industrial waste is an interesting and current topic. The waste residue after extraction can contain mainly dietary fiber (DF). The concept of antioxidant dietary fiber refers to that raw material with a high percentage of dietary fiber and appreciable amounts of natural antioxidants associated with the set of non-digestible compounds. Avocado peel (AP), mango peel (MP) and mango seed coat (MT) contain DF associated with PCs, that is known as antioxidant dietary fiber (ADF) [2]. In the literature there are few works regarding the characterization of ADF products. The objective of this work was to obtain the profile of PCs present in the ADF products from agroindustrial waste such as Avocado peel (AP), mango peel (MP) and mango seed coat (MT).

Experimental

The analysis of associated PCs in the selected dietary fibers was performed. The extraction and clean-up steps were established based on the characteristics of the sample, to minimize the interfering compounds present in such complex matrices. 100 mg of each sample was extracted by ultrasounds technique in two step: the first extraction was performed with methanol and water as solvent solution while the second extraction with acetone. The supernatant was collected, then a solid phase extraction (SPE) was performed as clean up. Targeted and semi-targeted approaches for the analysis of polyphenolic compounds were performed by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) [3]. To evaluate the antioxidant activity ABTS, FRAP and DPPH assays were also carried out.

Results

37 different PCs were identified in MP and AP: 16 phenolic acids, among which are hydroxybenzoic and hydroxycinnamic acids and 21 flavonoids, which are belong to isoflavonoids, flavones, flavanols, and glycosylated forms. MT have a poor profile of phenolic acid and flavonoids, but significative presence of lignans and stilbenes compounds. Moreover, the antioxidant activity *in vitro* of each extract was evaluated by antioxidants assay and the polyphenolic profile and they are in correlation with the antioxidant potential.

Conclusions

This work represents a contribution to the knowledge of the chemical composition, in particular, the PCs profile of agro-industrial waste and the ADF products. The preliminary results show that the ADF foods presents rich PCs profile associated to significative antioxidant activity. These ADF could have a potential use as a natural antioxidant ingredient in the formulation of meat products allowing the oxidative process of the meat, preventing the development of undesirable odors, stabilizing the color and retarding lipid oxidation during refrigerated storage of the meat, therefore increases its useful life [4].

References

1. Y. S. Velioglu, G. Mazza, L. Gao, and B. D. Oomah; *Journal of Agricultural and Food Chemistry,* 4113–4117
2. He, C., Sampers, I., Raes, K; *Food Hydrocolloids*, *111*, 106175.
3. H Oliva, E., Viteritti, E., Fanti, F., Eugelio, F., Pepe, A., Palmieri, S., ... Compagnone, D. J*ournal of Chromatography A*, *1651*, 462315.
4. Sáyago-Ayerdi SG, Brenes A, Goñi I LWT-Food Sci Tech. 2009; 42: 971-976.

P63

Set-up of a liquid chromatography–tandem mass spectrometric method for the quantitation of (poly)phenolic metabolites in human urine with on-line solid phase extraction

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Summary: *A high-throughput method based on on-line solid phase extraction coupled with liquid chromatography tandem mass spectrometry (SPE-LC-MS/MS) for the analysis of berry-related (poly)phenolic metabolites in human urine was developed and validated.*

Keywords: *(poly)phenols, nutrimetabolomics tandem mass spectrometry, biofluids*

Introduction

(Poly)phenols (PPs) are plant secondary metabolites found in a wide variety of foods and derived products [1]. After food intake, PPs are absorbed and metabolized by host gut microbial enzymes (Absorption-Distribution-Metabolism-Excretion, ADME), originating a complex mixture of derived metabolites that may additively or synergistically account for the reported beneficial health effects of phenolic-rich diets [2]. Analytical chemistry can effectively increase the knowledge about the dietary exposure of consumers to PP metabolites by the development of analytical platforms for their quantitation in human biofluids, to evaluate the bioavailable and excreted concentration levels. The aim of our research is to develop and validate an on-line SPE-LC-MS/MS method for the quantitative analysis in human urine of 34 structurally heterogeneous PP metabolites, selected accordingly to literature findings on the berry intake biomarkers [2]. First, the chromatographic behaviour of the selected analytes was investigated, focusing on the analytical challenging separation of isobaric metabolites (e.g., glucuronidated urolithins and quercetins), by testing different stationary and mobile phases. To develop a "wide-scope" method feasible to the targeted and quantitative analysis of PP metabolites in urine postprandial samples from a berry-based intervention study, the on-line SPE step was performed using a mixed-mode sorbent (i.e., Isolute ENV+), combining hydrophobic and polar interactions, capable to enrich a broad range of PP metabolites with different physicochemical properties.

Experimental

Urine samples were collected from volunteers after the intake of 25 g of bilberry (*Vaccinium myrtillus*) or blueberry (*Vaccinium corymbosum*) supplement mixed with 500 mL of drinking water [3]. Instrumental analysis was performed on a Shimadzu (Kyoto, Japan) chromatographic system coupled with a 5500 QTrap mass spectrometer (Sciex, Framingham, MA, USA), equipped with a Turbo V® interface with an ESI probe. On-line SPE and chromatographic analysis was

performed using a two position six-port switching valve connected to a Nexera X2 LC-30AD quaternary pump (for desorption and chromatographic separation), and one isocratic pump LC-20AD XR (dedicated to the on-line SPE cartridge loading and washing). The optimization of the on-line SPE extraction process on Isolute ENV+ sorbent phase was carried out by analysing three different conditions: (i) continuous desorption from the cartridge at room temperature, (ii) methanol assisted desorption followed by post-SPE dilution with water, and (iii) thermal-assisted desorption.

Results

Three stationary phases were tested to reach the best separation conditions, i.e., Kinetex (i) octadecyl, (ii) biphenyl, and (ii) phenyl-hexyl. The optimization of the chromatographic separation was carried out focusing on critical isobaric groups. Once the optimal conditions for the recovery of the 34 analytes were found, the on-line SPE-LC-MS/MS method was finally applied to the analysis of a pooled urine sample from the aforementioned study. The metabolites most present in the analysed samples (Fig. 1) were hydroxybenzoic acids and related sulfonate conjugates, contributing for the 62% of the total metabolic component, and among them, 2,5-Hydroxybenzoic acid exhibited the highest concentration value. Such result is not unexpected, since hydroxybenzoic acids are metabolic end-products of some major PP occurring in bilberries, i.e., anthocyanins and flavonols. Hydroxycinnamic acid sulfonates and urolithins with their conjugates showed comparable concentration values in the investigated sample, each group accounting for the 8.3-8.6% of the total metabolic contribution. Other hydroxycinnamic acids exhibited a relevant contribution to the metabolome, such as glucuronidated conjugates (3.6%), free acids and their derivatives (4.7%), i.e., Chlorogenic acid and 5-O-Feruloylquinic acid. Similar contributions were highlighted for hippuric acids (1.3%) and quercetin conjugates (1.0%), whereas the less present metabolites category was the one of resveratrol and their conjugates (0.2%), since such metabolites are mainly associated to wine and/or tea consumption, and its contribution may derive from pre- or post-intervention food sources. Finally, Abscisic acid and 3-Hydroxyphenilacetic acid were included in the “others” category, accounting for the 10.5% of the metabolic contribution. The relevant impact of 3-Hydroxyphenilacetic acid derives from the metabolic pathway of the major components of the supplements, i.e., anthocyanidins, ending with the formation of protocatechuic and phenylacetic acids.

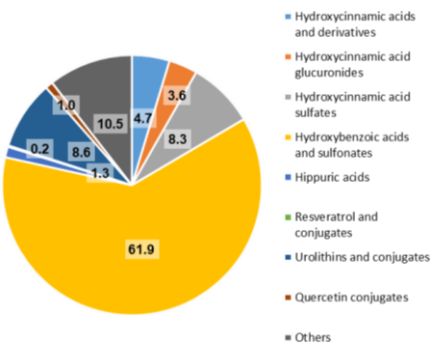

Figure 1. *Pie chart of the distribution of the target PP metabolites in the investigated pooled urine sample*

Future perspectives

Keeping in mind the critical aspects of both extraction and separation steps a Quality by Design workflow will be built, encompassing a screening procedure and an RSM optimization. The selected factors will be: Desorption Temperature, Exposure Time of the SPE cartridge to the chromatographic gradient, Loading solution pH, Wash Organic Percentage, Loading Flow, Wash Volumes and two different sorbent beds ISOLuteENV+ and Oasis HLB.

References

1. O. López-Fernández, R. Domínguez, M. Pateiro, P.E. Munekata, G. Rocchetti, J.M. Lorenzo; *Antioxidants*, 9 (2020), 479.
2. M. Ulaszewska, M. Garcia-Aloy, N. Vázquez-Manjarrez, M.T. Soria-Florido, R. Llorach, F. Mattivi, C. Manach; *Genes & Nutrition*, 15 (2020), 1-35.
3. C. Ancillotti, M. Ulaszewska, F. Mattivi, M. del Bubba; *Journal of the American Society for Mass Spectrometry*, 30 (2019), 381–402

P64

Extraction and characterization of alkaloids and polyphenols of a complex extract by HPLC-DAD-MS

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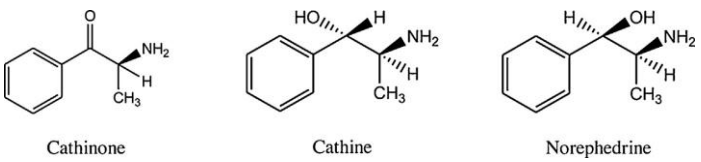
Summary: *Hydroalcoholic extraction of plant leaves and fractionation based on different polarity and acid-base properties allowed the separation of three classes of compounds: phenylpropanolamines, cathedulins, and flavonoids. HPLC-DAD-MS analysis enabled the chemical characterization of the main analytes by applying a single chromatographic method.*

Keywords: *cathedulins, flavonoids*

Introduction

The plant, commonly known as khat or qat, is a medium-sized evergreen tree belonging to the family of *Celastraceae*. The plant is cultivated in Arabian Peninsula, especially in Yemen, as well as in Eastern African countries, where is highly significant for the social and cultural life of the population [1]. It is estimated that 5 to 10 million people consumed khat daily by chewing the leaves to relieve fatigue, increase alertness, and reduce the sensations of hunger. The stimulant effects are mainly attributed to the alkaloid cathinone, present in fresh young leaves. This molecule is rapidly enzymatically reduced in mature leaves and after harvesting to the mild stimulant cathine and its non-active diastereomer (1R, 2S)-norephedrine (Fig. 1) [2]. In addition to the stimulant phenylpropanolamines, high molecular weight alkaloids, the cathedulins, have been isolated from the plant. These little-studied compounds are based on a polyhydroxylated sesquiterpene skeleton and are basically polyesters of euonyminol [3]. Several other phytochemicals such as flavonoids, steroids and triterpenoids, monoterpenes and volatile aromatic compounds, and other miscellaneous molecules like vitamins and amino acids have been found in khat [2]. Due to global migration and improved transportation facilities, as well as the application of preservation techniques (i.e. freezing, freezedrying), khat consumption has spread and become popular even in Europe [4].



Cathinone Cathine Norephedrine

Figure 1. *Phenylpropanolamines in khat*

The traditional use of the plant and its wide distribution make it interesting to study the chemical composition of the phytocomplex, which has not been

investigated in depth so far. Consequently, the contribution of the phytocomplex to the stimulating effects hitherto attributed to phenylpropanol-amines can be clarified.
In this context, this study aimed: i) to develop a comprehensive extraction method and a fractionation procedure that effectively separates the main classes of compounds; ii) to chemical characterize each fraction by HPLC-DAD-MS; iii) to study the chemical differences between dried and lyophilized leaves collected in different periods of time.

Experimental

Youngest leaves of the plant were harvested from plants from a nursery of Pistoia. The leaves were collected in November 2021 (dried leaves at room temperature, DL) and May 2022 (lyophilized leaves, LL). The extractions were conducted in triplicates according to the procedure reported in Fig. 2. Extracts were analyzed with HP 1260 Infinity II liquid chromatograph equipped with DAD detector and MS detector with API-ES interface (Agilent Technologies). A Poroshell 120 EC-18 column (2.7 µm, 3.0 x 150 mm, Agilent, USA) was used and a gradient method with water (pH 3.2 by HCOOH) and acetonitrile was applied. Chromatograms were registered at 200 nm (phenylpropanolamines), 225 nm (cathedulins), 330 nm (phenolic acids) and 350 nm (flavonoids). The MS analyses were carried out in positive and negative ionization modes, fragmentors 100, 150 and 200 V and the following ESI parameters: nitrogen flow rate 10.5 L min-1, drying gas temperature 300 °C; nebulizer pressure 1035 Torr; capillary voltage 3000 V, and acquisition performed in full spectrum scan (range 75–1300 m/z).

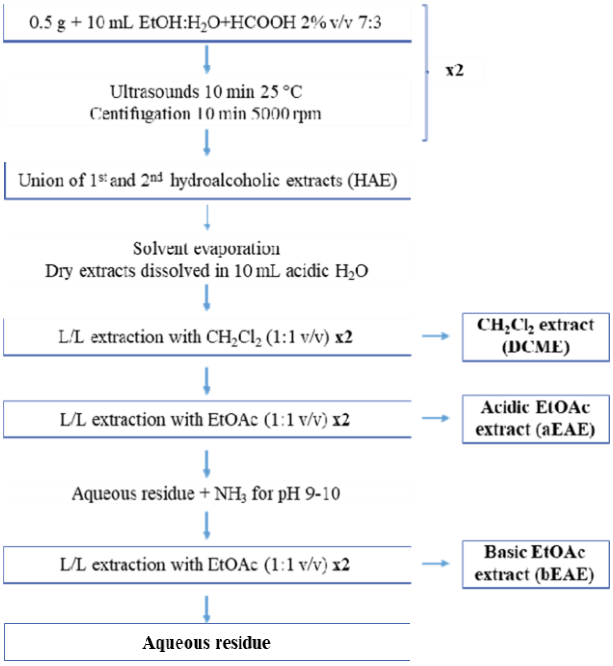


Figure 2. *Extraction and fractionation procedure applied on dried khat leaves*

Results

The extraction and fractionation procedure successfully allowed the separation of the main classes of compounds: the DCME contained almost only cathedulins, the aEAE was rich in flavonoids, and cathinone and derivatives were concentrated in the bEAE. The yields, calculated as dried extract on dry matter, are reported in Table 1.

Eighteen flavonoids and a caffeoylmalic acid were tentatively identified according to UV-Vis and MS spectra. Quercetin and myricetin glycosides were the most abundant, but luteolin, kaempferol, dihydroquercetin and catechin derivatives were also detected. The chromatographic method allowed the separation of 28 cathedulins and 16 of them were tentatively identified by MS. The spectra in positive ionization mode were characterized by the presence of both $[M+H]^+$ and $[M+2H]^{2+}$ species and by other fragmentation ions from the singly and doubly protonated precursors. These findings were in agreement with Kite et al. [5].

***Table 1**. Percentage yields of dried extract (DE) on dried leaves (DL) and lyophilized leaves (LL).*

	DL	LL
DCME	1.30 ± 0.09%	2.12 ± 0.08%
aEAE	5.00 ± 0.20%	6.20 ± 0.54%
bEAE	1.42 ± 0.08%	0.23 ± 0.21%

Cathinone and derivatives were poorly retained by the column and their separation was not optimal. Therefore, the relative abundance of cathinone and the two diastereomers cathine and (1R, 2S)- norephedrine was estimated by the area of the specific extract ions – 132 m/z for cathinone and 134 m/z for cathine and its diastereomer. The phenylpropanolamines content was about 8 times greater in LL harvested in May 2022 with respect to DL harvested in November 2021. This difference is probably mainly attributable to the different harvesting time. However, the combination of the harvesting time and the applied drying techniques did not affect the cathinone/cathine ratio (which was approx. 15/85). Finally, DL and LL showed a similar profile for flavonoids and cathedulins, with the same relative abundance of individual analytes with respect to their class.

Conclusions

The hydroalcoholic extraction and the proposed fractionation procedure is a simple and replicable method that enables the recovery of the main classes of khat metabolites. A single chromatographic method was applied to analyze the different extracts with good efficiency and resolution. The mass spectra were crucial for the chemical characterization of the three groups of phytochemicals investigated. Further studies are in progress to quantify the analytes in the different extracts and their variability in relation to harvesting time and drying techniques. In addition, further studies are desirable to assess the contribution of single classes of phytochemicals to the biological effects of the plant.

References

1. Feyissa, A. M., Kelly, J. P. (2008). *Progress in neuropsychopharmacology & biological psychiatry*, *32*(5), 1147– 1166. https://doi.org/10.1016/j.pnpbp.2007.12.033
2. Getasetegn M. (2015). *Phytochemistry Reviews, 15*, 907-920. https://doi.org/10.1007/s11101-015-9435-z
3. Engidawork E. (2017). *Phytotherapy research: PTR*, *31*(7), 1019–1028. https://doi.org/10.1002/ptr.5832
4. Pendl, E., Pauritsch, U., Kollroser, M., Schmid, M. G. (2021). *Forensic science international*, *319*, 110658. https://doi.org/10.1016/j.forsciint.2020.110658
5. Kite, G. C., Ismail, M., Simmonds, M. S., Houghton, P. J. (2003). *Rapid communications in mass spectrometry:RCM*, *17*(14), 1553–1564. https://doi.org/10.1002/rcm.1085

P65

Acmella oleracea (L.) R.K. Jansen: alkylamides and phenolic compounds in aerial parts and roots of *in vitro* seedlings

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Summary: *Secondary metabolites in the aerial parts and roots of seedlings of Acmella grown in vitro were evaluated. Similar chemical profiles were obtained from single seed and regenerating lines extracts. Spilanthol was prevalent in the leaves of seedlings from regenerating lines, while the roots contained double amount of phenols than the aerial parts.*

Keywords: *edible herbs, phytochemicals, HPLC-DAD-MS*

Introduction

Acmella oleracea (L.) R.K. Jansen (Asteraceae), popularly known as jambu and widely cultivated in the Brazilian Amazon region, represents an economically important medicinal and edible plant crop. The different anatomical parts of jambu, such as flowers, leaves and stems, are commonly used as food ingredients in typical Amazon cuisine, and as a traditional medicinal herb. It is a rich source of several bioactive compounds, notably alkamides, flavonoids and other phenolic compounds, used for the treatment of different human disorders [1]. A good approach to obtain a great number of plants, in a short period of time under selected standardized conditions of cultivation, is the use of *in vitro* plant cultures which allows rapid improvement of raw material and metabolite production [2]. This research involved the study of aerial parts and roots of *Acmella* seedlings grown *in vitro* and developed starting from two different techniques: from single seed and from regenerating lines derived by organogenesis. The study aimed to characterize the phytochemical profile of the extracts obtained from the two parts of the plants, focusing on the evaluation of its characteristic secondary metabolites, i.e. alkylamides and phenolic compounds, by using an HPLC-DAD-MS method. The variability of the quantitative results was evaluated by analysing more batches collected over time. The optimization of the extraction procedure and the analytical method was also performed.

Experimental

The samples are aerial parts and roots obtained from *in vitro* seedlings plants from seed and by regenerating lines derived by organogenesis of *Acmella oleracea*, according to Maggini *et al*. [2].
Three different extractive procedures were tested to evaluate the most efficient one for the recovery of phytochemicals. The most efficient was then applied to

all the samples. A fractionation step with hexane was then performed allowing the distribution of the recovered compounds in two extracts: a hydroalcoholic (HAE) and a lipophilic (HE).
The chromatographic analyses were conducted using an HP 1260 Infinity II liquid chromatograph equipped with DAD detector and MS detector with an API/electrospray interface (Agilent Technologies, Palo Alto, CA, USA). A RaptorTM ARC-18 column (150 × 3 mm, 5 µm, Restek, USA) was used, applying a flow rate of 0.4 mL min−1. Water at pH 3.2 by formic acid (solvent A) and acetonitrile (solvent B) were used. The MS analyses were carried out in positive and negative ionization mode with fragmentors 150 V and 200 V. Alkylamides were quantified at 229 nm by a five-point calibration curve of spilanthol, phenolic acids with a five-point calibration line of chlorogenic acid at 330 nm and flavonoids were expressed as quercitrin (quercetin-3-L-rhamnoside) used to build a five-point calibration line at 350 nm. The proposed analytical method was evaluated in terms of linearity, accuracy and precision (in terms of repeatability) from stock standard solutions.

