Società Chimica Italiana
Divisione di Spettrometria di Massa

Camerino (Italy), September 25-27, 2019

BOOK OF ABSTRACTS

PROCEEDINGS OF THE 6th MS FOOD DAY

September 25-27, 2019

Camerino - Italy

Scientific Committee

Gianluca Giorgi *(Chairman)*	*Università di Siena*
Giuseppe Avellone	*Università di Palermo*
Franco Biasioli	*Fondaz. Edmund Mach, S.Michele A/A (TN)*
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Lanfranco Conte	*Università di Udine*
Arnaldo Dossena	*Università di Parma*
Riccardo Flamini	*CREA-VE, Conegliano*
Emanuele Forte	*Ferrero, Alba*
Roberta Galarini	*IZS dell'Umbria e delle Marche*
Renzo Galli	*Fileni, Cingoli (MC)*
Ivana Gandolfi	*Parmalat, Parma*
Davide Garbini	*COOP Italia, Bologna*
Luciano Navarini	*illy caffè, Trieste*
Paola Pittia	*Università di Teramo*
Gianni Sagratini	*Università di Camerino*
Giovanni Sindona	*Università della Calabria*
Michele Suman	*Barilla, Parma*
Sauro Vittori	*Università di Camerino*

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Giovanni Caprioli	*Università di Camerino*
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Fabrizio Papa	*Università di Camerino*
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Daniele Scattolini	*Fileni, Cingoli (MC)*
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SCIENTIFIC PROGRAMME

Wednesday, September 25th, 2019

14:00 – 14:30 Registration and reception

14:30 – 15:00 **Welcome addresses**

Magnifico Rettore
Università degli Studi di Camerino

Fileni

Donatella Caruso
University of Milan, President of the Division of Mass Spectrometry of the Italian Chemical Society

Gianluca Giorgi
University of Siena, 6 MS Food Day Scientific Committee, *Chair*

15:00 **1st Session: Toxicants, allergens, pesticides**

Chairs: ***Donatella Caruso*** *(Univ. of Milan)* ***Gianluca Giorgi*** *(Univ. of Siena)*

15:00 – 15:40 **PL1: Mass spectrometry – An effective platform to assess toxicants and adulterants and their impact on human health**

Richard M. Caprioli

Mass Spectrometry Research Center, Vanderbilt University, Nashville TN (USA)

15:40 – 15:55 **OR1: LC-MS/MS and tetrodotoxins (TTXs): a preliminary study on mussels and *Vibrios* from NW Adriatic Sea**

Simone Bacchiocchi, Melania Siracusa, Debora Campacci, Sara Evangelista, Francesca Leoni, Arianna Piersanti

Centro di Referenza Nazionale Molluschi Bivalvi- Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", Ancona (Italy)

15:55 – 16:10 **OR2: Selection of proteotypic peptide markers tracing for six allergenic ingredients in incurred chocolate bar**

Rosa Pilolli, Christof van Poucke, Marc de Loose, Nathalie Gillard, Anne-Catherine Huet, Olivier Tranquet, Collette Larré, Karine Adel-Patient, Hervé Bernard, Chiara Nitride, E. N. Clare Mills, Linda Monaci

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

16:10 – 16:25 **OR3: Direct analysis of Glyphosate, AMPA, and other polar pesticides in food**

Lorenzo Zingaro, Anna Calì, Jerry Zweigenbaum, Derick Lucas

Agilent Technologies Italia, Rome (Italy)

16:25 – 16:40 **OR4: Cd and Pb time trends 2008-2018 in bivalve mollusks from the Adriatic Sea, FAO 37.2.1 area**

Tamara Tavoloni, Roberto Miniero, Gianfranco Brambilla, Paolo Palombo, Francesco Griffoni, Martina Ciriaci, Arianna Stramenga, Giampiero Scortichini, Arianna Piersanti

Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "T. Rosati", Ancona (Italy)

16:40 – 17:15 Coffee break

17:15 **2nd Session: wine & beer**

Chairs: ***Riccardo Flamini*** *(CREA-VE, Conegliano (TV)),* ***Roberto Larcher*** *(Fondaz. Edmund Mach, S. Michele a/A)*

17:15 – 17:30 **OR5: Searching for bioactive nutraceutical and functional food ingredients in *Vitis vinifera* leaves**

Marisa Maia, António E. N. Ferreira, Gonçalo Laureano, Ana P. Marques, Vukosava M. Torres, Anabela B. Silva, Ana R. Matos, Carlos Cordeiro, Andreia Figueiredo, Marta Sousa Silva

Faculdade de Ciências da Universidade de Lisboa (Portugal)

17:30 – 17:45 **OR6: High-resolution mass spectrometry metabolomics of different *Glera* grape clones used to produce D.O.C.G. Prosecco sparkling wine**

Mirko De Rosso, Marina Niero, Luca Aggio, Roberto Carraro, Christine Mayr, Massimo Gardiman, Riccardo Flamini

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

17:45 – 18:00 **OR7: An investigation on the reactivity of wine polyphenols towards SO_2**

Daniele Catorci, Fulvio Mattivi, Panagiotis Arapitsas, Federico Bonaldo, Graziano Guella

University of Trento, Trento (Italy)

18:00 – 18:15 **OR8: Volatile profiles of Italian monocultivar EVOOs during 12 months of storage: different trends associated to polyphenols and fatty acids content**

Eugenio Aprea, Emanuela Betta, Claudio Cantini, Flavia Gasperi

Fondazione Edmund Mach, Food Quality and Nutrition Department, IASMA Research and Innovation Centre, S. Michele all'Adige (TN, Italy)

18:15 – 18:30 **OR9: Metabolomics as a tool for the optimization of agronomical parameters of Ribolla Gialla for the production of sparkling wine**