Results
The proposed method allowed a satisfactory chromatographic resolution for all the target analytes in a short time. A total of 30 compounds (15 phenols and 15 alkylamides) were detected. The chromatographic profiles of aerial parts and roots extracts obtained from seed and regenerating lines presented the same pattern of secondary metabolites.
Aerial parts. The HAEs from seed showed the lowest content of phenolic compounds with a mean value of 3.40 mg/g DM. A significant higher concentration was found for the samples from regenerating lines, with a mean amount of 4.93 mg/g DM. The content of alkylamides in the HAEs ranged from 1.5 to 2.1 mg/g DM. In the HEs the average alkylamides content was below 2.4 mg/g DM. The spilanthol content in the aerial parts represented about 70-80% of the total alkylamides both in HAE amd HE extracts.
Roots. The HAEs showed a higher average phenolic content than that measured in the aerial parts, with a maximum value of 11.19 mg/g DM. Also for this tissue, seedlings obtained from seed showed the lowest phenolic content (below 8.0 mg/g DM). The total amount of alkylamides in the HAEs and HEs was less than 0.29 mg/g DM and 0.46 mg/g DM, respectively. The spilanthol content was approximately 36-60% of the total alkylamides, with values about ten to fifteen times lower than the concentration measured in the aerial parts.

Conclusions
In this study the content of alkylamides and phenols in the aerial parts and roots of *Acmella oleracea* plant obtained *in vitro* was evaluated for the first time. The chromatographic method allowed determining all the target molecules in a single step and resulted a useful tool for evaluating the natural variability of the secondary metabolites content in seedlings produced over time. The same pattern of secondary metabolites was observed in aerial parts and roots extracts obtained from seed and regenerating lines. The hexane fractionation step effectively recovered spilanthol and its derivatives in a concentrated extract. The aerial parts were confirmed to be richer in alkylamides and spilanthol, while low

amount of spilanthol was found in the roots. The data set collected for the first time for *Acmella oleracea in vitro* seedlings can be useful to define future steps focused on a highly controlled and reproducible raw material production in terms of phytochemical content.

References

1. L.E.S. Nascimento, N.D.A. Arriola, L.A.L. da Silva, L.G. Faqueti, L.P. Sandjo, C.E.S. de Araújo, M.W. Biavatti, J.L. Barcelos-Oliveira, R. Dias de Mello Castanho Amboni, *Food Chemistry,* 332 (2020).
2. V. Maggini, P. Bettini, F. Firenzuoli, P. Bogani, *Plants*, 10 (2021), 1–11.

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Evaluation of polyphenol content of corn tortilla chips with maya nut flour and mushrooms

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Summary: *In this work, the phenolic compounds in different formulations of tortilla chips with three flours (corn, Mayan nut and mushroom) were analyzed. Each snack shows a different phenolic profile, that allows the antioxidant evaluation in vitro and the choose of the best formulation for the functional foods.*

Keywords: *polyphenols, antioxidant activity, functional food.*

Introduction

Polyphenols compounds (PCs) are a group of bioactive compounds found in plants, characterized by more than one phenol group in their structure. Polyphenols have antioxidant potential and other biological properties, such as vasodilator, anti-inflammatory, antithrombotic and antiatherogenic action [1].
In the last years, food companies have focused their attention on the production of functional foods, enrichment with bioactive compounds that have beneficial effects on human health [2]. In Mexico, the Ramon tree (Brosimum alicastrum) has historical relevance, since it had economic and nutritional importance within the Mayan culture. Its fruit has a seed (known as a Mayan nut) that is used to produce edible flour for the preparation of various foods and drinks. The presence of phenolic compounds allows beneficial effects on human health, as reported in the literature [3]. Another particular food is Pleurotus ostreatus, an edible mushroom rich in bioactive compounds with beneficial impacts on human health. It has been reported in the literature that antioxidant activity is associated with PCs content. However, the PCs profile is little characterized in the literature [4].
The objective of this study is the analysis of the PCs compounds and the antioxidant activity in vitro in the tortilla chips produced with different formulations of corn flour, Maya nut seeds, and edible mushrooms to choose the best formulation for the food field.

Experimental

Ultrasound extraction, using methanol-water solvents, was used for the extraction of phenolic compounds from flours (corn, Maya nut, and mushrooms) and tortilla chips formulations. A targeted and semi-targeted approach for the

PCs profile was performed by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) [5]. The evaluation of antioxidant activity was performed with ABTS, DPPH, and FRAP assays.

Results

The PCs profile shows: 26 different phenolic compounds such as 10 phenolic acids, among which are hydroxybenzoic and hydroxycinnamic acids, and 16 flavonoids. The antioxidant activity of each extract was evaluated. The mayan nut flour had the highest antioxidant capacity in all the tests and this increased the antioxidant capacity and phenolic compounds in all the tortilla chips formulations, being the one with the best profile and antioxidant activity formulation 2, with 20% Maya nut.

Conclusions

The great diversity of phenolic compounds present in the analysed samples was determined. The presence of phenolic compounds gives the product health benefits and added value beyond its nutritional quality, compared to other snacks of the same type.

References

1. Motilva, M. J., Serra, A., Macià, A. *Journal of Chromatography A*, *1292*, 66-82.
2. Subiria-Cueto, R., Larqué-Saavedra, A., Reyes-Vega, M. L., de la Rosa, L. A., Santana-Contreras, L. E., Gaytán-Martínez, M., ... Martínez-Ruiz, N. R. *Foods*, *8*(12), 613.
3. K. Ozer, *International Journal of Food Properties*, 20,11, 2772–2781.
4. S. Selli, G. Guclu, O. Sevindik, H. Kelebek, *Food Chemistry*, vol. 354.
5. H Oliva, E., Viteritti, E., Fanti, F., Eugelio, F., Pepe, A., Palmieri, S., ... Compagnone, D. J*ournal of Chromatography A*, *1651*, 462315.

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New analytical approach to determination Ethylene Oxide and 2-Chloroethanol residues in food commodities

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Keyword: *Ethylene oxide; 2-Chloroethanol; Headspace-GC-MS*

Introduction

The Rapid Alert System for Food and Feed **(**RASFF) of the European Commission has received, in august 2020, a notification from Belgium about the exceeding of EU-MRL (maximum residue limit), 0.05 mg/Kg, of ethylene oxide (EO) in sesame seeds imported from India. As a consequence of this first alert many others have been notified to the RASFF, all of them concerning seeds imported from India [1-2].

EO has been banned in Europe since 1991 due to its toxicological concerns, nevertheless, is still used in Canada, USA, India and in different African's countries [3], as fungicide and biocide to prevent microbiological hazard, such as S*almonella,* in food commodities.

After fumigation, the interaction between EO with chloride and bromide ions leads to the production of 2-chloroethanol (2-CE), 2-bromoethanol and other compounds, which remain for a long time in foods, even after aeration [4]; so, these breakdown products can be used as footprint of EO-disinfections.

For this reason, in the 2008 the MRL-levels were defined as "Sum of ethylene oxide and 2- chloroethanol expressed as ethylene oxide".

Over the years, some analytical methods were proposed to determine residue levels of EO [5-6], but a recent method developed from EURL-SRM (European Union Reference Laboratory for Single Residue Methods) and based on the QUECHERS method seems to be the most versatile and adopted by many laboratories [2].

The purpose of this work has been to optimize and validate a fastest method based on headspace sampling and analyzed by GC-MS.

Experimental

To test the new analytical approach and compare with the one developed by EURL-SRM, three types of spices (ginger, curry and cinnamon) and two samples of homogenized fruit (apple and grape) were chosen. The performance of the method (repeatability, LOD, LOQ) was evaluated by adding EO and 2-CE at three different concentration levels (0.02, 0.05, 0.5 mg/Kg), on all the matrices above mentioned. Each experiment was carried out in triplicate and given the high volatility of EO (bp 10.4 °C) the addition operations were carried out in a cold room at -25 °C.

All measurements were performed by GC-MS instrument (Varian 450 GC coupled with Varian 300 MS TQ Mass Spectrometer) equipped with a Headspace sampling system (PAL-system, CTC Analytics, Switzerland). A Restek GC-Capillary column (Rxi-624Sil MS, 30 meter, 0,25 mm ID, 1,4 µm df)

was used and 1 ml was injected.

Results & Conclusions

The developed analysis approach, based on Headspace sampling, allowed to lower the detection limit by 5 times of the ethylene oxide compared with the EURL method, while for the 2-CE the results are comparable. In addition, the sample preparation is very fast, does not use extraction solvents and the manipulation of the sample is practically nil. The method performance data is compliant with the SANTE 11312/2021 directive [7] and the method thus developed could be a valid alternative to the EURL-SRM method for analyzing ethylene oxide and 2-chloroethanol in food products.

References

1. European Commission (2022) RASFF—food and feed safety alerts. In: Food safety (https://food.ec.europa.eu/safety/rasff- food-and-feed- safety-alerts_ en). Accessed 1 Jan 2022
2. EURL-SRM - Analytical Observations Report, EU Reference Laboratories for Residues and Pesticides, version 1.1 December (2020)
3. A. Dudkiewicz, P. Dutta, D. Kołożyn-Krajewska; *European Food Research and Technology*, 248 (2022), 1951-1958
4. J. Fowles, J. Mitchell, H. McGrath; *Food and Chemical Toxicology,* 39 (2001), 1055-1062
5. K.G. Jensen; *Z. Lebensm Unters Forsch,* 187 (1988), 535–540
6. X. Du, W. Zhang, B. Liu, T. Liu, Y. Xiao, M. Taniguchi, Y. Ren; *Journal of AOAC International*, Vol. 102 n°6 (2019), 1877-1883
7. Guidance SANTE 11312/2021-Analytical quality control and method validation procedure for pesticide residues analysis in food and feed

P68

Food as medicine: anti-inflammatory property of cinnamon extract, UHPLC-PDA-HRMS characterization of active compounds

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Summary: *Herbs and spices have been used since ancient times as natural antioxidants and anti-inflammatories. Among them, cinnamon is reported to exert also potent neuroprotective, hepatoprotective and cardioprotective effects. In order to identify the active compounds in cinnamon extract a full UHPLC-PDA-HRMS characterization was carried out before and after simulated digestion.*

Keywords: *Cinnamomum verum, polyphenols, anti-inflammatory*

Introduction

Herbs and spices are rich in natural bioactive substances with antioxidants and anti-inflammatory properties. Cinnamon with its 250 species is a wide-spread spice. Moreover, the anti-inflammatory power of cinnamon extract has been demonstrated in various models of inflammatory diseases such as colitis and arthritis [1]. In literature, the anti-oxidant and anti-inflammatory activity studies and the chemical characterization refer only to the spice extracts but not to the product of digestion, typical of oral assumptions. For that reason, in the present study it has been chosen to carry out an UHPLC-PDA-HRMS untargeted characterization of the cinnamon extracts not only before but also after gastrointestinal digestion.

Experimental

The pulverized Cinnamomum verum bark were extracted according to Cheng et al. work [2]. The gastrointestinal digestion was simulated in the 3 phases: oral phase, gastric phase and intestinal phase [3]. The digested cinnamon extract was administered to human epithelial colorectal adenocarcinoma (Caco-2) cell line cultured still showing anti-inflammatory effects. The obtained two aqueous extracts were investigated through UHPLC-PDA-HESI-HRMS Thermo Orbitrap Exploris 120 mass spectrometer. Spectra were recorded in full-mass mode within a range of 100-1500 m/z in positive and negative ionization, The Orbitrap resolution was set at 120000. Phenolic compounds were characterized according to the corresponding spectral characteristics (UV and MS/MS spectra), accurate molecular mass, characteristic MS fragmentation and libraries comparison in semi-automatic way through Thermo Scientific Compound Discoverer Software.

Results

UHPLC-PDA (extracted at 280 nm) chromatograms of cinnamon extract before (A) and after (B) digestion are reported in Fig. 1.

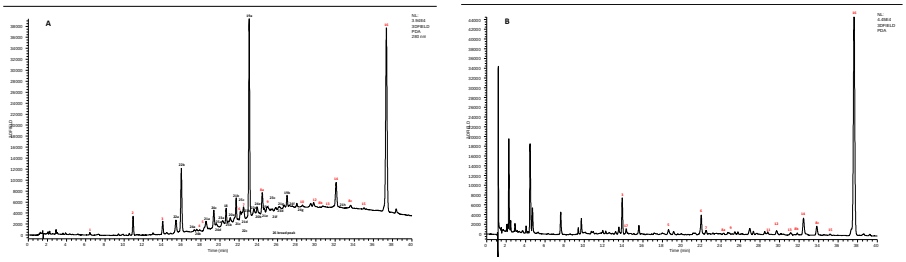

Figure 1. *UHPLC-PDA chromatographic separation (280 nm) of cinnamon extract before (A) and after (B) digestion*

All the identified molecules in undigested cinnamon extract belong either to the class of procyanidins [4] or they are derivatives of phenolic compound.
During the first ten minutes small and hydrophilic molecules such as: xylitol, raffinose and gluconic, quinic, malic, citric acid were eluted. All of them were identified on the bases of HRMS spectra and Compound Discoverer libraries. Furthermore, the chromatographic trace is characterized by two very intense peaks attributed to cinnamtannin B1 and trans-cinnamic acid, and by a broad peak starting at 18 minutes and ending at 40 minutes. This wide-ranging peak is ascribable to the procyanidins with different degree of polymerisation (from 2 to 8). After digestion (*Fig. 1B*) we can observe the complete disappearance of all catechin polymers, and an increasing of trans-cinnamic acid, from 250 to 4500 mg/l and other phenolic compounds such as 2-hydoxybenzoic acid, coumarin and hydroxybenzaldehyde which saw a tenfold increase in content. Proper to these molecules the anti-inflammatory activity must be attributed.

Conclusions

The aqueous extract of Cinnamomum verum bark exhibit virtuous anti-inflammatory activity, confirmed by scavenging tests. Through UHPLC-PDA-HRMS analysis the bioactive compounds were identified and quantified, after the digestion the molecules pattern undergoes a radical change with the disappearance of procyanidins. Concluding, the preservation of the anti-inflammatory activity in digested extract is probably due to the increased quantity of trans-cinnamic acid.

References

1. Hariri M., Ghiasvand R.; *Drug discovery from mother mature*, (2016), pp 1-24
2. Cheng D.M., Kuhn P., Poulev A., Rojo L.E., Lila M. A., Raskin I.; *Food Chem*, (2012) 2994-3002
3. Minekus M., Alminger M., Alvito P., Ballance S., Bohn T., Bourlieu C., Carrière F., Boutrou R., Corredig M., Dupont D., Dufour C., Egger L., Golding M., Karakaya S., Kirkhus B., Le Feunteun S., et al.; *Food and function,* (2014)
4. Symma, A. Hensel; *Phytochem*. (2022),. 809–833

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Oxidation of phytosterols: formation kinetics of phytosterol oxidation products (POPs) and evaluation of their purity and stability as related to storage

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Summary: The aim of the present work was to evaluate the oxidation kinetics of the main phytosterols and the changes in oxidised products (POPs) during low-temperature storage. Stigmasterol demonstrated higher resistance to early oxidation (1.11% drop) than both β-sitosterol and campesterol (53.75% and 50.62% drop, respectively) and a different oxidative behaviour

Keywords: *Oxidation, phytosterol, POPs, mass spectrometry*

Introduction

Phytosterols are bioactive compounds found in vegetable fats and foods of plant origin [1], known for their ability to reduce intestinal absorption of cholesterol [2]. However, the daily intake (300 mg/day) is not sufficient to achieve the cholesterol-lowering effect; in fact, 2 g are needed to reduce blood cholesterol level by 10% [3,4]. Various food products enriched in phytosterol are available to meet the consumers need, but the techniques that are usually used to incorporate phytosterols in food products such as microemulsion or microcapsule, expose these compounds to oxidation, leading to the formation of the Phytosterol Oxidation Products (POPs). POPs are reported as the onset of various diseases and negative health implications [1,5,6]. Therefore, it is required to reduce the oxidation of phytosterols by adopting the correct methods and monitor them properly. However, it must be emphasised that the lack of a validated analytical method as well as standard reference materials are the main problems that have remained unresolved to date. One of the approaches applied to overcome these, is to obtain POPs by oxidising pure phytosterols while monitoring their purity and stability to ensure correct analytical determination. To this end, the present work evaluated the kinetics of POPs formation and their stability during storage at low temperatures.

Experimental

For each commercial phytosterol standard, purity was first verified by gas-chromatograph coupled with Flame Ionization Detector (GC/FID). Known amounts of β-sitosterol, campesterol and stigmasterol after purification from the presence of any hydroperoxides, were dissolved in chloroform and then transferred to a previously weighed Petri dish. The solvent was evaporated and, as reported by Ansorena et al. [7], the reference phytosterol were exposed to high temperature (180 °C) under controlled humidity and oxygen, away from

light. The Petri dish were removed from the oven at different heating times (10, 20, 40 and 60 minutes). The oxidised phytosterols obtained were then collected and monitored by gas chromatography coupled with mass spectrometry (GC/MS, single quadrupole) [8]. The generated POPs were then stored at -30 °C and their purity was determined for a period of 4 months.

Results

Fig. 1 shows the oxidative behavior of the selected phytosterols. Stigmasterol was more stable than β-sitosterol and campesterol during the first 10 minutes of heat treatment. Moreover, β-sitosterol and campesterol showed a progressive decrease stabilised during the final stages of heating; furthermore, they were more susceptible to oxidation, reducing to a greater extent (83% and 81%, respectively) than stigmasterol (54%). The oxidative kinetic of each detected POP is shown in Fig. 2a, 2b, 2c.

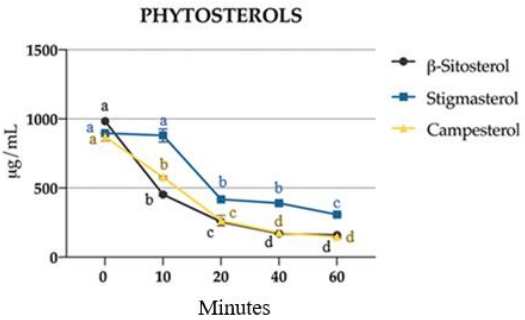

***Figure 1**. Phytosterols oxidative behavior reported as mean ± standard deviation of three independent replicates. Different letters denote statistically different means (Duncan's test p< 0.05)*

All isomers (7α/β-hydroxy, 7α/β-OH; 5,6α/β-epoxy; α/β-EP; and 7-keto, 7K) were identified and quantified after 10 min.

The 7k was the main isomer produced during the oxidation of all tested phytosterols. However, in the case of β-sitosterol it remained almost stable throughout the experiment; on the other hand, the 7k-campesterol and -stigmasterol underwent a significant ($p < 0.05$) increase after 40 min. The epoxide isomers, the second most isomers, abundantly formed during the oxidation of phytosterols. Specifically, for campesterol, a stable trend was observed during the heating; while on stigmasterol, a fluctuating trend was denoted for both isomers and the β-EP was less stable, since a significant decrease after 10 minutes was observed. The 7a/b -OH resulted in the smallest quantities. In general, for all phytosterols, the 7a-OH showed a significant increase at 40 minutes of heating. However, the 7b-OH did not significantly change in the case of β-sitosterol ($p > 0.05$), while decreased in case of campesterol and stigmasterol after 40 minutes ($p < 0.05$). The mixture oxidised up to 20 minutes was monitored during storage (-30 °C). About the b-sitosterol and campesterol, a significant decrease ($p < 0.01$) of both 7b-OH and a-EP, and

an increase of 7K was detected. Moreover, stigmasterol showed a different behaviour for all abovementioned isomers, except in the case of 7a-OH, which significantly increased.