Domen Škrab, Paolo Sivilotti, Urška Vrhovšek

Edmund Mach Foundation, Research and Innovation Centre, Department of Food Quality and Nutrition, San Michele all'Adige (TN, Italy)

18:30 – 18:45 **OR10: Mass spectrometry for free polyphenols in untreated samples of beer**

Paola Di Matteo, Paola Russo, Rita Petrucci

Sapienza Università di Roma, Dip. Scienze di Base e Applicate per l'Ingegneria Roma (Italy)

18:45 *End of session*

18:45 – 19:15 **Celebrating 10 years of MS Food Day!!**

Michele Suman, Luciano Navarini, Franco Biasioli, Lucia Bonassisa, Davide Garbini

19:30 Welcome cocktail & show

Thursday, September 26th, 2019

9:00 **3rd Session: MS ambient, PTR**

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

9:00 – 9:40 **PL2: Towards on-site food analysis by (trans)portable mass spectrometry**

Marco Blokland, Arjen Gerssen, Paul Zoontjes, Janusz Pawliszyn, Michel Nielen

Wageningen Food Safety Research (The Netherlands)

9:40 – 9:55 **OR11: Authenticity of honey: is DART-MS an effective screening tool?**

Tito Damiani, Nicola Dreolin, Sara Stead, Emiliano De Dominicis, Chiara Dall'Asta

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

9:55 – 10:10 **OR12: UniSpray™ a novel atmospheric pressure ionization source for LC-MS/MS for routine analysis**

Andrea Perissi

Waters Italia, Sesto San Giovanni (MI, Italy)

10:10 – 10:35 **KN1: Proton transfer reaction mass spectrometry and sensory techniques to investigate biotransformations of hop derived compounds in beer**

Patrick Silcock

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

10:35 – 10:55 **OR13: VOC profiling of raw and cooked gilthead sea bream (*Sparus aurata*) of different geographical origin by PTR-ToF-MS**

Iuliia Khomenko, Fabio Brambilla, Michele Pedrotti, Franco Biasioli

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

10:55 – 11:30 Coffee break

Poster session (even number posters)

11:30 **4th Session: VOCs, volatilome & isotopes**

Chairs: ***Franco Biasioli*** *(FEM, S. Michele A/A (TN))*, ***Davide Garbini*** *(Coop Italia, Casalecchio di Reno (BO))*

11:30 – 11:55 **KN2: High performing VOC phenomics to improve the horticultural production chain**

Brian Farneti

Genomics and Biology of Fruit Crop Department, Research and Innovation Centre, Fondazione E. Mach, San Michele all'Adige (TN, Italy)

11:55 – 12:10 **OR14: Volatilome fingerprinting and gene expression profiling of fresh produce: a multi-trait approach to identify markers of food quality**

Antonella Muto, Lucia Bartella, Innocenzo Muzzalupo, Leonardo Bruno, Leonardo Di Donna, Carsten Muller, Hilary J. Rogers, Laura McGregor, Antonio Ferrante, Adriana A. C. Chiappetta, Giovanni Sindona, Maria B. Bitonti, Natasha D. Spadafora

Department of Biology, Ecology and Earth Sciences, University of Calabria, Arcavacata di Rende (Cosenza, Italy)

12:10 – 12:25 **OR15: Coupling *in vivo* nose-space and sensory methods to investigate flavor release and perception**

Michele Pedrotti, Arianne van Eck, Iuliia Khomenko, Andrea Spaccasassi, Elke Scholten, Vincenzo Fogliano, Markus Stieger, Franco Biasioli

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

12:25 – 12:40 **OR16: Usefulness of HS-SPME-GC-MS quantification of volatile compounds for quality control of virgin olive oil**

Lorenzo Cecchi, Marzia Migliorini, Luca Calamai, Fabrizio Melani, Nadia Mulinacci

University of Florence – Department of NEUROFARBA, Sesto Fiorentino (Italy)

12:40 – 12:55 **OR17: Stable isotopes and elemental profiles for botanic and geographic characterisation of gum arabic**

Tiziana Nardin, Daniela Bertoldi, Matteo Perini, Silvia Pianezze, Giulia Ferrari, Roberto Larcher

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

13:00 – 14:30 Buffet lunch

14:30 **5th Session: food components, food testing and profiling**

Chairs: **Arnaldo Dossena** (Univ. of Parma, Parma), ***Giuseppe Avellone*** *(University of Palermo)*

14:30 – 15:10 **PL3: Novel insights in the occurrence and bioavailability of endocrine active food components**

Doris Marko, Benedikt Warth

University of Vienna (Austria)

15:10 – 15:25 **OR18: Exploring extra-terrestrial acceptability of inter-planetary food with the aid of mass spectrometry**

Jonathan Beauchamp, Andrew Taylor, Loic Briand, Victor Demaria Pesce, Martina Heer, Thomas Hummel, Scott McGrane, Christian Margot, Serge Pieters, Paola Pittia, Charles Spence, Inês Antunes

Fraunhofer IVV, Freising (Germany)

15:25 – 15:40 **OR19: Robust and integrated, analytical workflows delivering improved sensitivity and compliance in routine food testing labs**

Fausto Pigozzo, Richard Fussell

Thermo Fisher Scientific, Milan (Italy)

15:40 – 15:55 **OR20: Phospholipids profiling in San Andreas strawberries by using hydrophilic liquid chromatography coupled to ESI tandem mass spectrometry**

Mariachiara Bianco, Lachinkhanim Huseynli, Cosima D. Calvano, Andrea Viola, Mārtiņš Šabovics, Tommaso R.I. Cataldi