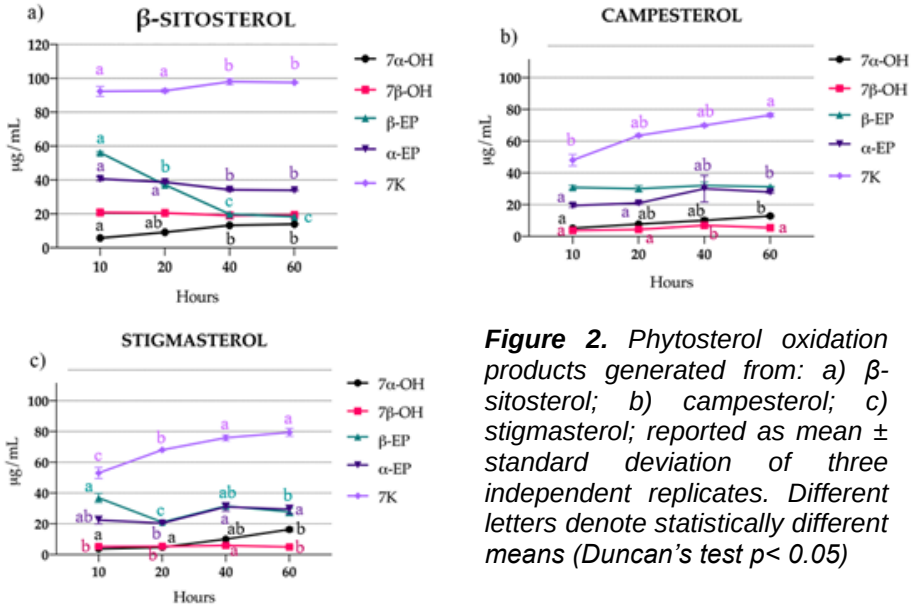

Figure 2. *Phytosterol oxidation products generated from: a) β-sitosterol; b) campesterol; c) stigmasterol; reported as mean ± standard deviation of three independent replicates. Different letters denote statistically different means (Duncan's test p< 0.05)*

Conclusions

The present work represents a preliminary study for better understanding the phytosterols oxidation, during both heat treatments and storage. Indeed, despite of the low stock temperatures of the POPs, a tendency to reach the equilibrium was observed. However, an evaluation with sensitive and robust methods including high-resolution mass spectrometry is required to identify secondary isomers, which could play a key role in the oxidation of phytosterol in complex food systems.

References

1. Gachumi, G.; Poudel, A.; Wasan, K.M.; El-Aneed, A. *Pharmaceutics* **2021**, *13*, 268.
2. Cuevas-Tena, M.; Alegría, A.; Lagarda, M.J. *Eur. J. Lipid Sci. Technol.* **2018**, *120*, 1800054.
3. Trautwein, E.; Vermeer, M.; Hiemstra, H.; Ras, R. *Nutrients* **2018**, *10*, 1262.
4. Cabral, C.E.; Klein, M.R.S.T. *Arq. Bras. Cardiol.* **2017**.
5. Scholz, B.; Guth, S.; Engel, K.-H.; Steinberg, P. *Mol. Nutr. Food Res.* **2015**, *59*, 1339–1352.
6. Wang, M.; Lu, B. *Trends Food Sci. Technol.* **2018**, *79*, 148–159.
7. Ansorena, D.; Barriuso, B.; Cardenia, V.; Astiasarán, I.; Lercker, G.; Rodriguez-Estrada, M.T. *Food Chem.* **2013**, *141*, 2757–2764.
8. Dutta, P. *Cholest. Phytosterol Oxid. Prod.* **2002**.

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Development and validation of LC-MS/MS method for the analysis of quinolizidine alkaloids in lupins

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Summary: *Quinolizidine alkaloids are compounds especially found in plants belonging to the Lupinus L. genus (Fabaceae family). In this work, a sensitive method involving the use of LC-MS/MS was developed in multiple reaction monitoring mode, with the aim of the simultaneous quantification and determination of thirteen alkaloids in Lupinus albus L.*

Keywords: *Quinolizidine alkaloids, Lupins, Multi Reaction Monitoring*

Introduction

Quinolizidine alkaloids (C_5NC_4 skeleton) are L-lysine derived compounds, having one or more nitrogen atoms usually contained in a heterocyclic ring system, which can be divided in bicyclic, tricyclic, and tetracyclic forms [1].

These compounds are especially found in plants belonging to the Lupinus L. genus (Fabaceae family), in which they impart the bitter taste and act as a defence mechanism against pathogens and herbivorous animals. There are almost 70 different quinolizidine alkaloids found in various lupin species, which levels and combinations vary according to botanical and geographic origin, but also to soil composition and climate. They can cause symptoms of poisoning in humans, affecting the nervous, circulatory, and digestive systems; for this reason, bitter lupin seeds are not suitable for human or animal consumption without a proper pre-treatment, in fact, it is necessary a debittering process [2].

According to this, Regulations in Australia, New Zealand, France and Great Britain require compliance with a maximum level of 200 mg/Kg of alkaloids in lupin flours and in the seeds themselves [3].

There are few methods in the literature about the quantification of alkaloids in this specific food matrix; most of them reported the use of gas chromatography coupled to mass spectrometry (GC-MS). In recent years, there has been an increase in the development of methods by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) to detect alkaloid compounds in various matrices, both food and biological [2].

In this work, a sensitive method involving the use of LC-MS/MS was developed in multi reaction monitoring (MRM) mode, with the aim of the simultaneous quantification of twelve different alkaloids. The analysis was conducted on different commercial forms of white lupin (L. albus L.), through the development of an efficient extraction procedure followed by a suitable clean-up step, performed by Solid Phase Extraction (SPE), in order to decrease the amount of interfering compounds and to obtain suitable recoveries. The presented analytical method was validated following FDA guidelines, which demonstrated the reliability and robustness of the procedure.

Experimental

200 mg of homogenized lupin samples were weighed and extracted in an homogenizer with 1 mL of methanol:water (MeOH:H_2O) 60:40 with 3 cycles of 10 sec at 7000 rpm with 45 sec stop between each cycle and they were centrifugated: 10 min at 4°C and 10000 rpm. 50 µL of supernatant was collected and diluted in 1 mL of 90:10 H_2O:MeOH; the resulting solution was loaded in a polimeric SPE cartridge for clean-up.
The sample was analyzed by means of Shimadzu Nexera LC 20AD coupled to Sciex Qtrap 4500 mass spectrometer equipped with ACE-Excel 2 C18-PFP 2.0 µm 100x2.1 mm column, operating in positive ESI mode.
The analysis was performed in multi reaction monitoring (MRM) mode.

Results

All the steps of the analytical method were developed and accurately tuned. First of all the ratios of inorganic and organic solvents in extraction solution were tested and the combination of 60:40 MeOH:H_2O was chosen. Moreover all the steps of clean-up by means of SPE were evaluated in order to obtain low matrix effect and good recoveries. Then the LC-MS/MS method was developed in order to obtain an efficient separation of the target analytes and a good peak shape to achieve a high signal to noise value, as shown in Fig. 1.
Finally the method was validated in according to international guidelines testing all the primary parameters, such as accuracy, precision, linearity, LOQs, LODs, carryover, recovery and matrix effect; all the results were in according to the chosen guidelines, and the method was applied on real samples.

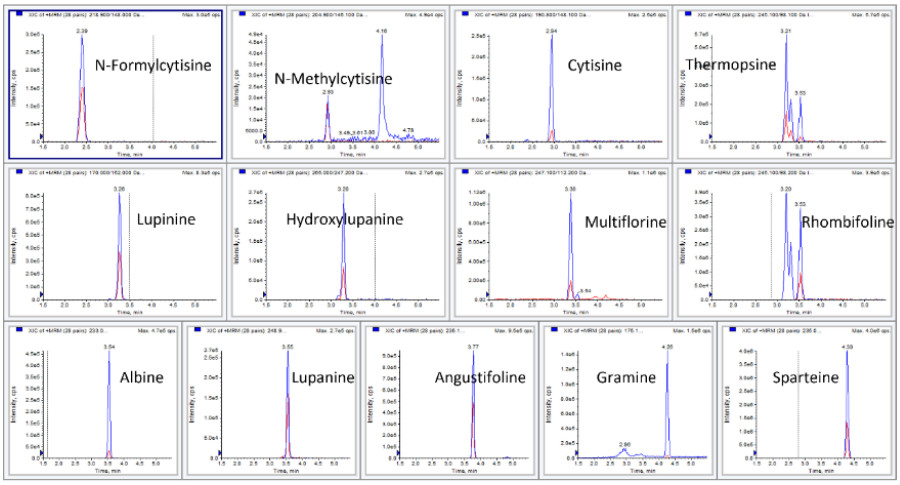

***Figure 1**. The various currents extracted from the selected analytes, obtained by means of C18-PFP Ace Excel 2 column*

Conclusions

In this work, a LC-MS/MS quantification method was developed and validated, together with a clean-up step providing good selectivity to the analyte detection. In according with validation results, a robust and reliable method was presented;

it was able to detect low concentrations of alkaloids in lupins with good precision and accuracy. Moreover, in our knowledge, for the first time a validated extraction and clean-up method for such wide range of analytes in LC-MS/MS analysis was presented.

References

1. T. Aniszewski, *Alkaloid Chemistry*, Chapter 2, (2015).
2. Federal Institute for Risk Assessment (BfR) Opinion No 003/2017.
3. D. Resta, G. Boschin, A. D'Agostina A. Arnoldi; *Mol. Nutr. Food Res*, 52, (2008), 490 –495.

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Determination of polycyclic aromatic hydrocarbons from dust deposition in wild edible plants by GC-q/MS analysis

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Summary: *Atmospheric dust generated by anthropogenic activities has a strong impact on human health and the environment, due particularly to the presence of organic micropollutants such as Polycyclic Aromatic Hydrocarbons (PAHs). This communication describes an analytical method useful for assessing the quantitative levels of PAHs in deposition on the leaf surface of wild edible plants.*

Keywords: *PAHs, atmospheric dust, contamination, wild edible plants, GC-q/MS*

Introduction

In 2004 the European Commission stated that the harmful effects of polycyclic aromatic hydrocarbons (PAHs) on human health and the environment are highly dependent on the deposition of settleable dust on the ground [1]. The latter mainly result from combustion and industrial and civil emissions. Once they reach the soil, persistent organic pollutants (POPs) such as PAHs can accumulate in the food chain through animals and plants [2]. Plants, and in particular wild edible plants (WEPs), could be used to assess the quantitative level of PAHs from settling dust and thus assess the contamination status of a given area. It is therefore necessary to have a simple and rapid analytical method for the determination of PAHs on the surface of WEPs. In the present study, *Portulaca oleracea* was used as a wild edible plant for the development of a method for the extraction and analysis of PAHs from deposition.

Experimental

The analytical protocol developed in the following work is divided into three phases. In the first step, 2 grams of fresh *Portulaca oleracea* leaves are placed in a beaker to which 30 mL of high purity cyclohexane (Romil, *Super Purity Solvent, SpS*) is added to minimise interference. Extraction is carried out in an ultrasonic bath at room temperature for 30 minutes. This is repeated one more time and finally the extract is filtered on filter paper and collected in a dark flask to avoid exposure to light. During the second step, the extract is concentrated to a volume of about 3 mL through a rotary evaporator set at a vacuum value of 235 mbar and a bath temperature of 40 °C. The concentrate is then passed over a column containing anhydrous sodium sulphate that has been previously dried in an oven for 24 h to remove the water present. Finally, the extract is reduced to a volume of 100 µL using a gentle stream of purified nitrogen. The last step involves analysing the extract by injecting 1 µL in gas chromatography single quadrupole mass spectrometry system (GC-q/MS).

Results

The proposed method was validated by determining the recoveries of six PAHs previously added to *Portulaca oleracea* samples. Two samples were added with 400 and 100 ng g^{-1}, respectively, and extracted through the proposed method. The average recovery rates (%), standard deviations (SDs) and relative standard deviations (RSDs) are reported in Table 1 whereas limits of detection (LODs) and limits of quantification (LOQs) are reported in Table 2. The linear dynamic range (LDR) of the method ranges between 50 and 500 µg kg^{-1}. All PAHs were quantified after a calibration curve performed for each molecule, using a mix of external standard. The calibration curves show r^2 > 0.99. The parameters reported showed the goodness of the methods for a sensitive and reliable determination of PAHs presents on the leaves surface of *Portulaca oleracea.*

Table 1. *Percentage recoveries along with standard deviations (SDs) and relative standard deviations (RSDs) of Portulaca oleracea samples spiked with 400 and 100 ng $g^{-1}$of PAHs.*

PAH	Recovery (%) at 400 ng g^{-1}	±SD	RSD (%)	Recovery (%) at 100 ng g^{-1}	±SD	RSD (%)
Benzo[a]anthracene	93.5	2.9	3.1	102.9	4.9	4.7
Benzo[b]fluoranthene	92.8	6.7	7.2	106.4	3.8	3.5
Benzo[j]fluoranthene	94.3	6.2	6.6	106.0	3.7	3.5
Benzo[k]fluoranthene	93.6	6.1	6.5	108.4	3.1	2.8
Benzo[a]pyrene	107.5	7.8	7.3	111.3	5.2	3.4
Indeno[1,2,3-cd]pyrene	93.9	17.4	18.5	92.3	4.5	4.1

Table 2. *Limit of detection (LOD) and limit of quantification (LOQ) of each PAH in the investigated WEP.*

PAH	LOD (ng g^{-1} fw)	LOQ (ng g^{-1} fw)
Benzo[a]anthracene	5	6
Benzo[b]fluoranthene	5	9
Benzo[j]fluoranthene	5	7
Benzo[k]fluoranthene	6	8
Benzo[a]pyrene	9	17
Indeno[1,2,3-cd]pyrene	10	15

Conclusions

The analytical determination of PAHs on the surface of the leaves of wild edible plants can be a useful tool for assessing the level of environmental and food contamination in a given area. Furthermore, it is possible to trace the emission source by studying the profile of the investigated PAHs. It is therefore essential to develop a simple and rapid method for a direct analysis of PAHs on the fresh leaves of WEPs. As the data show, the proposed analytical protocol is sensitive and accurate with excellent recovery levels and low standard deviation values. However, further studies will be carried out to assess the suitability of other

WEPs as indicators of environmental pollution.

References

1. Direttiva 2004/107/CE del Parlamento Europeo e del Consiglio del 15 dicembre 2004 concernente l'arsenico, il cadmio, il mercurio, il nickel e gli idrocarburi policiclici aromatic nell'aria ambiente.
2. A. Passuello, M. Mari, M. Nadal, M. Schuhmacher, J.L. Domingo; *Environmental International* 2010; 36(6):577-83.

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What's new on γ-oryzanol derivatives in millet and ancient wheat?

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Summary: *Steryl ferulates were investigated in twenty one ancient and modern wheat samples from Campania and Tuscany and twenty two millet samples belonging to Panicum, Pennisetum, Eleusine and Setaria genera. 10 steryl ferulates and 2 caffeoyl phytosterols were identified with campestanyl and sitostanyl as main steryl ferulates in wheat and millet.*

Keywords: *ancient cereals, steryl ferulates, high resolution tandem mass spectrometry*

Introduction

Steryl ferulates, esters of phytosterols with ferulic acid, can be considered a class of phenolic compounds, due to their phenolic moiety[1]. Research into steryl esters has mainly focused on rice and its γ-oryzanol content[2] because of its demonstrated beneficial effects on human health[3]. γ-Oryzanol, initially reported as single component, is now known to be a mixture of 10 molecules, with cycloartenyl ferulate, 24–methylene cycloartanyl ferulate and campesteryl ferulate as major components. The health benefits associated with γ-oryzanol include antinflammatory,antioxidant and chemopreventive properties[3-4]. It has also been proposed in the treatment of diabetes mellitus and prostate cancer[5.] Furthermore, free or esterified plant sterols/stanols have been recognized by the European Food Safety Authority for decreasing of plasma cholesterol and for maintenance of normal blood cholesterol levels[6].
Despite the beneficial properties of steryl ferulates, quantitative studies on cereals other than rice are currently rare.

Experimental

A total of 21 ancient and modern wheat samples from Tuscany and Campania and 22 millet samples belonging to different genera (Panicum, Pennisetum, Eleusine and Setaria), were investigated in terms of their steryl ferulates content. In addition one Nigerian sorghum, one commercial spelt from Garfagnana and one rice from Pavia were analysed. Steryl ferulates were determined starting from 5 g of grains and extracted thrice using 50 mL, 30 mL and 20 mL of CH_2Cl_2:MeOH (2:1 v/v). Each extraction was performed with the aid of ultrasonic bath for 20 min at 40 °C. The extracts were combined and dried under vacuum at 40 °C. The residue was re-dissolved in 1 mL of MeOH, centrifuged and analyzed using a HP 1200L liquid chromatograph equipped with a DAD detector

(Agilent Technologies, Palo Alto, CA, USA) with a Raptor ARC-18 column (150 × 3 mm, 5 µm, Restek, Bellefont, Pennsylvania, USA). An isocratic elution was applied with solvent A (CH_3OH) and solvent B (CH_3CN), 55:45 v/v. Total time of analysis 15 min, flow rate 0.6 mL/min and injection volume 20 µL. The same column and chromatographic conditions were performed for the HPLC-DAD-MS analyses using a Waters system (Milford, Massachusetts, USA) composed by 2695 HPLC, 2996 PAD and Quattro micro MS equipped with Zspray ESI source. The acquisition of the data was performed in negative ion mode from 150 m/z to 800 m/z, at 0.5 sec/scan rate. HPLC-ESI-HRMS and MS/HRMS analyses were performed according to Balli et al.,[7] on a LTQ-Orbitrap coupled to a Dionex Ultimate 3000 (Thermo Scientific, Bremen, Germany).

Results

The preliminary HPLC-DAD-MS analyses allowed identifying 12 compounds with different relative abundances depending on the analysed cereal. The MS/MS product spectra of compounds **1s**, **2s**, **5s**-**10s** showed a loss of 15 Da and a fragment at 193 m/z attributable to the ferulic acid moiety and a less intense fragment at 175 m/z. The MS/MS spectra of compounds **3s** and **4s** showed the loss of 15 Da and a fragment at 175 m/z. Compound **1*s** with a molecular weight of 562 Da and compound **3*s** with a deprotonated ion at 575 m/z showed a fragment ion at 179 m/z with empirical formula $C_9H_7O_4$, attributable to a caffeic acid moiety. These molecules were tentatively identified as sterol esterified with caffeic acid

Table 1. *Steryl ferulates composition in different cereals. R, rice; FM, finger millet; PM, pearl millet; MW, modern wheat; AW, ancient wheat; S, shorgum; SP, spelt.*

Steryl Ferulates	MW	$[M-H]^-$	Fragment ions (m/z)	Identified compounds	Matrices
1s	590	589	574;193;175	△7- Stigmastenyl ferulate	R
1*s	562	561	179	Caffeoyl phytosterol	S
2s	588	587	572;193;175	Stigmasteryl ferulate	R
3s	602	601	586;175	Cycloartenyl ferulate	R
3*s	576	575	179	Caffeoyl phytosterol	FM,PM,S
4s	616	615	600;175	24-Methylen-cycloartanyl ferulate	R,FM,PM,MW,AW
5s	576	575	560;193;175	△7- Campestenyl ferulate	R,MW,AW,SP
5§s	578	577		-	FM,PM
6s	576	575	560;193;175	Campesteryl ferulate	R,MW,AW,S
7s	590	589	574;193;175	Sitostenyl ferulate	R(trace)
8s	590	589	574;193;175	Sitosteryl ferulate	R,FM,PM,MW,AW,S,SP
9s	578	577	562;193;175	Campestanyl ferulate	R,FM,PM,MW,AW,SP
10s	592	591	576;193;175	Sitostanyl ferulate	R,FM,PM,MW,AW,SP

Campestanyl and sitostanyl were recognized as the main steryl ferulates in wheat and millet samples. Total steryl ferulates in Tuscan and Campania wheat samples ranged 37.6–62.3 µg/g dry weight and 14.4–56.6 µg/g dry weight, respectively. As concern millet samples, the lowest steryl ferulates content was found in Panicum, with values ranging from 2.98 µg/g to 8.72 µg/g. Foxtail millet and finger millet showed the highest amount with 46.07 µg/g and 85.29 µg/g, respectively. Noteworthy, finger millet was found to be rich in total steryl ferulates, with values similar to those obtained from rice. The results allowed pointing out different steryl ferulates compositions among different cereals, and suggested the possibility of using these compounds as markers for cereals differentiation.

References

1. Hakala, P., Lampi, A.M., Ollilainen, V., Werner, U., Murkovic, M., Wahala, K., Karkola, S., Piironen, V; (2002). *Journal of Agricultural and Food Chemistry, 50* (2002), 5300-5307.
2. Cho,Y.H., Lim, S.Y., Rehman, A., Farooq, M., Lee, D.J; *Journal of Cereal Science*, 88 (2019), 150- 481 156.
3. Burlando, B., Cornara, L.; *Trends in Food Science & Technology 40, 478* (1) (2014), 82-98.
4. Mattei, L., Francisqueti-Ferron, F.V., Garcia, J.L., Togneri Ferron, A.J., Vágula de Almeida, Silva, C.C…; *Molecular and Cellular Endocrinology, 537* (2021).
5. Son, M.J., Rico, C.W., Nam, S.H., Kang, M.Y.; *Journal of Food Science 76*(1) (2011)
6. Andersson, A.A.M., Dimberg, L., Åman, P., Landberg, R.; *Journal of Cereal Science, 59* (2014), 294-311.
7. Balli, D., Cecchi, L., Pieraccini, G., Innocenti, M., Benedettelli, S., Mulinacci, N.; *Journal of Food Composition and Analysis 109* (2022)

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Mineral elements in ethnic food purchased in the markets of southern Italy

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Summary: *Ethnic foods were analyzed in this study. The objective was to determine the content of six mineral elements and to determine by chemometric analysis a possible differentiation by type and geographical origin between the samples. The results showed optimal clustering by product type, but less efficient clustering by geographical origin.*

Keywords: *toxic elements, ethnic foods, food safety*

Introduction

In the last two decades, Oriental cuisine has undergone a great development, due to the globalization of the market and the increase of foreign communities in the European Union, reasons that have allowed the arrival of numerous ethnic products in Western countries [1]: in particular, seaweed and dried fish [2]. While these have beneficial properties for human health [3], negative concerns arise from their consumption in terms of food safety. The most relevant problem is the contamination of these products and, specifically, the possible presence of toxic elements derived from natural sources and/or anthropogenic activities [4].

Experimental

Therefore, in this study, the content of five toxic and potentially toxic elements (As, Cd, Cr, Hg, Pb, Tl) in seaweed and dried fish purchased from markets located in southern Italy was evaluated. In addition, iodine in seaweed was quantified.

Results

In the algae, the order of abundance was as follows: arsenic (mean = 8.19 ± 6.62 mg/kg), cadmium (0.38 ± 0.25 mg/kg), and lead (0.12 ± 0.10 mg/kg). In fish, As was also the most abundant element (mean = 0.47 ± 0.37 mg/kg), followed by Hg (0.12 ± 0.07 mg/kg).
Given the Cd limit set by the Centre d'Etude et de Valorization des Algues (CEVA, France) of 0.5 mg/Kg, only 5 out of a total of 26 seaweed samples had a cadmium content above this limit.
Among fish, on the other hand, only 1 out of 8 samples exceeded the maximum Pb limit imposed by Regulation (EC) No 1881/2006.
Correlations were observed between the different elements: Hg-As, Cr-Tl, Pb-Tl for seaweed, and As-Cd, As-Pb, Cd-Pb, As-Cr for fish.

The chemometric analysis produced an optimal grouping of samples by product type, while that by geographic origin gave less efficient results.

Conclusions

The mineral content determined in the fish and algae samples showed no results of concern. However, continuous monitoring of these products is essential to assess their contamination and to prevent risks to the consumer.

References

1. Mascarello G., Pinto A., Marcolin S., Crovato S., Ravarotto L., *Journal of Food Safety*, 37 (4), (2017), e12361.
2. Panebianco F., Nava V., Giarratana F., Gervasi T., Cicero N., *Journal of Food Composition and Analysis*, (2021).
3. Filippini, M., Baldisserotto, A., Menotta, S., Fedrizzi, G., Rubini, S., Gigliotti, D., Valpiani, G., Buzzi, R., Manfredini, S., Vertuani, S., *Chemosphere*, 263, (2021) 127983.
4. Di Bella G., Tardugno R., Cicero N., *Separations*, 5 (4), (2018), 51.

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Official controls for the determination of lipophilic marine biotoxins in mussels farmed along the Adriatic coast

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Summary: *Lipophilic marine biotoxins include okadaic acid, pectenotoxin, yessotoxin and azaspiracid groups. In this study, specimens of mussels were collected along the coasts of the Central Adriatic Sea during the years 2020-2021 and analyzed according to the European Harmonized Standard Operating Procedure.*

Keywords: *Marine biotoxins, LC-MS/MS, Mussels.*

Purpose

Marine biotoxins are produced by unicellular algae belonging to the genera *Alexandrium*, *Gymnodinium*, *Dinophysis*, *Prorocentrum* and *Pseudo-nitzschia* and they can accumulate in live bivalve molluscs as filter organisms. They are distinguished in water-soluble biotoxins, causing Paralytic Shellfish Poisoning and Amnesic Shellfish Poisoning, and lipophilic biotoxins mainly associated with Diarrhetic Shellfish Poisoning. According to the Regulation (EU) 627/2019 [1], the competent authorities have the task of periodically monitoring the production areas of bivalve molluscs, to verify the potential presence of toxic plankton and marine biotoxins. In this study, the results of official sampling carried out along the Adriatic coast from Martinsicuro (Teramo) to Termoli (Campobasso) in 2020-2021 were reported.

Methods

The sampling involved twenty mussel (*Mytilus galloprovincialis*) plants in Abruzzo and Molise regions (Central Italy), five of which located in province of Teramo, one in province of Pescara, four in province of Chieti and ten in province of Campobasso. A total of 159 and 175 samples were examined in 2020 and 2021, respectively. The samples were analyzed by the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise for the determination of okadaic acid (OA), dinophysistoxins 1 and 2 (DTX1, DTX2), pectenotoxin 1 and 2 (PTX1, PTX2), azaspiracid 1, 2 and 3 (AZA1, AZA2, AZA3), yessotoxin and its analogues (YTX, 1-Homo-YTX, 45-OH-YTX, 45-OH-homoYTX), applying the official method developed by the Community Reference Laboratory of Vigo (Spain) based on the use of mass spectrometry [2]. Briefly, the mussels were opened, separated from the shell, washed with running water to remove any residues, and pooled according to their origin, to constitute the global samples of 200 g. Then, 2 g of the homogenate were extracted with methanol, the extract was filtered and directly analysed by LC-MS/MS, to investigate the presence of

the above mentioned marine biotoxins. To determine the total content of OA group toxins, a preliminary alkaline hydrolysis from methanolic extract was required. The instrumental analysis was performed by UHPLC Exion LC coupled to Sciex Qtrap 6500^{+} equipped with IONDRIVE source set in the positive and negative mode. The chromatographic separation was obtained using a reversed-phase column X-Bridge C18 (50 x 2.1mm, 2.5µm), the flow rate was set at 0.3 ml/min and the injection volume was 20 µl. Two mobile phases (100% water with 2 mM ammonium formate + 50 mM formic acid and 100% acetonitrile with 2 mM ammonium formate + 50 mM formic acid) were used in gradient mode.

Results

The results of official controls for lipophilic marine biotoxins occurrence in mussels collected during the years 2020-2021 showed a good trend with regards to the compliance with the regulatory limits.

To express the results for each toxin group according to the European legislation (i.e., as µg equivalents/kg or mg equivalents/kg), the Toxicity Equivalent Factors (TEFs) were applied. Therefore, the individual content of each detected biotoxin was multiplied with the corresponding TEF before summarizing the total equivalents for the respective group toxin (European Food Safety Authority, 2009) [3].

The results showed the contamination of mussels only with OA and YTX. With regards to the first toxin group, a total of 49 (31%) and 25 (14%) positive samples were detected in the two years under investigation, respectively, at levels ranging from 41.6 to 159 µg equivalents/kg. Only 9 (6%) and 2 (1%) samples, in 2020 and 2021 respectively, were not compliant as they exceeded the regulatory limits (160 µg OA equivalent/kg) established by the Regulation (EC) 853/2004 [4]. These samples showed levels ranging from 163 to 269 µg OA equivalent/kg.

The presence of YTX was found in 28 (18%) and 56 (32%) samples in 2020 and 2021 respectively, at concentrations ranging from 0.060 to 0.284. These levels were always below the regulatory limit (3.75 mg YTX equivalent/kg).

Conclusions

The method applied in this study was able to determine the concentrations of lipophilic marine biotoxins, which must be routinely monitored to avoid the risk of consumer exposure with adverse health effects.

The modest quantities of lipophilic marine biotoxins found in the mussels analyzed in the present study demonstrated the good condition of the investigated marine areas as well as the safety of mussels harvested for human consumption.

References

1. Commission implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls. *Off. J. Eur. Union* L131:51.
2. EU-Harmonised Standard Operating Procedure for determination of

Lipophilic marine biotoxins in molluscs by LC-MS/MS (2015). *Version 5*, 1-33.
3. European Food Safety Authority (2009). Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on marine biotoxins in shellfish-summary on regulated marine biotoxins. *EFSA J.*1306, 1-23.
4. Regulation (EC) No 853/2004 of the European Parlamient and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs, *Off. J. Eur. Union* L139:55.

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Fresh or spoiled fish? Let's leave the word to biogenic amines (BAs) and mass spectrometry

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Summary: *Biogenic amines are indicators of freshness in food. The work presents an interesting method for the determination of BAs in fish. The method provide a solid phase extraction without derivatization coupled with LC-MS/MS analysis and achieves LOQ of 5 mg Kg^{-1} for spermine and 0.25 mg $Kg^{-1}$for the others amines.*

Keywords: *HPLC-ESI-MS/MS; biogenic amines; fish*

Introduction

Biogenic amines are low molecular-weight basic compounds found in various food such as fish, meat and fermented products (cheese, wine or beer) [1]. Prerequisites for the formation of biogenic amines include the presence of free-amino acids and suitable conditions for the growth of bacteria (low pH and high salt concentration) which have the ability to decarboxylate them. Fresh fish has high levels of free amino-acids and low levels of BAs which increases as decomposition progresses. The consumption of food containing high amine levels may lead to different food-born diseases (known as poisoning) due to their toxicity. Unfortunately most of BAs are heat and acid stable and they cannot be destroyed by cooking or freezing. The most important BAs are histamine, tyramine, putrescine, cadaverine, spermidine and spermine which are generated by decarboxylation of the free amino acids histidine, tyrosine, ornithine, lysine and arginine. Because of the structure of the precursor amino acids, BAs can have either aliphatic, aromatic or heterocyclic chemical structure. The analysis of BAs in fish tissues is of interest because of health risks and also because these compounds can be used as an indicator of food freshness or spoilage. Several researchers have proposed the Biogenic Amine Index (BAI) calculated from BAs levels in food and correlated with organoleptic qualities [2]. Due to their low volatility and lack of chromophores, biogenic amines are mostly analyzed by liquid chromatography with ultraviolet or fluorescence detection after derivatization with dansyl chloride, *o*-phtalaldehyde or other reagents [1]. However, derivatization reaction are time consuming and increase the risk of low recovery, analyte loss and contamination. In recent years LC-MS/MS has been used to quantify BAs without derivatization step. Neverthless the accurate determination of BAs with LC-MS/MS requires a sample clean-up step because of the large amount of interfering substances (matrix effect). Cleaning techniques usually involve solid phase extraction (SPE) that is generally carried out after pH adjustment because underivatized BAs have distinct chemical structure and pk_a values. The aim of this work was to develop an analytical method that simultaneously determinates six underivatized biogenic amines using a SPE followed by LC-MS/MS analysis.

Experimental

Biogenic amines, TCA and all the solvents were supplied by Sigma Aldrich, SPE STRATA X 33µ Cartridges 200 mg/6 ml were purchased by Phenomenex. Stock solution of each BA and Internal standard (IS) were prepared by dissolving 400 mg of each compound in 50 ml of HCl 0.1 M and were stored in glass bottles at 4°C. Working standard solution were prepared freshly by diluting the individual stock solution with HCl 0.1 M. Before being analyzed, the sample must be frozen to avoid decomposition processes. The extraction procedure was a slightly modified version of the method published in [1]. A representative portion (50 g) of fish was finely ground with a blender to homogenize it before extraction. A total of 5 g of fresh/frozen fish sample was weighed into a 50 ml polypropylene centrifuge tube and 100 µl of stock solution of IS was added. After the addition of 15 ml of 5% TCA, BAs were extracted with an Ultra turrax for 5 minutes at 10000 rpm. The extract was centrifuged and the supernatant was filtered. The residue was re-extracted with 10 ml of 5%TCA and the process was repeated. Then, both extracts were combined and 2 ml of these were brought to pH 11 by adding NH_4OH 28%. This extract was purified on SPE STRATA X cartridges, conditioned with 4 ml of methanol followed by 4 ml of water, at the rate of one drop every 10 seconds. The basic pH was used to make the compounds neutral and to allow the interaction with the cartridge. Due to the high stability of BAs at low pH, the passage of the extract through the cartridge should not take a long time. After sample loading was complete, cartridges were rinsed with 2 ml of MeOH/ H_2O (5/95 V/V) and dried under vacuum for 8 minutes to remove excess of water. It is very important that no water residues remain in the cartridges to avoid low recoveries. Analytes were eluted from the STRATA X sorbents with 2+2+1 ml of a solution of methanol/acetic acid (99/1 V/V). The elution solution was then diluted with 5 ml of HCl 0.1 M, filtered and injected into the LC-MS/MS. HPLC-MS/MS studies were performed using an Agilent 1260 Infinity series coupled to a triple quadrupole 6410 LC-MS/MS equipped with an ESI source operating in positive ionization mode. The separation of BAs was performed on a Synergy Hydro analytical column (250x4.6 mm ID particle size 4 µm) from Phenomenex and the LC conditions were as follows: injection volume 5 µl, flow rate 0.5 ml/min, column temperature 25 °C. The mobile phase consisted of ammonium formiate 15 mM and formic acid in water pH 3.3 (A) and methanol (B). The gradient program was 0 min 1%B ,0 -5 min 1 % B, 5-15 min 90%B, 15-20 min 1%B and 20-25 min 1%B.

Results

For the sample clean up step, were tested Strata X Cartridge 200 mg/6 ml and 100 mg/6ml. The former provided better recoveries and peak shape. In order to develop the LC-MS/MS method and the transitions of the molecules (precursor and product ions) an interesting study was performed by direct infusion of a solution of each amine (1 µg/ml). For this purpose, spectra were acquired in product ion mode. In our knowledge, many of the published articles propose only quantitative transitions instead the qualitative ones are not reported [1]. The selected qualitative and quantitative transitions are listed in the Table 1. The method was validating considering the following parameters: precision (RSD%), accuracy (recoveries), linearity and LOQ. Precision and accuracy were

determined by spiking blank tuna samples at different fortification levels of each BA (0,25, 1,3,5 and 10 mg/kg in matrix for all amines while 5,25 and 100 mg/kg in matrix for spermine). For each level of validation were performed 10 tests in order to determine the RSD% of each amine for each level of fortification. Accuracy was determined by calculating the average recovery for each level for each analyte. LOQ was estimated as the concentration of the analyte that gives a S/N equal at least to 10 and was calculated with a specific function of the Mass Hunter Agilent software which plans to inject 10 times the calibration standard which corresponds to the desired LOQ (5 mg/kg for spermine and 0.25 mg/kg for the others). In particular if the LOQ calculated is lower than the desired one it is possible to assume the latter as the LOQ of the method. One of the main problems of the method is that the presence of matrix components and the clean up step affect the response of BAs so it was necessary to use an IS (1,7 diamino heptane). For the same reason, calibration standards are not prepared in solvent but they are obtained by spiking know amounts of BAs on a blank sample. Due to dilution factor deriving from the extraction, the concentration of BAs in the matrix (mg kg^{-1}) will be diluted 25 times in the extract injected into the LC (µg ml^{-1}).Therefore, the BA concentration obtained from the calibration curve for both real and fortified samples must be multiplied by 25. Calibration curve of each BA, were constructed using quadratic least squares regression with a weighting factor of 1/x. All the calibration curves showed correlation coefficients $R^2 > 0,999$.

Table 1. *MRM transitions of biogenic amines*

Biogenic amine	**MRM transition (*m/z*)**	**Fragmentor (*eV*)**	**collision energy (*eV*)**
Cadaverine	103→**86**/69	22	**5**/10
Istamine	112→**95**/68	23	**10**/10
Putrescine	89→**72**/55	22	**5**/10
Spermidine	146→**72**/112/129	20	**10**/10/10
Spermine	203→**129**/112/84	48	**10**/25/25
Tiramine	138→**121**/103/77/91	20	**10**/10/38/10
I.S.	131→**114**/97	20	**10**/10

Conclusions

An analytical method for determining six underivatized BAs in fish by HPLC-MS/MS has been developed. It was validated by calculating repeatability, accuracy, LOQ and linearity. The method can be applied to the analysis of real samples and is useful for calculating the BAI index obtained as the ratio between different BAs.

References

1. G. Sagratini, M. Fernandez-Franzon, F. De Berardinis, G. Font, S. Vittori, J. Mañes; *Food Chemistry*, 132 (2012), 537-543.
2. V. Sirocchi, G. Caprioli, M. Ricciutelli, S. Vittori, G. Sagratini; *Journal of Mass Spectrometry*, 49 (2014), 819-825.

P76

APCI and HESI source evaluation to investigate nitrosamine formation in meat

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Summary: *Meat is a very complex matrix and the legal limits for some nitrosamines are extremely low (2 ug/kg). A method with adequate cleaning of the matrix and maximum sensitivity in ionization was then evaluated using an APCI – HESI source couple with a HQOMS.*

Keywords: *meat, nitrosamine, APCI, HESI, Orbitrap*

Introduction

Nitrosamines are known as carcinogens and could potentially formed in cured meat as result of the reaction of amines with nitrites, which are generally used as a preservative [1].

Experimental

In this study 4 samples of hums have been produced differently in order to encourage or not the development of nitrosamines. Various extractions were tested to find the best recovered of these compounds and the best matrix purification using acetonitrile:acetone (50:50, *v/v*) and EXtrelut NT combined with Florisil. Different columns were tested for correctly separate the compounds, finding in the use of Acclaim Vanquish 2.7 µm PA2 2,1x150 mm the best choice. As eluents water and acetonitrile with the 0.1% of formic acid were used in gradient and a sample SPE on-line pre-treatment was performed to also allow the injection of 100 µL. APCI source and HESI source, in positive and negative mode, were tested to evaluate the most performing ionization and high resolution mass spectra were acquired, scanning from *m/z* 50 to 500, in profile mode through full MS-data dependent MS/MS analysis (resolution 70,000 FWHM for m/z 200, 3 Hz). The maximum injection time (IT) was set at 100 ms and the automatic gain control (AGC) target at $3 \cdot 10^6$ ions. Data-dependent mass spectra were collected at a resolution of 17,500 FWHM (defined for m/z 200, 12 Hz, IT of 50 ms, AGC target of $1 \cdot 10^5$ ions).