Department of Food Technology, Latvia University of Life Sciences and Technologies, Jelgava (Latvia)

15:55 – 16:35 Coffee break

Poster session (odd number posters)

16:35 **6th Session: metabolomics**

Chairs: ***Paola Pittia*** *(Univ. of Teramo),* ***Sauro Vittori*** *(Univ. of Camerino)*

16:35 – 17:15 **PL4: Applications of MS-based metabolomics to investigate the host:microbiome co-metabolic processing of food components**

Fulvio Mattivi, Marynka Ulaszewska, Kajetan Trost

University of Trento, Trento (Italy)

17:15 – 17:30 **OR21: Untargeted metabolomics approach and antimicrobial assays to characterize chemical profiles of different *Satureja montana* L. essential oils**

Alessandro Maccelli, Luca Vitanza, Anna Imbriano, Maria Elisa Crestoni, Federica Rinaldi, Catia Longhi

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

17:30 – 17:45 **OR22: Comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry featuring tandem ionization: adding an extra-dimension to hazelnuts (*Corylus avellana l.*) primary metabolome fingerprinting**

Marta Cialiè Rosso, Cecilia Cagliero, Erica Liberto, Patrizia Rubiolo, Barbara Sgorbini, Carlo Bicchi, Chiara Cordero

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

17:45 – 18:00 **OR23: Polar pesticides method: a new and unique solution for both food complex matrix and environmental samples**

Alessandro Armandi, Stefano Fiorina, Samuele Scurati

Sciex, Milano (Italy)

18:00 – 18:15 **OR24: Determination of the polyphenolic fraction of food real-world samples by comprehensive two-dimensional liquid chromatography coupled to photodiode array and mass spectrometry detection**

Francesco Cacciola, Katia Arena, Francesca Rigano, Paola Dugo, Luigi Mondello

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

18:15 End of session

20:30 Social dinner

Friday, September 27th, 2019

9:00 **7th Session:proteins, peptides, isotopes**

*Chairs: **Renzo Galli** (Fileni, Cingoli (MC)), **Angelo Visconti** (Bonassisa Lab., Foggia)*

9:00 – 9:40 **PL5: Protein oxidation and glycation compounds in food: targeting individual structures by MS techniques**

Michael Hellwig, Thomas Henle

Technische Universität Dresden, Dresden (Germany)

9:40 – 9:55 **OR25: Phosphopeptide profile of kefir as affected by the production technology**

Sandra Pati, Maria Luisa Savastano, Antonio Bevilacqua, Maria Rosaria Corbo, Maurizio Quinto, Antonio Rizzuti, Monika Pischetsrieder, Ilario Losito

Department of Agricultural, Food and Environmental Sciences (SAFE), University of Foggia, Foggia (Italy)

9:55 – 10:10 **OR26: HPLC-MS/MS method for fast and comprehensive quantification of sesame lignans**

Anna Rathgeb, Zana Jamal Kareem, Andargie Mebeaselassie, Petr Karlovsky

Molecular Phytopathology and Mycotoxin Research Unit, University of Goettingen (Germany)

10:10 – 10:25 **OR27: Characterization of Italian authentic saffron by using stable isotope ratio analysis**

Silvia Pianezze, Luana Bontempo, Federica Camin, Luca Ziller, Angelo Antonio D'Archivio, Matteo Perini

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

10:25 – 11:00 Coffee break

11:00 **8th Session: MS & coffee**

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

11:00 – 11:15 **OR28:** Shimadzu

11:15 – 11:30 **OR29: Development of a new analytical method for 30 bioactive compounds quantification in Spent Coffee Ground**

Simone Angeloni,Giovanni Caprioli, Gulzhan Khamitova, Luciano Navarini, Gianni Sagratini, Sauro Vittori

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

11:30 – 11:45 **OR30: Polyphenols in coffee by-products extracts: determination and cytoprotection in an undifferentiated neuroblastoma cell line**

Cristina Juan, Gaia de Simone, Jordi Mañes, Gianni Sagratini, Giovanni Caprioli, Ana Juan-García

Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Burjassot, València, (Spain)

11:45 – 12:00 **OR31: Extraction of espresso coffee by changing particle size distribution and evaluation of bioactive compounds through HPLC-VWD and HS-SPME/GC-MS**

Gulzhan Khamitova, Simone Angeloni, Giovanni Caprioli, Lauro Fioretti, Gianni Sagratini, Sauro Vittori

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

12:00 – 12:30 Closing ceremony. Announcement of next MS Food Day

12:30 – 14:00 Farewell light buffet

POSTER COMMUNICATIONS

P1 Organic (PBDEs, NDL-PCBs) and Inorganic (Pb, Cd, Hg, As, Ni, Mn) contaminants in hunted wild boar from Central Italy

Tamara Tavoloni, Arianna Stramenga, Tommaso Stecconi, Massimiliano Giannotti, Paolo Palombo, Martina Ciriaci, Elisabetta Manuali, Valeria Castro, Michela Conquista, Arianna Piersanti

IZS-Umbria e Marche "Togo Rosati", Ancona (Italy)

P2 Extraction and characterization of bioactive compounds from agro-industrial by-products for spray packaging applications to extend the shelf-life of highly perishable foods

Maria Grimaldi, Antobella Cavazza,Thekla Leone, Michele Antonio Papadopoli, Claudio Corradini

Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, Parma (Italy)

P3 Characterization of new putative polyphenols in post-harvest withered grapes (*V. vinifera L.*) by high-resolution mass spectrometry

Mirko De Rosso, Annarita Panighel, Antonio Dalla Vedova, Riccardo Flamini

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

P4 Determination of co-eluted isomers in wine samples by application of MS/MS deconvolution analysis