Results

N-Nitrosodimethylamine (DMNA), N-Nitroso-N-methylethylamine (NMEA), N-Nitrosopyrrolidine (NPYR), N-nitrosodiethylammine (NDEA), N-Nitrosopiperidine (NPIP), N-Nitrosomorpholine (NMOR), N-Nitrososarcosine (NSAR), N-Nitrosodi-N-propylamine (NDPA), N-Nitroso-L-Proline (NPRO), N-Nitrosodi-N-butylamine (NBUT), 3-Nitroso-4-thiazolidinecarboxylic Acid (NTCA), N-Nitroso-2-methylthiazolidine-4-carboxylic Acid (NMCA), N-Nitrosonornicotine (NNN), N-Nitrosodiphenylamine (NDPhA), 4-(N-Nitrosomethylamino-1(3-pyridyl)-1-butanone (NNK) were tested. In solvent only NSAR best respond with APCI interface with a LOD of 0.2 µg/L while in HESI with a LOD of 2.5 µg/L. In meat

matrix analysis instead, the nitrosamines DMNA, NSAR, NMOR, NPRO, NMEA, NTCA, NDEA, NMCA and NPPI responded with 0.5-2 orders of magnitude higher, NPYR, NDPA and NBUT had no variation and only NNN, NNK and NDPhA showed a minor response.
In only one ham sample was found nitrite concentration up the LOQ, but nitrate was always present. Finally, the sample with nitrite was the only one the showed nitrosamine concentrations higher than LOD, NSAR at 6 µg/kg, NTCA at 140 µg/kg and NMCA at 403 µg/kg.

Conclusions

The HPLC-HQOMS has proved to be an excellent technique for the analysis of non-volatile and volatile nitrosamines. To evaluate all the tested nitrosamines with a low LOD both the source APCI and HESI must be use.

References

1. Lehotay, S. J., Sapoahnikova, Y., Han, L., Johston, J. J., Journal of Agriculture and Food Chemistry (2015), 63 (47), 10341-10351.

P77

Realignment of human saliva metabolites patterns in a diet-intervention study: the potential of GC×GC-TOF MS combined to fingerprinting to unravel the Advanced Glycation End-products effects

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Summary: *2D Salivary metabolome fingerprints acquired with different GC×GC-TOF MS setups are realigned using an easy-to-apply strategy. The UT fingerprint of the realigned dataset is used to evaluate the impact of Advanced Glycation End-products (AGEs) rich diets compared to a raw food control.*

Keywords: *Saliva metabolome, Fingerprinting, GC×GC-TOF MS*

Introduction

The use of saliva in clinical diagnostics is historically under-explored compared to other bio-fluids like urine or blood; the first can be considered superior because of its non-invasive sampling and the minimal training required for sampling/collection. In this study, GC×GC-TOF MS is adopted as fingerprinting platform to reveal saliva metabolite patterns resulting from a diet intervention study with meals rich in advanced glycation end-products (AGEs). In particular, a strategy is designed to effectively re-align 2D chromatographic images and enable reliable cross-comparative analysis. The crucial role of MS (implemented with a variable ionization energy source – i.e., tandem ionization) is discussed in relation to the features alignment and specificity of the matching.

Experimental

Saliva samples from healthy subjects were collected after 3 days of low AGEs (raw food) and high AGEs diet (baked products and grilled food) conducted at the University of Dresden [1]. Saliva metabolites were converted in trimethylsilyl derivatives by applying a standardized protocol before GC×GC-TOF MS analysis.

Seventy-one features effectively cross-aligned between chromatograms were found, of them fifty-one were putatively identified using the combination of MS spectral similarity matching (above a threshold of 700 DMF) and linear retention index (I^T) with a tolerance of ± 10 units. A selection of amino acids, mono- and di-saccharides, organic acids and amines were also analyzed as pure standards for identity confirmation and system performances evaluation.

Twenty-seven features were sub-dived in 3 groups based on their response relative intensity (Signal-to-Noise ratio) to guide the process parameters optimization. As a metric of positive performances, the rate of false negative matches was considered as resulting from the combination of MS similarity

threshold, template pattern distance threshold, and Signal-to-Noise ratio threshold [2].
Moreover, the 2D chromatograms generated within the study were acquired with different system setups to simulate long-term studies, which generate relative and absolute misalignments between the datasets – misalignment is illustrated in Fig. 1.

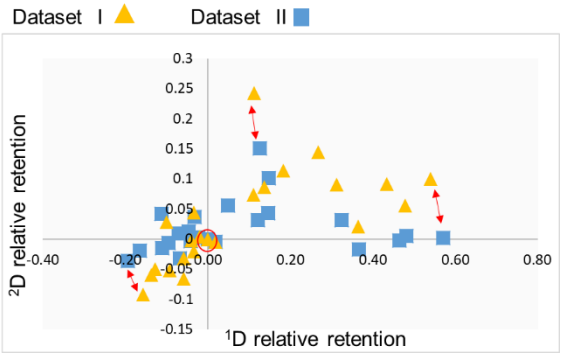

***Figure 1**. Relative misalignment in the two-chromatographic dimensions between two datasets acquired in a two-year time frame. Relative values are calculated taking Serine 3TMS as a reference centroid 2D peak*

The strategy used to compensate for the severe pattern misalignment of the datasets included the sub-division of the features template into four temporal regions with relative reference 2D-peaks (i.e., 1,4-Dibromobenzene, Proline, Oxoproline, and Palmitic acid) as local centroids to guide the template transformation – Fig. 2.

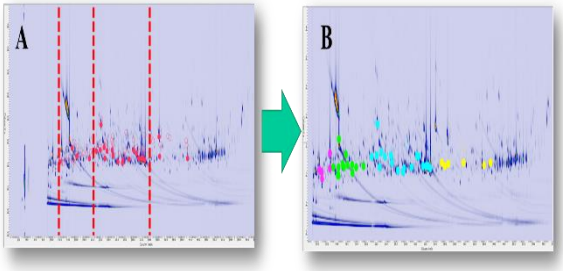

***Figure 2**. Template matching before (A) and after (B) the local realignment. The empty circle stands for an unmatched peak, while the filled circle stands for a matched peak. The different colours in B represent the four temporal regions*

Results

The realignment strategy allowed to increase the percentage of positively matched peaks from 51% (26/51 identified peaks) to 84% (43/51 identified peaks). The combined untargeted and targeted fingerprinting (*UT* Fingerprinting[3]) approach on saliva samples generated 430 UT features, which

were used to cross-compare saliva metabolite patterns of the two diet groups. A PCA on percent response data showed two natural clusters with partial overlap due to the higher intra-group variability of the high AGEs cluster – Fig. 3.

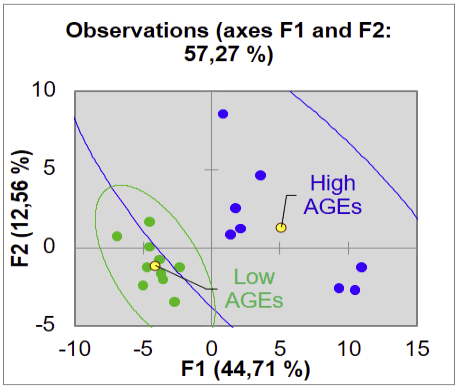

***Figure 3**. UT Fingerprint PCA (18 samples x 430 variables) showing two partially overlapping clusters*

PLS-DA was carried out to identify the analytes with the highest impact on the discrimination; among the 430 UT variables, 26 obtained a Variable Importance on the Projections (VIPs) score greater than 1, thus considered significant by the classification algorithm. In particular, amino acids such as Alanine, Aspartic acid, Glutamic acid, Proline, Serine, and Threonine all showed higher concentration in the low AGEs cluster, while free fatty acids such as Oleic acid, Palmitic acid, Palmitoleic acid, and Stearic acid were more abundant in the high AGEs cluster.

Conclusions

In this study severe pattern misalignment generated by different chromatographic setups were compensated by tuning processing parameters of template matching algorithm; local re-alignment with multiple centroids combined with the TOF MS spectra information resulted in a successful combination for effective cross-comparative analysis toward targeted and untargeted features. Despite the limited number of samples, by this study a proof-of-concept about the impact of a AGEs rich diet have an on the salivary metabolome is provided with greater yet specific impact on amino acids and both saturated and unsaturated fatty acids.

References

1. F. Manig, M. Hellwig, F. Pietz, T. Henle, PLoS One. 14 (2019) e0220208. https://doi.org/10.1371/journal.pone.0220208.
2. S.E. Reichenbach, P.W. Carr, D.R. Stoll, Q. Tao, J. Chromatogr. A. 1216 (2009) 3458–3466. https://doi.org/10.1016/j.chroma.2008.09.058.
3. M. Cialiè Rosso, F. Stilo, S. Squara, E. Liberto, S. Mai, C. Mele, P. Marzullo, G. Aimaretti, S.E. Reichenbach, M. Collino, C. Bicchi, C. Cordero, Anal. Bioanal. Chem. (2020) 403–418. https://doi.org/10.1007/s00216-020-03008-6.

P78

Semi-untargeted approach by HPLC-MS/MS for the identification of conjugated forms of PCs in different food matrices

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Summary: *Polyphenols (PCs) are a numerous class of bioactive molecules and are known for their antioxidant activity. In this work, EPI and NL scan modes were combined by using an IDA approach in order to investigate the polyphenolic content of different vegetal matrices (green coffee, saffron, hop).*

Keywords: *NL-IDA-EPI, polyphenols, HPLC-MS/MS*

Introduction

Polyphenols (PCs) are natural occurring secondary metabolites with significant physiological importance in plants and different biological functions on human health: they are known for their potential in the prevention and treatment of diseases related to oxidative stress. [1].

PCs are generally grouped into two main categories based on their structure and properties: non-flavonoid compounds, such as phenolic acids, stilbenes, lignans, and flavonoids.

Due to their complexity, a complete analysis of this compound is a challenging task, despite numerous analytical approaches are used for the determination of PCs in plants, such as liquid chromatography (LC), generally coupled to different detectors as UV-vis, DAD and MS. With LC-MS it is possible to work either in targeted mode [3], generally coupled to low resolution MS (LRMS), or in untargeted mode, usually with high resolution MS (HRMS) [4], respectively for quantitative and identification analysis.

As PCs are present in fruit and vegetables also in conjugated forms with sugar residues [2], we attempted investigate the conjugated forms of PCs in different food matrices by means of targeted and semi-untargeted approaches. The potential of the quadrupole/linear ion trap hybrid mass spectrometer (LIT-QqQ) was exploited to develop a semi-untargeted method for the identification of polyphenols in different food matrices: green coffee, Crocus sativus L. (saffron) and Humulus lupulus L. (hop). Moreover, thanks to semi-untargeted approaches several conjugate forms of flavonoids and hydroxycinnamic acid were detected using neutral loss (NL) as a survey scan coupled with enhanced product ion (EPI) scan based on information-dependent acquisition (IDA) criteria. Also a reliable and sensitive quantification method was developed and validated, showing good performance on several PCs.

The presented approach is focused on a specific class of molecules and provides comprehensive information on the different conjugation models that are related to specific base molecules, thus allowing a quick and effective identification of all possible combinations, such as mono-, di-, or tri-glycosylation or another type of conjugation such as quinic acid esters.

Experimental

The HPLC–MS/MS analysis of PCs was performed following Oliva et al. [3]. Briefly, a Nexera XR LC system (Shimadzu, Tokyo, Japan) was coupled to a Qtrap 4500 mass spectrometer (Sciex, Toronto, ON, Canada) equipped with a heated ESI source. Analytes were separated using an Excel 2 C18-PFP (10 cm x 2.1 mm ID) column.

Conjugated forms of PCs, not present in the target list, were identified using the NL-IDA-EPI mode to elucidate their structures based on known fragmentation patterns. With the survey scan (NL), it was possible to select all precursor ions characterized by a common moiety, such as the glycosidic fraction in flavonoids or quinic acid esters moiety in hydroxycinnamic acids; in particular, the following ions were selected: 132 amu corresponding to the mass of a pentose, 146 amu for rhamnose and coumaroyl units, 162 amu a hexose, and caffeoyl groups, 174 amu for quinoyl groups, 176 amu for feruloyl unit, and 308 amu for rutinose unit. The data obtained in the first phase of acquisition of the masses selected in the NL scan were then used to trigger EPI-dependent scans, using the IDA criteria pre-set, which made it possible to obtain characteristic fragmentation patterns for each analyte identified as showed in Fig. 1.

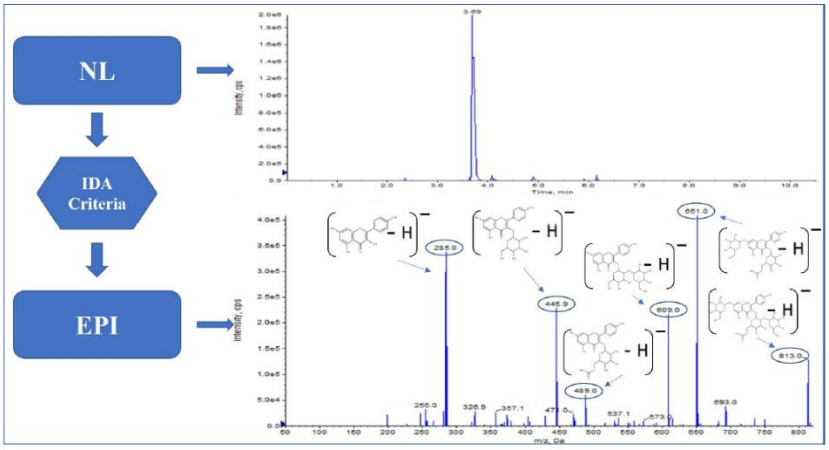

Figure 1. *Kaempferol-glucosyl-(1->2)-(6"-acetylgalactoside)-hexoside fragmentation pattern in Saffron*

Results

MRM analysis was used for the identification and quantification of PCs in the three plant matrices, The analysis allowed us to identify and quantify both phenolic acids and flavonoids and their respective glycosidic derivatives. The method showed a sensitive and robust quantitative analysis of the target analytes, providing LOQs ranging between 0.0004 and 0.06 ng mg^{-1}. Furthermore, the precision and accuracy of the method were suitable, with values included between ±10% near LOQs. The validation of the method was performed considering the LOQs, LODs, accuracy, precision, and linearity parameters.

For the identification of the conjugated forms of the PCs, NL-IDA-EPI scans were

then used with common losses both for the glycosidic forms of flavonoids and quinic acid esters of hydroxycinnamic acid, with neutral loss m/z of 132, 146, 162, 174, 176 and 308, corresponding to the mass of a pentose unit, rhamnose and coumaroyl units, a hexose and caffeoyl unit, quinoyl units, feruloyl unit, rutinose units, respectively.

Conclusions

By means of a rational exploitation of LIT-QqQ, it was possible to obtain a putative identification of different conjugated forms of the main flavonoids; EPI and NL scan modes were combined by using an IDA approach in order to investigate the polyphenolic content of different vegetal matrices (green coffee, saffron, hop) and identify a large number of mono-, di- and tri-glycosylated forms, as well as esters of quinic acid. Several compounds not belonging to the target list were detected in the selected matrices.

References

1. T A. Basli, N. Belkacem, I. Amrani; *Phenolic Compounds – Biological Activity – Chapter 10*, London 2017.
2. M. Morandi Vuolo, V. Silva Lima, M.R. Maróstica Junior; *Bioactive compounds: Health Benefits and Potential Applications - Chapter 2* (2019); pp 33-50
3. E. Oliva, E. Viteritti, F. Fanti, F. Eugelio, A. Pepe, S. Palmieri, M. Sergi, D. Compagnone *J. Chromatogr. A*, 1651 (2021), 462315.
4. O. López-fernández, R. Domínguez, M. Pateiro, P.E.S. Munekata, G. Rocchetti, J.M. Lorenzo, *Antioxidants,* (2020), 1–27.

P79

Monitoring of phytocannabinoids in hemp seeds and their products

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Keywords: *phytocannabinoids, hemp seeds and oils, UHPLC-HRMS/MS*

Introduction

Hemp (*Cannabis sativa* L.) is an annual plant containing a wide range of biologically active substances, the most important of which are the so-called phytocannabinoids. These unique secondary metabolites have various therapeutic effects on humans (antioxidant, neuroprotective etc.).[1] Dozens of phytocannabinoids are currently known, but among those legally regulated with psychotropic effects are phytocannabinoids from the tetrahydrocannabinol group.

The hemp plant has a wide range of uses, for example, in the textile, construction, pharmaceutical, and, of course, food industries.[2] In the case of specific plant parts, hemp seeds and products thereof, such as hemp oils or flours, are very popular. The seed lipids are rich in polyunsaturated fatty acids (linoleic and linoleic acids) and antioxidants such as carotenes and tocopherols. The seeds themselves are free of all phytocannabinoids, but contamination including the psychotropic delta-9-tetrahydrocannabinol (Δ^9-THC) may occur during contact with the resin contained in stalked glandular trichomes located on female flowers trichomes.[3,4]

The European Food Safety Authority (EFSA) has carried out an acute dietary exposure assessment for Δ^9-THC, which indicates that there is a health risk as far as the Acute Reference Dose (AfRD) of 1 µg/kg body weight is exceeded. In connection with the need to monitor the content of these psychotropic substances, there is a new proposal for a maximum limit that relates to the sum of Δ^9-THC and its acidic precursor delta9-tetrahydrocannabinolic acid (Δ^9-THCA). The maximum limit is then expressed as the sum of Δ^9-THC and Δ9-THCA adjusted value of 0.877.[5]

In 2022, the European Commission approved an amendment to Commission Regulation (EC) No. 1881/2006, which sets maximum limits for certain contaminants in food, including Δ^9-THC. This is 3 mg/kg for hemp seeds, products obtained from hemp seeds, and 7.5 mg/kg for hemp oil.

The aim of the work was the monitoring of phytocannabinoids in samples of hemp seeds and oils. The obtained results of Δ^9-THC content were then compared with the Acute Reference Dose (ARfD) according to EFSA. And the authenticity of individual samples is critically evaluated. The analysis was performed using ultra-high performance liquid chromatography coupled with tandem high-resolution mass spectrometry (UHPLC-HRMS/MS).

Experimental

In the first phase of the experimental part, the content of 17 phytocannabinoids

was determined in 10 samples of hemp seeds and 10 samples of hemp oil available on the Czech market. Hemp seeds were homogenized, weighed, and an appropriate amount of dichloromethane-methanol was added. The suspension was shaken, filtered, and collected for analysis. A mixture of deuterated internal standards was added to the sample taken in this way. Quantification was performed using an external matrix calibration where the matrix was without phytocannabinoids. In the case of hemp oils, only the weighed sample was dissolved in ethanol. The sample prepared this way was also taken for analysis with the addition of a mixture of internal deuterated standards. Quantification was performed using an external solvent calibration.
The analysis was carried out using the technique of reverse-phase ultra-high performance liquid chromatography coupled with tandem high-resolution mass spectrometry (UHPLC-HRMS/MS). Electrospray (ESI) operated both in positive and negative mode and was used for ionization. A quadrupole-orbitrap was used as a mass analyzer. The identification of the analyzed analytes was carried out on the basis of matching the retention times with the retention times of the analytical standards, then using the exact mass m/z of the ions of the analytes and the analytes isotopic.

Results

The total content of phytocannabinoids in hemp seeds ranged from 3.56 to 49.17 mg/kg. When evaluating the content of the sum of Δ9-THC and Δ9-THCA adjusted by a value of 0.877 expressed as THC, the content ranged from 1.03 to 7.04 mg/kg.
The highest finding of the total content of phytocannabinoids in hemp oils was 1437 mg/kg. Such a high finding was only in 2 samples when other samples did not exceed the level of total phytocannabinoid content of 100 mg/kg. When evaluating the content of the sum of Δ9-THC and Δ9-THCA adjusted by a value of 0.877 expressed as THC, the content ranged from 0.9 to 27.5 mg/kg. Carboxylic forms of phytocannabinoids, especially CBDA, cannabigerolic acid (CBGA), and ∆9-THCA, were the most represented in the oil samples as in the hemp seeds.
In the case of one sample purchased at the Czech market, none of the targeted phytocannabinoids were detected, though their traces are present in all common hemp oils. It might be due to the very effective purification of seeds used for processing or, more probably, the oil was not authentic. The authenticity of suspect oil can be based on a comparison of suspect oil and authentic hemp oil.