Marta Menicatti, Massimo Ricciutelli, Roberta Galarini, Simone Moretti, Michele Mari, Gianni Sagratini, Sauro Vittori, Simone Lucarini, Giovanni Caprioli, Gianluca Bartolucci

Dipartimento Neurofarba dell'Università di Firenze (Italy)

P5 Alkaloid profiling of food tannins using high resolution mass spectrometry

Roberto Larcher, Pietro Apostoli, Giorgio Nicolini, Tiziana Nardin

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

P6 Characterization of Iris pallida Lam. rhizomes cultivated in Chianti area by HPLC-DAD-MS, HS-SPME-GC-VUV and HS-SPME-GC×GC-TOF analysis

Francesca Ieri, Pamela Vignolini, Chiara Vita, Fabio Villanelli, Annalisa Romani

QuMAP Laboratory, PIN Polo Universitario Città di Prato, Prato (Italy)

P7 Anchovy waste-derived fish oil loaded on periodic mesoporous silica for nutraceutical applications

Claudia Lino, Rosaria Ciriminna, Antonino Scurria, Giuseppe Avellone, Mario Pagliaro

Istituto per lo Studio dei Materiali Nanostrutturati, CNR, Palermo (Italy)

P8 Characterisation of the methanolic extracts from the ancient apple variety ''Mela Rosa dei Monti Sibillini''

Joice G. Nkuimi Wandjou, Laura Lancioni, Fabrizio Papa, Gianni Sagratini, Sauro Vittori, Massimo Bramucci, Massimo Ricciutelli, Giovanni Caprioli, Filippo Maggi

School of Pharmacy, University of Camerino (Italy)

P9 Influence of boiling cooking on phytochemical profile of orange cauliflower (*Brassica Oleracea* L. var. *botrytis*)

Alessandra Giardinieri, Guillem Campmajó, Ancuta Nartea, Elena Bartolucci, Michele Balzano, Deborah Pacetti, Natale Giuseppe Frega, Javier Saurina,Oscar Núñez

Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Barcelona (Spain)

P10 Honey discrimination by Volatile Organic Compounds analysis: comparison between GC-IMS and GC-E-Nose

Fernando Gottardi, Cesare Rossini, Maria Teresa Rodriguez-Estrada, Dario Mercatante, Fabio Battaglia, Nicole Mei

LabService Analytica s.r.l, Bologna (Italy)

P11 Analytical method for determination of glyphosate and other polar pesticides in vegetables and honey using Ion Chromatography coupled with High Resolution Mass Spectrometry (IC-HRMS)

Barbara Angelone, Enrica Ferretti, Mara Gasparini

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

P12 UHPLC-HRMS polyphenolic profile for the characterization and classification of nuts

Guillem Campmajó, Javier Saurina, Oscar Núñez

Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Barcelona (Spain)

P13 Preliminary characterization of a candidate reference material for poly-and perfluoroalkyl substances

Roberta Galarini, Simone Moretti, Carolina Barola, Danilo Giusepponi, Fabiola Paoletti, Giorgio Saluti, Gianfranco Brambilla

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

P14 Perfluoroalkylated pollutants in liver of farm animals by LC-Q-Orbitrap: method development and validation

Carolina Barola, Simone Moretti, Danilo Giusepponi, Fabiola Paoletti,[1] Giorgio Saluti, Severyn Salis, Cecilia Testa, Roberta Galarini

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

P15 Fish oil from anchovy by-products via direct and green extraction: characterisation via mass spectrometry of nutraceutical ingredients

Antonino Scurria, Rosaria Ciriminna, Claudia Lino, Giuseppe Avellone, Mario Pagliaro

Istituto per lo Studio dei Materiali Nanostrutturati, CNR, Palermo (Italy)

P16 Evaluation of volatile profile of ten Italian tomato cultivars and relative processed products

Sara Savini, Mirella Bandini, Anna Sannino

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

P17 Transfer of a multiclass method for over 60 antibiotics in food from a high resolution tandem mass spectrometry platform to a low resolution one

Fabiola Paoletti, Danilo Giusepponi, Carolina Barola, Simone Moretti, Giorgio Saluti, Federica Ianni, Roccaldo Sardella, Roberta Galarini

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

P18 In depth characterization of dried rings and flakes from Emilia Romagna authocthon onion (Allium cepa L.) by GC-MS and GC×GC/TOF

Lorenzo Cecchi, Francesca Ieri, Pamela Vignolini, Nadia Mulinacci, Annalisa Romani

University of Florence, Department of NEUROFARBA, Sesto Fiorentino (Italy)

P19 Differentiation of Italian land snails (Helix aspersa) from foreign samples, using C and N stable isotopes analysis: preliminary results

Simona Altieri, Vincenzo Guida, Gionata De Vico, Carmine Lubritto

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

P20 A comprehensive analysis of volatile profile of Cannabis sativa L. variety Futura essential oil by TotalFlowUnderVacuum GCxGC-TOF and GC-VUV

Eligio Sebastiani, Fabio Villanelli, Francesca Ieri, Luca Calamai, Annalisa Romani

SRA Iinstruments SpA, Cernusco S/N Milano (Italy)

P21 Chemotype diversity of commercial essential oils

Filomena Monica Vella, Roberto Calandrelli, Domenico Cautela, Immacolata Fiume, Gabriella Pocsfalvi, Bruna Laratta

Consiglio Nazionale delle Ricerche (CNR), Istituto di Ricerca degli Ecosistemi Terrestri (IRET), Napoli (Italy)

P22 Fully automated determination of 3-MCPD and glycidol in edible oils and fats by GC/MS based on the commonly used methods ISO 18363-1, AOCS Cd 29(a&c)-13, and DGF C-VI 18