Conclusions

The total content of phytocannabinoids, as well as their relative ratios, differed significantly across the sample set. This may be due to the choice of different varieties of cannabis plants, the method of cultivation, or the method of collection or purification of the seeds themselves. Carboxyl forms of individual phytocannabinoids such as CBDA, CBGA, or Δ9-THCA prevailed. The same factors as the hemp seeds themselves may have caused the variability between results in the hemp oils.
Of all the samples tested, 2 samples of hemp seeds and 5 samples of hemp oils

did not comply with the newly proposed legislative limits for the sum of Δ9-THC and Δ9-THCA with an adjusted value of 0.877 expressed as THC. the possible exceeding of ARfD for Δ9-THC was considered, too. Supposing consumption of 10 g portion of by a person with body weight, then in case of 2 samples, and in the case of the sum, this dose would be exceeded in 5 samples, even by more than 6x. Exposure to such doses may pose a risk mainly for groups such as breastfeeding women, children, or the elderly.

Acknowledgement
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References

1. Li, H.; Liu, Y.; Tian, D.; Tian, L.; Ju, X.; Qi, L.; Wang, Y.; Liang, C. *European Journal of Medicinal Chemistry* 2020, *192*
2. Andre, C.M., Hausman J.-F., Guerriero G., Frontiers in Plant Science, 2016*, 17*
3. *Report Scientifically Safe Guidelines THC Food nova EIHA*. 2015 [cited 2022 8.6.]; Available from: http://eiha.org/media/2015/08/15-07-24-Report-Scientifically-Safe-Guidelines-THC-Food-nova-EIHA.pdf.
4. Citti, C.; Pacchetti, B.; Vandelli, M. A.; Forni, F.; Cannazza, G. *Journal of Pharmaceutical and Biomedical Analysis* 2018, *149*, 532–540.
5. Arcella, D.; Cascio, C.; Mackay, K. *EFSA Journal* 2020, *18* (1)

P80

Chemical characterization and evaluation of antioxidant activity from different cultivars of Cannabis sativa L. of Abruzzo's region

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Summary: *In this work, the chemical composition and the antioxidant evaluation of the inflorescences from 12 Cannabis sativa L. monoecious cultivars (Carmagnola Lemon CL, Ferimon F, Gran Sasso Kush GSK, Antal A, Carmagnola C, Kompolti K, Futura 75 F75, Villanova V, Tiborzallasi T, Finola FL, Kc Virtus KV and Pineapple P) cultivated at the same condition, were investigated. GC-MS analysis was carried out to evaluate the volatile fraction, while HPLC-MS/MS was used for cannabinoids and polyphenolic compounds. The evaluation of antioxidant activity was carried out using ABTS, Trolox equivalence antioxidant capacity (TEAC), ferric reducing antioxidant property (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays in vitro. The dataset was analyzed by univariate and multivariate analysis.*

Keywords: *Cannabis sativa L.; HPLC-MS/MS analysis, Cannabinoids*

ntroduction

In the last years, Cannabis sativa L. has been one of the most studied plants over the world [1]. The attention has usually been focused only to non-psychoactive cannabinoids; however, also other secondary metabolites as terpenoids, phenolic acids, flavonoids, glycosides, promote biological activities in the plant and, despite they are considered minor constituents of Cannabis sativa L., are a good potential source of bioactive compounds [2]. Among biological activities, these compounds are recognized as important antioxidant compounds
, then as potential natural food preservatives or possible candidate in pharmaceutical field [3]. In fact, the interest in Cannabis sativa L. is growing in the agricultural field, as this species can also be considered as an important source of antioxidant compounds; farmers aim to obtain new cultivars that are more resistant and easier to cultivate, with peculiar characteristics so that they can introduce these new genotypes in the food or also in the phytotherapeutic field [4]. In this work we present the characterization of different bioactive compounds and the antioxidant activity evaluation in vitro of different industrial hemp cultivars from Abruzzo territory cultivated in the same agronomic condition.

Experimental

The extracts were prepared using the procedure of Wang et al. (2018) with slight changes [5]. Briefly, before the extraction, every sample was homogenized. Fine powder of plant material (10 mg) was accurately weighed into a 1.5 mL Eppendorf vial and extracted with 1 mL of ethanol in an ultrasonic water bath for 30 min, followed by centrifugation at room temperature at 10,000 rpm for 15 min. Prior to HPLC analysis for the analysis for cannabinoids the supernatant was passed through a 0.2 µm PTFE filter, diluted 2000 times and collected in an HPLC vial while for polyphenols the supernatant involved in a second step of clean up by means solid phase extraction (SPE) [6].
The analysis of terpenes was analysed by SPME coupled to GC- MS [7]. The antioxidant activity (ABTS, FRAP and DPPH) was evaluated following the procedure in the literature [7].

Results

The results of SPME/GC–MS analyses on the volatile fraction obtained from hemp extracts shows a total of 28 compounds: 19 monoterpenes and 9 sesquiterpenes. Each cultivar shows a peculiar terpenoid profile, where FL and K have the higher relative amount of sesquiterpenes than the other cultivars, which are recognized for antioxidant activity. Concerning the chemical characterization of phenolic acids and flavonoids compounds, using HPLC-MS/MS, 19 compounds were analyzed: 9 flavonoids and 10 phenolic acids. The results of chemical composition of the hemp cultivars exhibited significant differences among cultivars because of the presence of specific phenolic acids or flavoinds. For the flavonoids content were distinguished F75 with high content of quercetin, apigenin, luteolin and diosmetin, CL with hiperoside and isoquercitin, GSK and F with significant concentration of rutin and T with significative content of catechin. Regarding phenolic compoundswere distinguished T for a richer profile of phenolic acids than the others. The cannabinoids profile shows high content of varines in CL, while KV, C and T presented significative concentration of acid cannabinoids CBGA and CBDA, the latter in common with GSK. The evaluation of antioxidant activity was correlate with the chemical profile of 12 cultivars showing a highest antioxidant activity of CL cultivar than the others, justified by synergic activity of the polyphenolic components, in particular the flavonoids, with the monoterpenes fraction and the cannabinoids. Following the antioxidant potential of CL, F75 present a good antioxidant activity for DPPH and FRAP assay due by a chemical profile rich in diosmetin, apigenin, quercetin and CBD content. GSK and F presented both good antioxidant potential, characterized by high content of gallic acid and, in addition, have the same terpene profile for D-limonene and β-myrcene recognized as strong antioxidant compounds. Finally, C, K, A, T, KV, V, P and FL have low antioxidant response, justified by a poor profile in terms of quantity of the cannabinoids and polyphenols.

Conclusions

In this work, 12 cultivars of Cannabis sativa L. belong to Abruzzo’s territory, grown and stored using the same agronomic approach, were analyzed to investigate their chemical composition, such as terpenes, cannabinoids and

polyphenols and the antioxidant potential. Our results indicated an interesting chemical profile for all the cultivars and the different composition of bioactive compounds is correlated to different antioxidant activity. In particular, CL, F75, GSK and F, presents a rich chemical profile correlated to a strong antioxidant capacity than the others. Except for F75, these cultivars are classified as a recent hybrid cultivar, selected by farmers to improve the content of the bioactive compound, and so increase the biological potential due to the synergism of all secondary metabolites. Therefore, this work shows as different cultivars of Cannabis sativa L. can be considered an excellent source of bioactive compounds, with good in vitro antioxidant activity and then considered promising candidates for different applications in food or pharmaceutical fields.

References

1. M.M. Radwan, 2017, *Springer*. (2017), 161-182.
2. R. Mechoulam, L.O. Hanu, R. Pertwee, A.C. Howlett, Nat Rev Neurosci. 15(11), (2017), 757–764.
3. C. Schillaci, R. Nepravishta, A. Bellomaria, *Albanian Journal of Pharmaceutilac Science* 1, (2014), 9–15.
4. R. Pavlovic, S. Panseri, L. Giupponi, V. Leoni, C. Citti, C. Cattaneo, M. Cavaletto, A. Giorgi, *Front Plant Sci*ence 10, (2019), 1265.
5. Y.-H- Wang, *Planta medica,* 84(04), (2018), 260-266.
6. E. Oliva, F. Fanti, S. Palmieri, E. Viteritti, F. Eugelio, A Pepe, M. Sergi, *Molecules*,*27*(10), (2022), 3089.
7. S. Palmieri, M. Pellegrini, A. Ricci, D. Compagnone, C. Lo Sterzo, Foods,*9*(9), (2020), 1221.

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Characterization of volatile compounds of hemp (*Cannabis sativa* L.) seed oil by SPME/GC/MS

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Summary: *Hemp seed oil is experiencing a growing interest, mainly due to its richness in healthy molecules. Several compounds contribute to its overall flavor. In our research the volatile fraction of cold-pressed oil of eight cultivars was evaluated by SPME/GC/MS. Differences were observed between the different cultivars.*

Keywords: *hemp seed oil, volatile profile, SPME/GC/MS*

Introduction

Recently there has been a renewed interest in low THC hemp (*Cannabis sativa* L.) in the food industry due to the beneficial properties provided by hemp seeds and oil as well. Hemp oil is extremely rich in linoleic (18:2 w6) and a-linolenic (18:3 w3) essential fatty acids and has an w6/w3 ratio between 2:1 and 3:1, which is considered to be optimal for human health [1,2]. Moreover, it is rich also in antioxidants, such as tocopherols and phenolic compounds [3].

The volatile compounds deriving from the plant can influence hemp seed oil aroma and the technological procedures such as pressing and storage could affect the volatile profile of hemp seed oil [4,5]. Among the different classes of molecules present in the volatile profile of hemp plant, terpenes contribute to the characteristic aroma. Terpenes, including monoterpenes and sesquiterpenes, are secondary metabolites contained into the trichome structures of the *Cannabis* plant and are responsible for the defence and interaction with pollinators and pests [6]. β-myrcene, α-limonene, α-pinene, β-pinene and β-caryophyllene are the most common terpenes detected in the essential oil of *Cannabis* [7]. The presence of terpenes was identified also in hemp seed oil and was attributed to the contamination from residuals of leaves and inflorescences. Although they are present as contaminants, these molecules add beneficial value to the hemp seed oil because they have anti-inflammatory, anti-cancer, and antioxidant activities [8,9].

To our knowledge, few data are available in literature on the volatile composition of hemp seed oil [4,5], while there are no studies on the characteristics of oils from different cultivars. In our research the volatile profile of cold-pressed oil obtained from eight hemp seed cultivars was characterized by solid phase micro-extraction coupled with gas chromatography-mass spectrometry (SPME-GC/MS).

Experimental

Hemp seeds from eight cultivars: Felina 32 (FEL), Futura 75 (FUT), Finola (FIN), Carmagnola (CAR), Carmagnola selection (CARM SEL), Uso 31 (USO),

Santhica 27 (SAN), Secuieni Jubileu (JUB) were cold pressed by a small-scale oilseed press. The extracted hemp seed oil was stored in amber glass bottles at 4°C under N_2 atmosphere, until the volatile compound analysis.
A DVB/CAR/PDMS, 50/30 um, 2-cm-long fiber was used to collect volatile fractions by SPME (Solid Phase Micro Extraction). The fiber was conditioned at 270°C for 30 min in a split/splitless GC injector before analysis. One mL of hemp oil was placed in a sealed glass vial with a pierceable septum. The hemp oil sample was kept at ambient temperature to equilibrate for 5 min and then the fiber was exposed to the headspace for 50 min. The extraction of volatile compounds from pressed hemp seed oil was performed in duplicate. The gas chromatographic analysis of volatile compounds was carried out according to Rizzolo et al. [10]. The identification of volatile compounds was performed by the following criteria: comparison with the mass spectra of the Wiley library, injection of authentic standards analysed under the same GC/MS conditions, calculation of Retention Indices (RI) and comparison with those obtained from both authentic standards and literature. Data were expressed as area unit $\times 10^{-6}$.

Results
The main chemical classes of volatiles detected were ketones, aldehydes, alcohols, free fatty acids, terpenes, and hydrocarbons (Table 1). Differences were observed among the cultivars as regards both the quantitative and qualitative composition of the oil. FIN oil showed the highest content of volatiles, consisting mostly of terpenes (about 90% of the total profile). A possible explanation of this result can be found in the small size and the higher surface to volume ratio of FIN seeds, that can cause an increase in the contamination from other parts of the plant that are rich in these molecules. CARM SEL and CARM demonstrated an abundant volatile fraction, as well, with a predominance of terpenes (about 50% of the total profile), followed by alcohols and free fatty acids respectively. FEL, FUT, JUB, USO and SAN were characterized by a less abundant volatile fraction, and only in FEL e FUT terpenes accounted for the half of the total volatiles. In SAN, JUB and USO a more balanced composition among the different classes was observed. Within terpenes the most abundant molecules were a-pinene and b-myrcene, followed by b-pinene and limonene, although differences were found among cultivars. Some authors report variations in the terpene composition of *Cannabis* essential oil deriving from different cultivars and their observations could support our results on seed oil [11].

Conclusions
Our work improves the knowledge of the composition of volatile profile of hemp seed oil. The preliminary data suggest that the cultivar can influence the composition of the volatile fraction of hemp seed oil. Terpenes found in hemp seeds could enrich the final product and characterize its origin and sensory properties.
This work was carried out within the project: "Enhancement of the hemp supply chain through product and process innovation" (CANAPRO), co-financed by the FEASR Operation 16.1.01 "PEI Operational Groups" of the Lombardy Rural Development Program 2014-2020.

Table 1. *Content of volatile compounds, grouped according to their chemical class, in hemp seed oils (values are expressed as area units x 10^{-6})*

	SAN	CARM	CARM SEL	FEL	FUT	FIN	JUB	USO
ketones	57,7	127,6	129,2	21,4	28,5	16,5	184,2	22,5
aldehydes	58,2	312,1	298,6	3,8	16,5	63,7	103,0	5,7
alcohols	301,9	328,2	1483,6	475,0	376,9	321,6	379,4	394,6
free fatty acids	162,9	455,7	202,9	96,3	66,6	223,8	393,0	134,3
terpenes	335,9	1808,2	2918,7	710,9	783,7	7309,2	402,7	444,9
hydrocarbons	159,3	342,9	273,5	268,3	101,0	138,9	209,3	192,1
total	1076,0	3374,6	5306,4	1575,7	1373,3	8073,8	1671,7	1194,1

References

1. A.P. Simopoulos, A. Leaf, N. Salem; *Prostaglandins Leukotrienes and Essential Fatty Acids,* 63(3) (2000), 119–121.
2. J.C. Callaway; *Euphytica*, 140 (2004), 65–72.
3. F. Blasi, C. Tringaniello, G. Verducci, L. Cossignani; *LWT - Food Science and Technology*, 158 (2022).
4. A. Gaca, E. Kludská, J. Hradecký, J. Hajšlová, H. H. Jele; *Molecules* 26, 285 (2021).
5. M. Tura, D. Ansorena, I. Astiasarán, M. Mandrioli, T. Gallina Toschi; *Antioxidants* 11, 490 (2022).
6. S. R. Sommano, C. Chittasupho, W. Ruksiriwanich, P. Jantrawut; *Molecules*, 25, 5792 (2020).
7. A. S. Wanas, M. M. Radwan, S. Chandra, H. Lata, Z. Mehmedic, A. Ali, KHC Baser, B. Demirci, M. A. ElSohly; *Natural Product Communications*, 15 (5): 1 (2020).
8. C.M. Andre, J.-F. Hausman, G. Guerriero; *Frontiers in Plant Science*, 7 (2016).
9. C. Leizer, D. Ribnicky, A. Poulev, S. Dushenkov, I. Raskin; *Journal of Nutraceuticals, Functional & Medical Foods* 2(4) (2000) 35-51.
10. A. Rizzolo, G. Bianchi, M. Povolo, C.A. Migliori, G. Contarini, V. Pelizzola, T.M.P. Cattaneo; *Food Packaging and Shelf Life,* 8 (2016) 41–49.
11. G. Micalizzi, F. Alibrando, F. Vento, E. Trovato, M. Zoccali, P. Guarnaccia, P. Dugo, L. Mondello; *Molecules* 26, 1588 (2021).

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Salting-out Assisted Liquid-Liquid Extraction for the rapid and simple simultaneous analysis of pyrrolizidine alkaloids and related *N*-oxides in aromatic herbs

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Summary: *the aim of this work was to develop a sustainable analytical methodology SALLE-UHPLC-MS/MS to monitor the presence of the 28 PAs/PANOs suggested by the EFSA in seven different aromatic herbs in order to broaden knowledge about the occurrence of these contaminants in aromatic herbs and ensure their food safety.*

Keywords: *pyrrolizidine alkaloids, aromatic herbs, UHPLC-MS/MS*

Introduction

PAs are natural toxins produced by the secondary metabolism of plants as a defense mechanism against herbivores and insects. Currently, more than 600 different types of PAs and their PANOs have been identified in a wide variety of plant species (>6000), but the great majority of them (about 95%) belong to the families of Asteraceae, Fabaceae, Boraginaceae, Orchidaceae and Apocynaceae [1]. 1,2-Unsaturated PAs are defined as hepatotoxic, genotoxic, and carcinogenic agents and they are one of the most significant hepatic phytotoxins class. The major sources of PAs consumption in humans seem to be products contaminated with these PAs-producing plants. in the last two years, these alerts have noticeably increased for other products such as spices and aromatic herbs, highlighting the striking number of alerts raised for the relatively high amounts of PAs found in oregano [1].

Experimental

SALLE was evaluated as extraction and clean-up technique for the analysis of PAs/PANOs in aromatic herbs. UHPLC-MS/MS was selected as highly sensitive and selective multiresidue method. The proposed method was validated and applied to aromatic herbs samples.

Results

The analytical method was properly validated, with extraction efficiency from 75 to 105% and satisfactory intra-day (< 11) and inter-day (< 13) precisions. The method proved to be a sustainable analytical strategy which meets green analytical chemistry principles as it reduced use of organic solvents and used green solvents. Its feasibility was verified through the analysis of 105 aromatic herbs samples. Of the samples analyzed, 95% were contaminated. Lasiocarpine, lasiocarpine N-oxide, europine, europine N-oxide, senecivernine, senecionine, lycopsamine N-oxide and intermedine N-oxide were the alkaloids which significantly contributed to the contamination of the samples.

Conclusions

A sustainable and green analytical methodology based on the SALLE procedure combined with UHPLC-MS/MS analysis was successfully developed and properly validated to monitor the presence of the 28 PAs and PANOs suggested by the EFSA in seven different aromatic herbs. The excellent performance, simplicity, speed and sustainability make it suitable for PAs and PANOs monitoring and occurrence studies in aromatic herbs, in order to guide future regulations for these products.

References

1. Casado, N., Morante-Zarcero, S., Sierra, I. (2022). *Trends in Food Science & Technology.*

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Ready biodegradability test and UHPLC-qToF analysis of Metarecod, a 100% natural therapeutic substance based medical device

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Summary: *Ready biodegradation test (RBT) represents a first-tier screening test in the assessment of environmental exposure of chemical and pharmaceutical products. In this work the RBT was implemented by applying advanced analytical techniques based on UHPLC-qToF. The new procedure was used to study Metarecod, which was proved to be a readily biodegradable therapeutic product.*

Keywords: *UHPLC-qToF, Metarecod, Environment.*

Introduction

Biodegradation is the process by which organic substances are decomposed by microorganisms into the simplest natural building blocks (eg CO_2, H_2O, NH_3) that can be integrated into the natural biogeochemical cycles. Anthropic and industrial activities have led to the emergence of a series of new polluting compounds and their release into the environment is responsible of a number of adverse effects (as they enter into the food cycle), therefore their correct evaluation is essential for a good environmental risk assessment (ERA). One of the tests more used to evaluate biodegradation of pure chemical and pharmaceutical compounds is the ready biodegradability test (RBT) [1], which was introduced into regulatory testing over 30 years ago. Currently, the RBTs protocols most used are published by OECD, comprising OECD 301 test series [2]. RBTs are known to have a number of well-documented limitations [3]; To overcome them we proposed to implement this test by adding more accurate measurements based on UHPLC-qToF technique [4].
In this work we applied for the first time this new analytical approach to study *Metarecod*- a medical device based on natural ingredients useful for the rebalancing of one or more metabolic parameters such as cholesterol, triglycerides, glycaemia up to the treatment of Metabolic Syndrome.