Andrea Carretta, Federico Sacco, Dominik Lucas, Andreas Hoffman, Carlos Gil

SRA Instruments SpA, Cernusco S/N Milano (Italy)

P23 Quality assessment of Tuscan extra virgin olive oil by HS-SPME-GC-MS analysis of volatile compounds, HPLC-DAD analysis of phenolic compounds and chemometrics

Lorenzo Cecchi, Pujun Xie, Maria Bellumori, Fabrizio Melani, Nadia Mulinacci

Università di Firenze, NEUROFARBA Department, Sesto F.no, Florence (Italy)

P24 Determination of ten mycotoxins on Moroccan medicinal and aromatic plants by liquid chromatography coupled to tandem mass spectrometry

Aicha El Jai, Ana Juan-García, Jordi Mañes, Abdellah Zinedine, Cristina Juan

Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Burjassot, València (Spain)

P25 16-O-methylated diterpenes in green *Coffea arabica*: UPLC-MS/MS method optimization and validation

Elena Guercia, Silvia Colomban, Luciano Navarini

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

P26 UHPLC-MS/MS method for rapid quantification of chlorogenic acids in roasted coffee

Silvia Colomban, Elena Guercia, Luciano Navarini

illycaffè Spa, Trieste (Italy)

P27 Omega-3 enriched *Hermetia illucens* as novel ingredient for insect-based food for the future: influence of growth substrate based on coffee-roasting by-product and microalgae

Cristina Truzzi, Anna Annibaldi, Matteo Antonucci, Silvia Illuminati, Giuseppe Scarponi, Paola Riolo, Sara Ruschioni, Matteo Zarantoniello, Ike Olivotto

Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona (Italy)

P28 Tips and tricks for handling high purity water in the LC-MS laboratory

Anastasia Domanov, Matteo Cazzaniga, Stephan Altmaier, Martin Lange, Stéphane Mabic

Merck, Advanced Analytical, Milan (Italy)

P29 Innovative sampling approaches for drinking water in the analysis of emerging pollutants by liquid chromatography - tandem mass spectrometry

Marina Di Carro, Emanuele Magi

Dipartimento di Chimica e Chimica Industriale, Università di Genova, Genova (Italy)

P30 Analysis of Bisphenol A in foods using solid phase micro-extraction with an overcoated fiber

Maurizio Sacchetti, Katherine Stenerson, Leonard Sidisky, Craig Aurand, Lisa McCombie

Merck Spa, Milan (Italy)

P31 Analysis of pesticides in paprika - Development of an SPE cleanup method

Maurizio Sacchetti, Hugh Cramer, Katherine Stenerson, Christine Dumas

Merck Spa, Milan (Italy)

P32 Flavonoids annotation using a product ion-dependent MS^n data acquisition method on a Tribrid Orbitrap mass spectrometer

Igor Fochi, Reiko Kiyonami, Iwao Sakane, Seema Sharma, Graeme McAlister, Caroline Ding, Andreas Huhmer

Thermo Fisher Scientific, Rodano (Italy)

P33 Multidisciplinary approach to determine botanical and geographical origin of durum wheat, semolina and pasta

Elisa Gritti, Elena Poloni, Emiliano De Dominicis, Federica Cattapan, Samim Saner, Giuditta Gambardota

Mérieux NutriSciences Research, Resana (TV, Italy)

P34 LC simulation software employment for complex chromatographic method development in food and pharmaceutical analysis

Anna Beghetto, Elisa Gritti, Valeria Meli, Gianluigi Boschello, Emiliano De Dominicis, Riccardo Arduini

Mérieux NutriSciences Research, Resana (TV, Italy)

P35 Characterization of the main phenolic compounds in agri-food by-products by HPLC-DAD-MS

Mohamad Khatib, Lorenzo Cecchi, Enrica Bargiacchi, Maria Bellumori, Marzia Innocenti, Nadia Mulinacci

Università degli Studi di Firenze, NEUROFARBA Dep, Sesto F.no, Florence (Italy)

P36 Characterization and valorization of ancient cereals by HPLC-UV and ICP-MS

Marta Radaelli, Elisa Scalabrin, Marco Roman, Gabriele Capodaglio, Gabriella Buffa

Department of Environmental Sciences, Informatics and Statistics, University Ca' Foscari Venice, Venezia-Mestre (Italy)

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Cristiana Labella, Luca Tommasi, Angelo Visconti, Lucia Bonassisa

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Eleonora Oliva, Federico Fanti, Johannes Delgado Ospina, Clemencia Chaves, Alessia Pepe, Manuel Sergi, Dario Compagnone

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Andrea Giorgi, Luigi Giannetti, Elisa Gennuso, Francesco Necci, Valentina Gallo, Francesca Marini, Bruno Neri

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome (Italy)

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Elisa Gennuso, Luigi Giannetti, Valentina Gallo, Andrea Giorgi, Francesco Necci, Francesca Marini, Bruno Neri

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P67 Qualitative and quantitative determination of ethyl-2-hydroxy-4-methylpentanoate (ethyl leucate) in wine

Mauro Paolini, Valeria Bianchin, Giorgio Nicolini, Tomas Roman, Loris Tonidandel, Roberto Larcher

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P68 Discrimination of saffron samples using MS-based techniques (IRMS and GC-MS) and peptide gas sensors array (E-Nose)

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ABSTRACTS

Plenary, Keynotes, Orals

PL1

Mass spectrometry – An effective platform to assess the impact of toxic compounds in foods on human health

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Our society has become well aware of health and nutrition and the potential long-term effects of the molecular constituents in foods. Although many of these constituents are beneficial and necessary for life, some termed 'toxicants' are detrimental to good health and can be acutely poisonous. Toxicants can be derived from natural sources or can arise from food preparation processes. Moreover chemicals used in every step of food processing including production, harvesting, packing, transport, marketing, and consumption can have unwanted health effects. This presentation will address two approaches that utilize modern analytical technologies to assess and identify constituents in foods that may have deleterious effects on human cells. The first involves a multi-omics approach that addresses perturbations that can occur through exposure and ingestion of toxicants considering both dose and temporal effects. Measurements of proteins, lipids, metabolites using LC-MS/MS together with RNA-seq after exposure of human cells to specific toxicants can be assessed in terms of their effects on metabolic pathways. The second approach to the analysis of toxicants in food addresses the spatial component, i.e., these compounds do not effect organs uniformly nor are they necessarily present if foodstuffs uniformly. Here Imaging Mass Spectrometry provides unique data on many hundreds of compounds by providing molecular maps of cellular components in samples. Within these descriptions, a discussion of the necessary bioinformatics will be addressed that enables effective assessment of the potential health effects of these compounds.

OR1

LC-MS/MS and tetrodotoxins (TTXs): a preliminary study on mussels and *Vibrios* from NW Adriatic Sea

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Summary: *Tetrodotoxins are neurotoxins responsible of lethal poisoning upon ingestion of pufferfish. Recently TTXs have been found in European shellfish. We report the development of a LC-MS/MS method to TTXs determination in shellfish. The procedure was used for a preliminary study of TTXs contamination in mussels and Vibrios of Marche coasts.*

Keywords: *mussels, Tetrodotoxins, LC-MS/MS*

Introduction

Tetrodotoxins (TTXs) are potent neurotoxins well known, especially in indo-pacific regions, as responsible of the fatal poisoning associated with pufferfish eating.

They act as muscular and nervous cells sodium channels blockers, stopping the nervous signal transmission.

The primary TTXs environmental sources are thought to be marine bacteria, mainly belonging to *Vibrio* genus and poliketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) were identified as possible pathways genes in their biosynthesis [1].

TTXs can accumulate in various edible marine species including fish, gastropods and bivalve molluscs entering in the food chain and becoming a potential threat to humans.

Starting from 2015 TTXs have been found in shellfish in United Kingdom, Greece and The Netherlands, raising European authorities and scientific community concerns, because of lack of a maximum permitted level (MPL) [2]. In 2017 the EFSA delivered a scientific opinion on the risks related to the presence of TTXs in marine bivalves and gastropods. A preliminary reference level of 44 µg TTX eq kg^{-1} was proposed, but more data were requested in order to provide a better exposure assessment. Recently, for the first time, TTXs were detected in Italian shellfish from Syracuse bay (Sicily) and Marano Lagoon (Friuli Venezia Giulia) [3, 4]. The Istituto Zooprofilattico Sperimentale Umbria e Marche (IZSUM) is in charge of the official Marine Biotoxins monitoring in mussels from Marche coasts, therefore the need to develop a LC-MS/MS method to assess TTXs contamination in mussels and in symbiotic *Vibrio* isolates.

Experimental

An in house method based on the European Union Reference Laboratory for Marine Biotoxins LC-MS/MS protocol was developed, performance tested and applied to field samples [5]. MS experiments were carried out on a hybrid triple-quadrupole/linear ion trap 3200 QTRAP mass spectrometer (ABSciex, Darmstadt, Germany).

Method performances in terms of linearity, precision (repeatability), accuracy, detection limit (LOD), quantification limit (LOQ) were assessed.

Mussels were collected in Marche breeding farms and wild sites (NW Adriatic Sea) from January till August 2018. All mussel samples were also tested for Vibrio spp. by means the Leão et al. modified protocol [1]. Vibrio isolates, confirmed at species level by PCR, were cultured.

Bacterial pellets were extracted following the modified protocol of Turner et al. [6] and analysed by LC-MS/MS.

Results

The implemented LC-MS/MS method showed good linearity, precision and accuracy while sensitivity was variable and not sufficiently high in general (LOD 8-25 µg kg^{-1} and LOQ 75 µg kg^{-1}), mainly as consequence of a dramatic matrix effect (80-90%). However the protocol is suitable to reveal TTXs at EFSA guidance level. All the mussel samples analysed showed TTX levels below the LOQ and only 3 of them, collected in July 2018 from Pesaro wild sites, showed detectable TTXs traces (> LOD) (Fig. 1). Literature data support this finding, since few µg kg^{-1} of TTX were quantified also in Italy [3] and in Europe [2]. Moreover Turner et al. [7] described some environmental factors which may promote TTXs accumulation in mussels such as water temperature ≥ 15 °C, water depth <5 m, high irradiance and low salinity. The Pesaro wild sites have environmental features that may correspond to the once described by Turner.

Microbiological and molecular analysis of the 100 samples brought to the isolation of 89 *V.alginolyticus* strains. None of the isolates showed the presence of detectable TTXs (<LOD).

V.alginolyticus was isolated only from one of the three TTX contaminated mussel samples.

Until now TTXs biological producers are not yet clearly identified and the correlation between TTXs contamination and isolation of TTXs producing bacteria are still not completely understood. Further studies are surely necessary to better explain the TTXs synthetic and accumulation pathways [1].

Conclusions

The present study shows that TTXs are not yet a threat in Marche coasts. The implemented LC-MS/MS can be used for TTXs mussels monitoring and toxins profile assessment but further methodological improvement should be implemented.

The chemical, microbiological and molecular integrated approach is a useful and powerful tool to study the TTXs mussel contamination phenomenon in its complexity. The study of the possible climatic changes may also be useful to better understand the topic.