Experimental

Samples collected from the vessels at the beginning (T0) and at the end (T28) of the RBT were randomized and analysed by means of a UHPLC 1290 coupled to a qToF 6545 (Agilent) using RP18 chromatographic column, in high resolution (2GHz) by means of AIF MS/MS DIA acquisition technique. Positive and negative ion modes were acquired at two different collision energies: 0, 30 eV. The raw data were handled and a peak list with m/z values and the corresponding peak intensities was generated. Confidence level was set at 95%,

the model validity and predictivity was assessed by classical multivariate statistical tests (R^2X, Q^2, T^2-Hotelling, DModX). Principal component analysis (PCA) was carried out according to the method of the co-variance. Hierarchical Cluster Analysis (HCA) was carried out according to Ward's linkage method and Euclidean distances.

Results

*Metarecod***,** a complex product 100% natural, is a medical device based on "NeoPolicaptil", a mixture of natural ingredients capable of making a gel that delays the absorption of nutrients including glucose, cholesterol and triglycerides.

The ready biodegradability test was carried out according to OECD 301F, characterized by the measurement in continuous of the oxygen consumed by a microfauna (collected from sediments and sewage treatment plants). The test is considered passed if the 60% of the theoretical oxygen demand removal is achieved. This level must be reached in a 10-days window, within 28 days. Chemicals that achieve this goal later, are not considered to be readily biodegradable. In our case, *Metarecod* reached the 77% of biodegradation by the 28th day. In order to investigate the behaviour of our product at the molecular level, samples collected at the beginning (T0) and at the end (T28) of the OECD 301F test were studied by acquiring fingerprints using the UHPLC-qToF. The untargeted study (figure 1) by means of the PCA analysis showed typical clusters. The samples at T28 are arranged in the space closer to the medium samples to attest that within 28 days the product was biotransformed.

Figure 1. *Untargeted study_ PCA (Principal Component Analysis) of Metarecod.*

A targeted investigation was carried out to evaluate some specific compounds representative of the product such us Stevioside, Rebaudioside A and Rebaudioside C. Therefore, by the extract ion chromatograms of ions at m/z 804.3774 $(M-H)^-$, m/z 966.4302 $(M-H)^-$ and m/z 950.4353 $(M-H)^-$, respectively, their presence was checked. After 28 days from the beginning of the RBT neither Stevioside, Rebaudioside A and Rebaudioside

C nor their direct derivatives were detected, confirming the results obtained previously.
The same analytical approach was used to test another therapeutic product, referred as *Product A,* based on *Metformin* a hypoglycaemic API with a widespread use in Europe and in the world to treat dysglycidemia. It is common to several pharmaceutical brands and was detected in the environment since early 2000s [5]. *Product A* didn't pass the OECD 301F criteria, resulting not readily biodegradable. We suppose that this was due to *Metformin*, already classified as not readily biodegradable (tests OECD 301D and OECD 301F) [5]. However, conflicting data on the biodegradation of *Metformin* are available [6a and 6b]. Therefore, to better investigate its behaviour UHPLC-qToF fingerprints of samples at T0 and T28 form the RBT were collected and the untargeted study by means of the PCA was performed. Samples at T28 were arranged in the space distant from the medium samples to attest that within 28 days the product was not biotransformed. The targeted investigation carried out by extracting the ion chromatogram (EIC) at m/z 130.1102 $(M+H)^+$ revealed the presence of Metformin at the end of the RBT (T28). To better appreciate the effect due to biodegradation during the test, metformin was semi-quantificated by calculating the normalized area% at T0 and T28. The results showed that *Metformin* at T28 is practically not biodegraded with an area% of 98.48%, while *Metformin* metabolites, Guanylurea, Methylbiguanide, 2,4-AMT/4,2,1-AIMT, were found at a very low intensity. In fact, the sum of the area% of these compounds was about 0.09%.

Conclusions

The results of this study could encourage updating RBT tests with new analytical techniques based on accurate mass spectrometric measurements, useful for holistic evaluation of test complex mixture of complex formulated therapeutical products. The new procedure, applied for the first time to Metarecod and *Product A,* has shown that Metarecod it is readily biodegradable while *Product A* based on *Metformin* was not.
In conclusion, these results may open up the opportunity to extend the concept of risk/benefit assessment of therapeutic products to the environment, which is in line with the current strategies of the European green deal.

References

1. European Medicines Agency (CHMP), EMEA/CHMP/SWP/4447/00 corr2, 2006.
2. OECD Test No. 301, Ready Biodegradability. 1992.
3. A. Kowalczyk, T.J. Martin, R.A. Egmond, C.J. Finnegan, H. Schäfer, R.J. Davenport, G.D. Bending, *Ecotoxicol Environ Saf.,* 111 (2015), p. 9-22.
4. L. Mattoli, G. Proietti, C.M. Quintiero, G. Fodaroni, M. Burico, M. Gianni, E. Giovagnoni, Vo. Mercati, C. Santi, *Environ. Sci. Adv.*, (2022), DOI: 10.1039/d1va00038a
5. C. Trautwein, K. Kümmerer, *Chemosphere*, 85 (2011), 765-773.

6. a) M. Markiewicz, C. Jungnickel, S. Stolte, A. Białk-Bielińska, J. Kumirska, W. Mrozik, *Journal of Hazardous Materials*, 324 (2017), 428-435.; b) O.J. Straub, D.J. Caldwell, T. Davidson, V. D'Aco, K. Kappler, P.F. Robinson, B. Simon-Hettich, J. Tell, *Chemosphere*, 216 (2019), 844-854.

P84

Optimization of glucosinolates ultrasound-assisted extraction from *Camelina sativa* L. by-products and their activity on human cancer cells

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Summary: *Waste produced by the food chain is a source of bioactive molecules. In this work, Camelina sativa by-product was used as a source of glucosinolates. For this purpose, a green extraction method was developed to increase their recovery and the optimized extract was tested in vitro to evaluate its activity.*

Keywords: *Camelina sativa by-product, experimental design, ultrasound-assisted extraction*

Introduction

Cruciferae are plants responsible for numerous activities; intake of these plants appears to reduce the risk of developing lung, stomach and colorectal cancer[1-2]. Glucosinolates (GLSs) are some of the metabolites contained in these plant families that contribute to their protective effect. Several studies are known to highlight the activity of these biomolecules, for example against heart disease or neurodegenerative diseases[3-4]. One of the Brassicaceae containing GLSs is *Camelina sativa* Crantz. (L.), cultivated mainly for the oil produced from the seeds that is rich in fatty acids[5]. Following the pressing of the seeds, a seed-press cake by-product remains that could be a source of GLSs and other beneficial metabolites on human health. The aim of the work is to optimize an environmentally sustainable extraction method for the recovery of GLSs from the by-product of *C. sativa*.

Experimental Design

The first phase of the work involved the identification of GLSs present in the *C. sativa* by-product extract, obtained following the ISO method[6], using ultra-pressure liquid chromatography (UPLC) coupled with a high-resolution mass spectrometry (HRMS). Next, a green extraction method based on ultrasound-assisted extraction (USAE) was optimized to reduce the time, cost and toxic solvents of the process through an experimental design using a response surface design. Finally, the optimized extract was purified by solid phase extraction (SPE) to concentrate the GLSs, and the purified extract was tested on healthy and cancerous colon cells.

Results and Discussion

Chemical characterization of ISO extract identifying 11 phytochemical compounds belongs to phenol and glucosinolate class. The main analytes in *C. sativa* seed-press cake by-product are three glucosinolates: glucoarabinin,

glucocamelinin and homoglucocamelinin[7]. Given the interesting content of GLSs in *C. sativa* seed-press cake, their recovery by ultrasonic extraction was optimized considering all process parameters (n° of the cycle, % ethanol, and extraction volume). The optimization was evaluated by a Box-Behnken 2 factor interaction design. The result showed a maximum recovery of all three GLS using 65% of Ethanol, 5 mL of solvent and 2 cycles. USAE optimized extraction improves the recovery of glucoarabinin, glucocamelin and homoglucocamelin by 501%, 878% and 234%, respectively than conventional ISO method. The extract optimized was then purified to obtain an extract enriched in GLSs to test their effect in vitro. The results on cells showed an interesting antiproliferative activity, the extract rich in GLSs increases in antioxidative metabolism in cells without toxic effects on healthy lines.

Conclusions

The study allowed the development and optimization of a green extraction method for the recovery of GLSs from the by-product of *Camelina sativa*, underlining its use as a possible source of active molecules. In addition, the extract obtained showed promising antioxidant activities indicating its possible use for the preparation of nutraceutical products promising against cellular oxidative stress for the prevention of cancer forms.

References

1. Verhoeven D.T., Goldbohm R.A., van Poppel G., Verhagen H., van den Brandt P.A. *Cancer Epidemiol Prevent Biomark*. (1996) 5:733–48.
2. Higdon J.V., Delage B., Williams D.E., Dashwood R.H. *Pharmacol Res.* (2007) 55:224–36.
3. Vig A.P., Rampala G., Thind T.S., Arora S. *LWT Food Sci Technol.* (2009) 42:1561–72.
4. Traka M.H. *Adv Botanic Res.* (2016) 80:247–79.
5. Zubr J. Oil-seed crop: camelina sativa. *Industr Crops Prod.* (1997) 6:113–9.
6. Norm ISO. *ISO* (1992) 9167:1–9.
7. Berhow M.A., Polat U., Glinski J.A., Vaughn S.F., Isbell T., et al. *Industr Crops Prod.* (2013) 43:119–25.

P85

Alkyl pyrazines determination in roasted hazelnut pastes by gas chromatography – ion mobility spectrometry

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Summary

Alkyl pyrazines are VOCs generated in foods by Maillard reaction during thermal treatments, which play an important role in the aromatic profile of roasted hazelnuts. GC-IMS is a rapid analytical technique with arising importance in food chemistry. In this study a quantitative approach based on GC-IMS to determine the content of alkyl pyrazines was developed and applied to roasted hazelnut pastes from different geographical origins.

Keywords: *GC-IMS, alkyl pyrazines, targeted*

Introduction

The characteristic aroma of roasted hazelnuts is the key-driver of their consumption and industrial use. Pyrazines are Maillard reaction products generated during thermal treatment processes, such as roasting. They are responsible for roasty and earthy notes characteristic of roasted food matrices, and several alkyl pyrazines have been reported as key-odorants of roasted hazelnut (*Corylus avellana* L.). Due to their low odour-thresholds, they are crucial to determine the aromatic profile even though their concentrations in kernels is low (ng/kg) [1].

Gas chromatography coupled with ion mobility spectrometry (GC-IMS) is an emerging analytical technique which is rapidly gaining popularity in food flavour analysis due to its robustness, high sensitivity and the second-dimension separation provided by IMS [2]. Most of the published studies are based on untargeted fingerprinting and qualitative approaches, while little research is focused on quantitative studies targeting specific classes of aroma compounds [3]. This is due to two peculiarities of the IMS working principle that makes the quantification challenging: (i) the formation of multiple ionized species (monomer and dimer) from a single analyte, and (ii) the non-linear detector response.

In this study, we focused on alkyl pyrazines and their content determination in roasted hazelnuts applying GC-IMS technology for a targeted and quantitative approach.

Experimental

A FlavourSpec GC-IMS system (G.A.S., Dortmund, Germany) coupled with a headspace autosampler HT2000H (HTA, Brescia, Italy) was used in the two phases of this study. In the first phase, we studied the concentration-response

curves of 8 alkyl pyrazines over a 3 order of magnitude range of concentrations (0.1-100 µg/g). Two different model matrices (a mix of medium chain triglycerides – MCT – and a hazelnut paste physically treated to remove the majority of volatiles components – deodorized hazelnut paste) were tested to evaluate the impact of the matrix effect of hazelnut paste on the target analytes. In the second phase, hazelnut paste samples obtained by roasting kernels from different geographical regions (Italy and Turkey) have been analyzed. The roasting process was carried out in a pilot scale infrared roaster at 140°C. Starting from the results of the first phase, a quantification protocol based on external standard calibration has been optimized and applied to determine the content of the identified analytes. The ion (monomer or dimer) used for the quantification was carefully selected depending on the analyte concentration and the presence of coeluting peaks.

Results

The results of the first phase showed a non-negligible matrix effect, explained by the different fat percentage in MCT and hazelnut paste. Moreover, a relevant impact of the pyrazine ring substitution pattern on the concentration-response curve trends was observed, highlighting the need of an external standardization approach to perform a reliable quantification. Five of the target alkyl pyrazines (2-methyl, 2,5-dimethyl, 2,6-dimethyl, 2-ethyl, and 2,3,5-trimethylpyrazine) have been identified and quantified in the hazelnut paste samples. 2-methylpyrazine and 2,5-dimethylpyrazine are the most abundant pyrazines, while 2,6-dimethylpyrazine concentration is under the limit of quantification.

Conclusions

The implementation of a quantitative approach extends the GC-IMS applicability for targeting specific aroma compound classes. This methodology could be successfully applied for the characterization of food flavour compounds in the agro-industrial field.

References

1. J. Kiefl, P. Schieberle; *J. Agric. Food Chem.*, vol. 61, no. 22, 5236–5244, 2013.
2. S. Wang, H. Chen, B. Sun; *Food Chem.*, vol. 315, no. January, p. 126158, 2020.
3. W. Zhu, F. Benkwitz, B. Sarmadi, P. A. Kilmartin; *J. Agric. Food Chem.*, 2021.

P86

Untargeted screening of perfluoroalkyl substances in food contact materials and total oxidizable precursor assay: hidden PFAS in food contact materials

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Summary: *Poly- and perfluoroalkyl substances (PFASs) are a challenging issue for many laboratories. Often, they cannot be determined by classical analytical methods, requiring often a pretreatment of the sample such as the total oxidizable precursor assay (TOP). An untargeted approach is fundamental for an exhaustive screening of the wide number of compounds used by the industry.*

Keywords: *PFAS and sample preparation, screening approach, instrumental library*

Introduction

Poly- and perfluoroalkyl substances (PFASs) are a family of several thousand fluorinated aliphatic compounds used in hundreds of industrial products such as fire-fighting foams, coatings and paper products for food packaging [1].
Liquid chromatography tandem mass spectrometry is often used for the determination of PFAS in food contact materials because of its ability providing high sensitivity and linearity in PFAS determination [2]. LC-QqQ, because of its low resolving power and slow acquisition speed, is limited to a target analysis which requires to know in advance the types of compounds that are in the samples. On the other hand, Quadrupole time-of-flight mass spectrometry (qTOF-MS) provides a high resolving power, selectivity, mass accuracy and high acquisition speed necessary for the discovery of novel PFAS for which no reference standards are yet available [3]. Nowadays, more than 9,000 PFAS have been identified, and their number is still growing fast. Therefore, untargeted approach with high resolution mass spectrometry is needed [4]. And a specific instrumental library is a fundamental tool.
Early studies focused on perfluoroalkyl acids (PFAA), like perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). In 2006 the European Commission limited the use of these substances [5], in the same year US EPA launched a global stewardship program inviting companies to reduce PFOA [6]. Following these regulations that limited their use, industries searched for alternative fluorinated compounds, like perfluoroalkyl sulfonates and perfluoroalkyl ether carboxylic acids (e.g., GenX, ADONA and F-53B)[7]. Other emerging categories of compounds are fluorotelomers and fluorinated polymers, substances with high molecular weight that can degrade over time and lead to the formation of PFCAs [8]. They cannot be determined by classical analytical methods, requiring a pretreatment of the sample such as the total oxidizable precursor assay (TOP) that uses hydroxyl radicals produced by thermolysis of persulfate under basic pH. These conditions convert hidden PFAS to

corresponding oxidation products detectable in liquid chromatography tandem mass spectrometry [9].

Experimental

An untargeted approach with a 9000 perfluorurate compounds list was used in methanolic extract of pan coatings in order to identify possible perfluorinated compounds that could migrate into foods during their cooking. Furthermore, this approach allows to identify the compounds that could contaminate the water used in manufacturing and related wastewater. These possible contaminants can be searched through target analysis in order to monitor wastewater and prevent contamination of the environment.

In the coating 8468 µg/kg of 2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)-propanoic acid (GenX), 15 µg/kg of perfluorononanoic acid (PFNA), 22 µg/kg of perfluoroundecanoic acid (PFUnA) and 140 µg/kg of 1H,1H,2H,2H-Perfluorooctane sulfonic acid were found (6:2 PFOS). (Fig. 1)

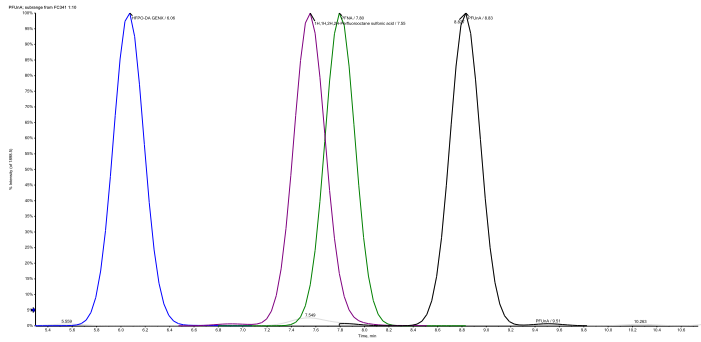

Figure 1. *Extraction masses of GenX [M-CO2]⁻, 6:2 PFOS [M-H]⁻, PFNA [M-H]⁻, PFNUnA [M-H]⁻ in coating's extract*

Wastewater was analyzed for the substances found in the untargeted screening of the coating and GenX, and 6:2 PFOS were found again in concentration of 0.42 µg/L and 0.16 µg/L. Also PFOA was found at concentration of 0.02 µg/L. All analytes were confirmed matching retention time and MS-MS spectrum with corresponding references standards. (Fig. 2). The presence of perfluorooctanoic acid in wastewater could be the result of degradation of other perfluorinated compounds.

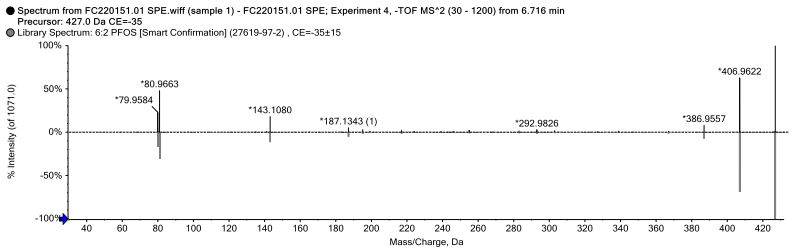

Figure 2. *HRMS-MS spectrum of 6:2 PFOS from wastewater and HRMS-MS spectrum of 6:2 PFOS from relative standard*

Often in paper and board samples the total fluorine content obtained by combustion of the sample and determination of fluoride anion (TOF assay) does not match the sum of the perfluorinated substances found in the routine analysis of PFAS. Through the TOP analysis it is possible to determinate these hidden PFAS through oxidation of the complex perfluorinated molecules not detected in standard testing. A demonstration of the importance of this approach can be seen in the analysis of a cellulose dish that did not release classical pfas but showed high level of fluoro anion in TOF analysis. The dish was examined again via TOP assay and high level of PFAS were detected, the difference is shown in Table 1.