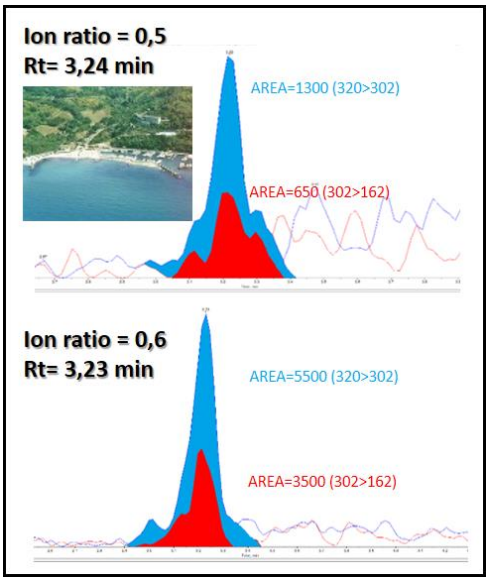

Fig. 1. *LC-MS/MS profile of a contaminated mussel sample from a Pesaro wild site (top) and of the same sample spiked with TTX at 25 µg Kg^{-1} (down). Rt =Retention time. In red and blue mass transitions monitored.*

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OR2

Selection of proteotypic peptide markers tracing for six allergenic ingredients in incurred chocolate bar

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Summary: *In this communication we will describe the identification of reliable peptide markers tracing for six allergenic ingredients in incurred chocolate matrix. The marker selection was accomplished with a dual approach combining the critical evaluation of proteotypic peptides already reported in the existing literature and their validation by discovery analysis on the specific incurred matrix under investigation.*

Keywords: *food allergen detection, peptide markers identification*

Introduction

Mass spectrometry (MS) techniques have been widely exploited in the last ten years to trace allergens in foods with the highest confidence. Despite the need for expensive equipment and trained personnel, the chance to provide multiplexing and unequivocal allergen identification accounts for the overall strength of the MS based approaches compared to previously established methods, such as immunoassays and DNA based methods. Nonetheless, some gaps in the development of MS-based quantitative methods still need to be addressed, mostly in light of the harmonization in method development and validation for reliable results comparability [1].

In this frame, the European project ThRAll "Detection and quantification of allergens in foods and minimum eliciting doses in food allergic individuals", funded by the European Food Safety Agency will actively contribute to the advancement in harmonization of MS-based method for food allergen detection by developing a prototype quantitative reference method for the multiple detection of food allergens in standardized incurred food matrices [2]. In the project, six main allergens, namely cow's milk, hen's egg, peanut, soybean, hazelnut and almond, have been prioritized in light of their higher occurrence in food recalls for undeclared presence with serious risk decision. Two model matrices will be produced *ad hoc* in a food pilot plant, namely a chocolate bar and a broth powder. The allergenic ingredients will be incorporated into the food before being processed to mimic as closely as possible the manufacturing process.

In this communication, we will describe the preliminary results obtained in method development aimed at the identification of reliable peptide markers the

six target allergenic ingredients incurred in chocolate matrix.

Experimental

The chocolate bar was prepared in a food pilot plant by inclusion of six allergenic ingredient before food processing. The following ingredients were sourced for incurred matrix preparation: skim milk powder (MoniQA, SMP-MQA-092014), spray-dried whole egg (NIST, RM 8445), light roasted and partially defatted peanut flour (LGC, LGCQC1020), enzyme active and non-toasted full fat soy flour (Soja Austria), not roasted hazelnut flour (Hazelz, New Zealand), Blanched almond flour (Just Almonds, California, USA). The total protein content for each ingredient was assayed by Kjeldhal method, whenever not specified by the producer. The incurred chocolate bars were prepared at different inclusion levels and, in particular, the 40 ppm and 1000 ppm ($\mu g_{TOT\ protein}/g_{matrix}$) levels were used for discovery experiments. After grounding and sieving (1 mm), the chocolate matrix was extracted with a denaturing buffer (Tris·HCl 200 mM pH 9.2 + Urea 5M) and different sample preparation protocols, were applied for comparison [2-3]. The enzymatic digestion was carried out with trypsin (gold mass spectrometry grade, Promega) and the resulting peptide pool analyzed by *data dependent*™ acquisition mode on a hybrid quadrupole/Orbitrap™ MS platform (Q-Exactive plus, Thermo Fisher Scientific). The MS/MS spectra acquired were processed with a commercial software for protein identification (Proteome Discoverer, v. 2.1, Thermo Fisher Scientific).

Results

The current literature on MS-based allergen detection aiming at collecting all available information about proteins and peptide markers validated in independent studies for the six allergenic ingredients of interest. Several critical aspects affecting peptide markers reliability were discussed and the initial list was refined according to specific features, namely sequence length, matrix similarity with the ThRAll project, kind of investigation and occurrence of amino acid residues prone to endogenous and exogenous modifications. The peptides that were not validated in either chocolate or incurred highly processed matrices were rejected. This preliminary literature-review based list was validated in chocolate bar incurred matrix prepared within the ThRAll project by discovery experiments with untargeted HR-MS/MS analysis.

Different technical aids were compared for protein extraction and purification, such as sonication (either probe or bath), size exclusion chromatography and/or C18 solid phase extraction. The effect of the sample preparation on the final list of identified proteotypic peptides was evaluated, guiding in the selection of a preferential protocol to maximize the protein coverage and populate the list of potential peptide markers, with several options. The peptides identified by discovery analysis were screened according to the same reliability criteria already established for reviewing the literature and finally the information provided by the dual approach was combined. Most of the proteotypic peptides already selected for their high consensus in the previous literature were confirmed by the HR-MS/MS validation in the incurred chocolate matrix. Interestingly, also new options for same allergenic ingredients were highlighted from our experiments that will be included in the targeted method for further validation.