Table 1. *Difference of Pfas Content with/without oxidation process.*

	Methanolic extract of cellulose dish (µg/kg)	**TOP assay (µg/kg)**
HFBA	ND	140
PFPeA	ND	1864
PFHxA	82	252
PFHpA	3	60
Total PFAS	85	2316

References

1. B. Göckener, A. Fliedner, H. Rüdel, I. Fettig, J. Koschorreck, Science of The Total Environment, 782 (2021)
2. S.Taniyasu, Kurunthachalam Kannan, Man Ka So, A. Gulkowska, E.Sinclair, T. Okazawa, N.Yamashita, Journal of Chromatography A, 1093 (2005) 89-97
3. A. Kaufmann, P. Butcher, K. Maden, S. Walker, M. Widmer, Analytica Chimica Acta, 673, (2010) 60-72
4. National Institute of Environmental Health Sciences
5. European Commision, 2006
6. US EPA, 2006
7. S H Brandsma, J C Koekkoek, M J M van Velzen, J de Boer, Chemosphere, 220, (2019) 493-500
8. L.Schultes, G. F. Peaslee, J. D. Brockman, A. Majumdar, S. R. McGuinness, J. T. Wilkinson, O. Sandblom, R. A. Ngwenyama, and Jonathan P. Benskin, Environmental Science & Technology Letters, 6, (2019) 63-68
9. E.F. Houtz, D. L. Sedlak, Environmental Science & Technology, 46, (2012) 9342−9349)

P87

In vivo monitoring of flavour release using PTR-MS: effect of chewing rate and food composition

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Summary: *Coupling dynamic sensory methods with in vivo nose space analysis by high sensitivity direct injection mass spectrometry allowed a better understanding of the way Volatile Organic Compounds (VOCs) are released from the food into the mouth and nasal cavity, and how they are perceived during consumption.*

Keywords: *aroma release, flavour perception, oral processing behaviour*

Introduction

Flavour analysis has shown to be a major application area for PTR-MS. The way Volatile Organic Compounds (VOCs) are released from the food into the mouth and nasal cavity during consumption and the corresponding organoleptic sensation have proven to be critical for the sensory perception and preference of food. To date, most of the published works that have attempted to relate flavour release and sensory perception are far away from mimicking the real consumption context, as assessments often don't consider food oral processing of the consumers during mastication [1-4].

The aim of the study was to investigate the effect of chewing rate, food structure and composition on aroma release and sensory perception of foods by coupling dynamic sensory methods with simultaneous nose-space analysis by high sensitivity direct injection mass spectrometry.

Experimental

Two carriers (bread and sponge cake) were combined with three formulations of strawberry jams varying in pectin and sugar content (Low pectin/high sugar; Low pectin/low sugar; High pectin/high sugar). All jams were spiked with citral 0.4% (w/w) and limonene 0.4% (w/w).

Using a standardized fast and slow chewing protocol (chewing for 25s with a chewing rate of 1.33 or 0.66 chews/s), release of the target volatiles was measured in exhaled nostril air using a commercial PTR-ToF-MS (Proton Transfer Reaction Time-of-Flight Mass Spectrometer, Ionicon Analytik, Innsbruck, Austria), while its corresponding perception (citrus flavour intensity) was rated using Time-Intensity (TI) profiling using a panel consisting of 8 women, in triplicate.

Results

In general, carrier addition showed an increase in aroma release while displaying

a decrease in aroma perception, highlighting the presence of cross modal interactions.
Differences in oral structural breakdown induced by different chewing rates led to some differences in flavour release and perception, stressing the importance of individual characteristics.

Conclusions

Coupling dynamic sensory methods with nose space analysis by high sensitivity direct injection mass spectrometry and oral processing behaviour proved to be an optimal approach to have a better understanding of the effects that texture-aroma interactions and level of oral breakdown have on dynamic aroma perception and flavour release of real complex food matrices during mastication. Ultimately, this will help in the design of successful food products tailored to specific populations, with specific characteristics.

References

1. I. Déléris, A. Saint-Eve, F. Dakowski, E. Sémon, J.-L. Le Quéré, H. Guillemin, I. Souchon;*Food Chem*. 127 (2011) 1615–1624.
2. S. Heenan, C. Soukoulis, P. Silcock, A. Fabris, E. Aprea, L. Cappellin, T.D. Märk, F. Gasperi, F. Biasioli; *Food Chem*. 131 (2012) 477–484
3. J. Mesurolle, A. Saint-Eve, I. Déléris, I. Souchon; *Molecules*. 18 (2013) 6035–6056.
4. A. van Eck, M. Pedrotti, R. Brouwer, A. Supapong, V. Fogliano, E. Scholten, F. Biasioli, M. Stieger; *J. Agric. Food Chem.* (2021).

P88

Former foodstuffs in animal feed: How to distinguish authorized ingredients from prohibited one using mass spectrometry?

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Summary: *Former foodstuffs (FFS) constitute a promising alternative feed that could contribute to the sustainability of the food chain. However, their use remains limited notably from a safety point of view. This work propose a MS-based proteomics method to detect prohibited animal proteins and distinguish them from the presence of authorized ingredients.*

Keywords: *Former foodstuffs, animal proteins, proteomics*

Introduction

According to the Commission Regulation (EU) N° 2017/1017 [1], former foodstuffs (FFS) are foodstuffs, other than catering reflux, which were manufactured for human consumption but which are no longer intended for human consumption. These products are typically produced based on biscuits, bread, breakfast cereals, pasta, snacks or sweets. As circular economy is one of the major issues promoted by the European Union and in order to increase their use, FFS containing ruminant collagen and/or gelatine was recently authorized in non-ruminant feed [2]. FFS have to follow the same rule that other products of animal origin for their use in animal feed [3]. Their high content in ruminant DNA coming from authorized by-products such as dairy products and gelatine make them challenging to be distinguished from the use of prohibited proteins [4]. Mass spectrometry-based proteomics has proofed to be a power method perfectly adapted to animal-by products regulation requirements by providing information about the tissue and species of origin based on the detection of specific peptide sequences.

The objective of this work was to optimize the sample preparation protocol to increase the sensitivity and the specificity of bovine processed animal proteins (PAPs) detection while distinguishing them from the presence of authorized ingredients. The analysis on the sediment fraction, as used in the official light microscopy method, was evaluated.

Experimental

Pig feed adulterated with 0.1 % (w/w) of bovine PAPs were used to evaluate the sensitivity of the protocol. Specificity was verified by adding pure bovine gelatine or former foodstuffs containing or not gelatine to the feed. Samples were prepared following the two sample preparation procedures, standard [5] or optimized method. First procedure was based on sample extraction in a buffer containing 200 mM TRIS-HCl pH 9.2, 2 M urea followed by trypsin digestion and purification with tC18 SPE (Waters). The optimized one includes preliminary sedimentation of the feed using high density solvent (tetrachloroethylene). Analyses were performed by liquid chromatography (Acquity UHPLC system, Waters) coupled with a triple quadrupole mass spectrometer (Xevo TQ-XS,

Waters). Peptide markers identified in previous studies, targeting haemoglobin and collagen of ruminant origin, were simultaneously monitored.

Results

Results obtained with the two approaches (standard v/s optimized method) were compared. Using the standard method, collagen peptides were detected indifferently in samples containing PAPs or gelatine. Haemoglobin peptides were not detected. The optimized method was able to detect bovine PAPs at the 0.1 % (w/w) level based on haemoglobin and collagen peptides co-detection. This level corresponds to the LOD of the official methods for animal proteins detection. Samples containing only gelatine were negative for haemoglobin and gelatine was sometimes detected. This co-detection of bovine haemoglobin and collagen allows distinguishing the presence of PAPs from the use of gelatine.

Conclusion

In the context of the circular economy, FFS is an interesting source of nutriment for animal feed. However, due to the presence of some ingredients, interference giving false suspicion of prohibited materials can be observed. By using the optimized sample preparation presented in this poster, MS-based proteomics is able to detect prohibited material while avoiding false suspicion due to authorized ones.

References

1. European Commission, Commission Regulation (EU) 2017/1017 of 15 June 2017 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials. Official Journal of European Union, 2017. L159: p. 48-119.
2. European Commission, Commission Regulation (EU) 2021/1372 of 17 August 2021 amending Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the prohibition to feed non-ruminant farmed animals, other than fur animals, with protein derived from animals. Official Journal of European Union, 2021. L295: p. 1-17.
3. European Union, Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. Official Journal of European Union, 2001. L 147: p. 1-40.
4. O. Fumiere, J. Zagon, and M.C. Lecrenier, Re-authorization of gelatin and collagen of ruminant origin in non-ruminant feed: a new analytical challenge for the control of the feed ban? submitted, 2022.
5. M.C. Lecrenier, et al., A mass spectrometry method for sensitive, specific and simultaneous detection of bovine blood meal, blood products and milk products in compound feed. Food Chemistry, 2018. 245(Suppl. C): p. 981-988.

P89

Proteins from *Tenebrio molitor*: an interesting functional ingredient and a source of ACE inhibitory peptides

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Summary: *The angiotensin-converting enzyme (ACE) inhibitory potential of the main protein fractions from Tenebrio molitor larvae (TML) was examined to evaluate their use as a novel antihypertensive functional food. LC-HRMS/MS investigation coupled with in silico studies and organic synthesis led to the identification of three novel ACE inhibitory peptides.*

Keywords: *edible insects; Tenebrio molitor; Functional foods*

Introduction

The use of insects for food purposes is a low-cost, smart and eco-sustainable solution. Insects provide quality nutrients, in particular proteins for more than 50% of their weight using limited resources. Unlike most plants, insects are a complete source of digestible proteins and essential aminoacids, moreover, they contain many natural bioactive peptides endowed with antihypertensive, antitumor, antiobesity and antioxidative activities.

In our previous work [1] we disclosed the inhibition of ACE by the water-soluble protein fraction of these insects after gastrointestinal *in vitro* digestion. Efficacy studies carried out on spontaneously hypertensive and age-matched normotensive rats confirmed their promising activity [2].

In 2013 Dai and colleagues disclosed a tripeptide (Tyr-Ala-Asn, YAN) from these insects after alkalase-mediated hydrolysis and determined its ACE inhibitory activity (IC_{50} 0.017 mg/mL) [3].

In the present work, YAN has been synthesized and used as a standard for LC-MS/MS quantification in TML extracts. Interestingly, low yields of YAN from TML did not explain adequately the activity of the whole protein fraction. With the purpose to identify the components responsible for the potent ACE inhibitory activity, we investigate the entire protein fraction, identifying three novel ACE inhibitory peptides.

Experimental

TML used for the experiments were obtained from the mass rearing established at CREA-DC (Florence, Italy). Proteins were extracted from 1g of dried insects and were hydrolyzed by the gastrointestinal enzymes pepsin, trypsin and α-chymotrypsin. ACE inhibitory activity was measured by an indirect assay method based on the quantity of hippuric acid (HA) released by ACE from *N*-hippuryl-L-

histidyl-L-leucine (HHL). HA quantity was determined by RP-HPLC. Crystal structure of human Angiotensin-Converting Enzyme in complex with lisinopril (PDB ID: 1O86) was obtained from the Protein Data Bank and subjected to refinement procedures using Protein Preparation Wizard of Schrödinger Maestro 2017-2 suite [4]. Ligand and peptides were prepared using Maestro 2017-2 [4] and processed using LigPrep [5] tool. The docking procedure was conducted using Glide docking. Peptides were synthesized using Liberty microwave-assisted automatic peptide synthesizer (CEM, Matthews, NC) and analysed by LC-MS and NMR. LC-HRMS/MS analysis of the hydrolyzed protein containing active components of TML was carried out using a liquid chromatography system (Ultimate 3000, Thermo Scientific) coupled with a hybrid quadrupole-Orbitrap mass spectrometer (Q-Exactive, Thermo Scientific).

Results

YAN tripeptide, already reported by other authors, has been synthesized and used as a standard for LC-MS/MS quantification in TML extracts. YAN was quantified and its ACE inhibitory activity confirmed. Interestingly, the low quantity found was not sufficient to rationalize our previous data.

With the purpose to identify the active ACE inhibitory components, we fractionate the mixture, evaluating in parallel the ACE inhibitory activity and the protein content. As a result, we identified a very promising fraction for additional experiments. Extensive LC-HRMS/MS analysis was carried out, identifying additional peptide sequences with MW of 438 and 536 Da. Starting from the crystal structure of human Angiotensin-Converting enzyme in complex with lisinopril (PDB ID: 1O86), extensive *in silico* studies were carried out to prioritize the synthesis of sequences, choosing the ones characterized by the most profitable interactions within the ACE binding site.

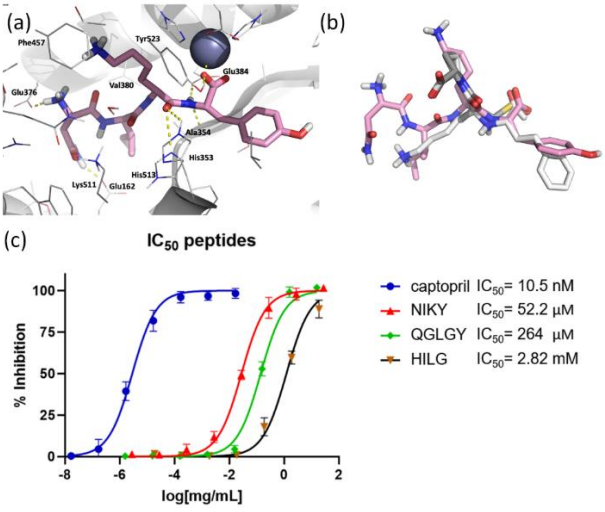

Figure 1. *(a) Binding mode of NIKY peptide; (b) Superposition of NIKY peptide (pink) with captopril (gray) and lisinopril (white); (c) ACE inhibitory percentage for captopril, NIKY, QGLGY, HILG. Captopril was used as a reference*

As a result, three additional peptides were synthesized and tested *in vitro*. HILG tetrapeptide suggested as a potentially active component with MW of 438 Da, was characterized by the worst activity of the series (IC_{50}=2.8 mM). Among the two peptides with MW=536, NIKY and QGLGY, the first one had the lower IC_{50} value of 52 µM, 5-times lower than the latter and 3-times lower than that calculated for YAN.

Conclusions

The multidisciplinary approach described herein highlights that TML benefits exceed their nutraceutical value, paving the way for their use as an antihypertensive functional food. Additional studies are currently ongoing to characterize the other fractions of the hydrolyzed TML extracts in search of other minor compounds with ACE inhibitory effects.

References

1. Cito, A, Botta, M., Francardi, V., Dreassi, E*. Journal of Insects as Food and Feed,* 4 (2017), 231–240.
2. Pessina, F.; Frosini, M.; Marcolongo, P.; Fusi, F.; Saponara, S.; Gamberucci, A.; Valoti, M.; Giustarini, D.; Fiorenzani, P.; Gorelli, B.; Francardi, V.; Botta, M.; Dreassi, E. *PLOS ONE,* 5 (2020**)**, e0233788.
3. Dai, C.; Ma, H.; Luo, L.; Yin, X. *European Food Research and Technology*, 236 (2013), 681–689.
4. Schrödinger. Release 2017-2: Maestro. New York, NY 2017.
5. Schrödinger. Release 2017-2: LigPrep. New York, NY 2017.

P90

Real-time monitoring of the hood removal efficiency of indoor air pollution due to cooking

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Summary: *This work presents the online monitoring of VOC release during cooking of chicken burgers by means of PTR-MS. The cooking process was used as a model to assess the efficiency of different types of VOC abatement systems. PTR-MS allowed to monitor the release profile of several known cooking-related compounds in sensitive, rapid and repeatable fashion.*

Keywords: *air purification systems, PTR-MS, real time monitoring, cooking.*

Introduction

Many different forms of volatile organic compounds (VOCs) are emitted into the atmosphere during cooking process of food, which has an impact on indoor air quality.

There air filtration systems currently available on the market use different technologies to reduce the levels of VOCs released in air.

The effectiveness of air filtration systems created to reduce VOCs can be assessed using official procedures.[1] These entail the use of climatic cells having different volumes and made of different materials as polyethylene or stainless steel.

Instruments based on mass spectrometry methods are typically used to measure V O C concentrations. The TD-GCMS is the method typically utilized to do these analyses. This technique entails sampling the air using cartridges constructed with certain adsorbent materials for the VOCs that will later be thermally desorbed to inject them into the GC-MS.

The main instrument used to measure VOCs concentrations during our tests is the PTR-MS.

Based on these characteristics, employing the PTR-MS as a measuring tool has the following advantages:

- have an elevated sensitivity
- measurements may be made in real time and quickly.

Our research aims to assess the effectiveness of several air purification systems that employ various technologies for the reduction of VOCs.

The systems we tested were based on two distinct purification principles:

- Photocatalysis: a process that uses catalysts made of Titanium Oxide (TiO_2), which irradiated with ultraviolet radiation, oxidize the VOCs present in the air into CO_2.

- VOCs are removed from the air using activated carbon filters, a process that relies on the adsorption principle.

Experimental

Test With standard solution

The air purification system has been tested in both of its 2 configurations: the first, which just installs activated carbon filter technology, and the second, which also installs a photocatalytic system.

The initial experiments were carried out in a 1 m^3 stainless steel climate cell with both system setups.

The primary stages for doing the test are outlined below:

- Place the air purification device with the predetermined configuration in the test chamber's center.
- Inject the standard a mixture made up of 2,5 µl of acetaldehyde, 3 µl of acetone, and 4,5 µl of toluene was injected into the chamber, in order to achieve a concentration of 1000 ppb per chemical.
- Turn on the purification system, and monitor the VOCs concentration trend throughout the test.

Test with hamburger

After the testing with the mixes of standard VOCs were established, further experiments were conducted on both conformations of the system utilizing the heating of a chicken burger as a source of VOCs.

These tests were conducted in a bigger, 8 m^3 chamber with polyethylene walls.

Because the PTR-MS detected an elevate number of VOCs from such a complicated matrix, only the peaks with the highest intensity were taken into consideration. For each peaks, the test findings are presented as the percentage difference between the starting concentration and the ending concentration over the course of the test.

Tenax cartridges, which have a high rate of adsorption for a variety of VOCs types, were utilized to sample the air at several points throughout the test in order to provide a more thorough study.

The VOCs mixture that has been adsorbed on the tenax is subsequently desorbed using a solvent, and the GC-MS is then used to extract additional data that the PTR-MS does not offer.

Results

Figures 1 and 2 display the outcomes of the tests conducted on both air purification system setups. The graphs display the first four peaks that were more intense than the rest produced during the cooking of the hamburgers as measured by the PTR-MS in normalized cps. In order to assess the effectiveness of the air purification system configurations over time, the ratio of Ct to C0 was computed for each of these peaks every 20 minutes after the system was turned on.

Time 0 is the system's ignition point and the beginning of the tests, which began after the food had been cooked.

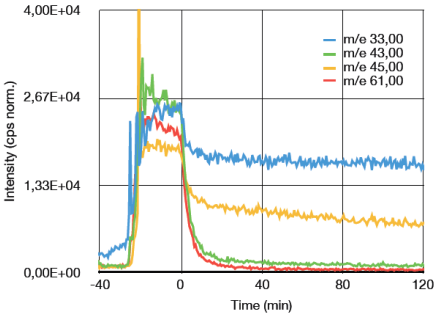

Figure 1. *Configuration system: active carbon*

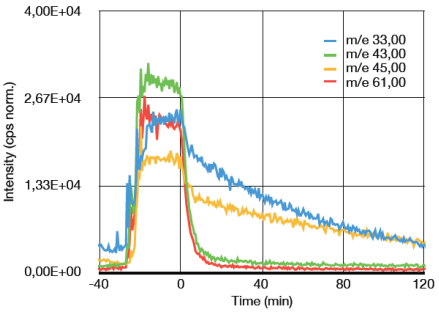

Figure 2. *Configuration system: active carbon and photocatalysis*

Table 1*. Test Results: active carbon*

	Ct/C0 t = 0	Ct/C0 t = 40	Ct/C0 t = 80	Ct/C0 t = 120
m/e 33	100%	66%	68%	66%
m/e 43	100%	6%	5%	5%
m/e 45	100%	51%	43%	42%
m/e 61	100%	3%	3%	2%

Table 2. *Test Results: active carbon and photocatalysis*

	Ct/C0 t = 0	Ct/C0 t = 40	Ct/C0 t = 80	Ct/C0 t = 120
m/e 33	100%	48%	29%	19%
m/e 43	100%	4%	4%	3%
m/e 45	100%	46%	36%	25%
m/e 61	100%	3%	2%	2%

Conclusion

The research presented here shows how the theory of photocatalysis applied to an air purification system in conjunction with activated carbon technology boosts the effectiveness in the field of VOC abatement.

According to the test results, the chemicals m/e 45 and m/e 33, that have a low tendency to adsorb on activated carbons, are more effectively degraded when the photocatalytic component is included in the system.

References

1. Norma Europea EN 16846-1/2017, Norme Internazionale IEC 63086.

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