Conclusions

In this communication, we presented the identification of reliable peptide markers tracing for six allergenic ingredients in incurred chocolate matrix according to a dual approach designed in the ThRAll consortium. The critical evaluation of proteotypic peptides already reported in independent works and their validation by discovery analysis on the specific chocolate incurred matrix under investigation provided a final list of highly reliable signature peptides to build up a selected-reaction monitoring-based prototype reference method for multiple allergen detection.

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OR3

Direct analysis of Glyphosate, AMPA, and other polar pesticides in food

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Summary: *Glyphosate direct analysis by Ion exchange LC/MS/MS with the Agilent 1260 Infinity II bio-inert LC and the Agilent 6495 triple quadrupole LC/MS/MS*

Keywords: *Glyphosate, Triple quadrupole, direct analysis,*

Introduction

A direct method with LC/MS/MS using triple quadrupole technology was developed to provide the sensitivity and selectivity needed for low ppb detection of glyphosate and its metabolites AMPA, HEPA, and MPPA, along with glufosinate and its metabolite N-acetyl glufosinate in food. The direct ion exchange separation was achieved using a quaternary amine bound to a polyvinyl alcohol column.

Glyphosate is the active ingredient in the popular herbicide Roundup,and is used throughout the world. Glyphosate is a broad-spectrum systemic herbicide that interferes with the shikimic acid pathway by binding to the 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) enzyme1. It is an organophosphorus compound, specifically a phosphonate. Recently, its safe use has come into question with a report issued by the IARC2. However, the European Commission has approved the use of glyphosate for another five years in a final decision to a European Citizen's Initiative3. This decision was made public citing the conclusions of the European Food Safety Authority4. Most recently, a USA lawsuit against the producer of Roundup resulted in a large jury award5. This has heightened the demand for a sensitive method at the low ppb level for food, and even lower levels for environmental water analysis.

Reliable sample preparation and analysis are needed to routinely analyze glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA). However, glyphosate and its metabolites have high polarity and chelating properties, so they can be challenging for extraction from food and for analysis. Of concern is the affinity of these compounds to stainless steel and other surfaces, making system-to-system reproducibility difficult. This Application Note shows the analysis of glyphosate, its major metabolite, plus six other metabolites and polar pesticides, as shown in Figure 1. All the pesticides are phosphonates. Fosetyl aluminum, a postharvest fungicide, is also important to include, as it can be mistaken for AMPA. We used the Agilent 1260 Infinity II bio-inert LC coupled to the Agilent 6495A triple quadrupole LC/MS for analysis of food samples of plant origin to 10 ppb.

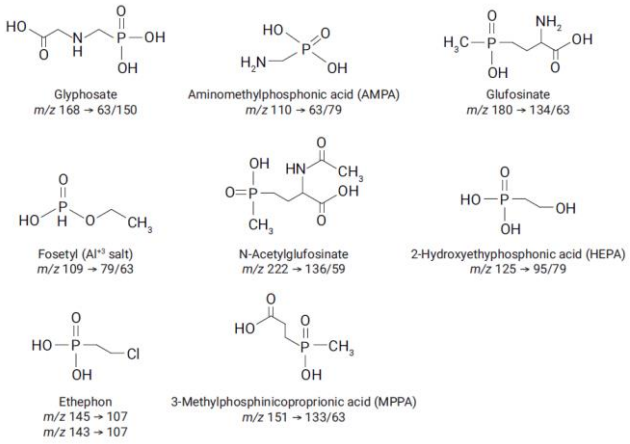

Fig. 1. *Structure of the polar pesticides analyzed in the application*

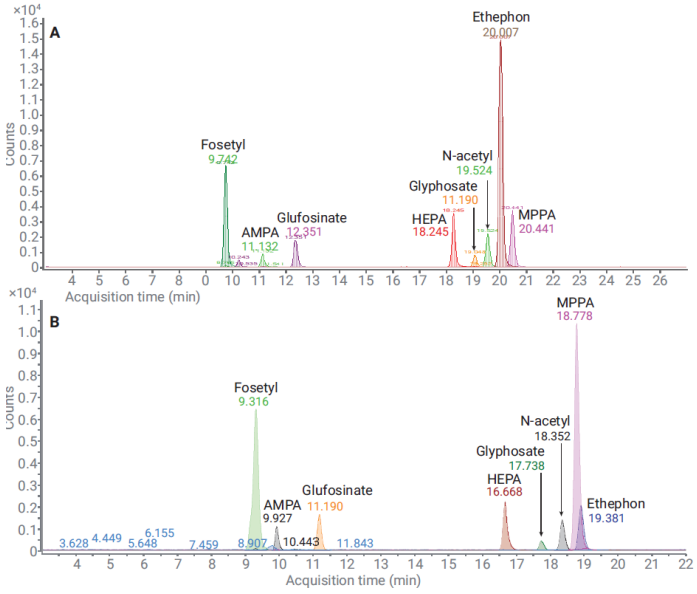

Fig. 2. *Chromatographic separation achieved with the described method of A) 25 ppb standard in ultrapure water and B) 10 ppb standard in 50:50 water: 0.1 % formic acid/methanol.*

Conclusion

A method for the detection of glyphosate and its metabolite AMPA was developed and shown to provide detection at 10 ppb in foods of plant origin. The method requires all surfaces in contact with the sample to be either plastic or PEEK, including the use of polypropylene sample containers. A PEEK and ceramic sample path through the instrument was achieved with the 1260 Infinity II bio-inert LC. High-density polyethylene mobile phase containers were used to reduce sodium in the analytical method. Using the 1260 Infinity II bio-inert LC coupled to the 6495A triple quadrupole LC/MS enables routine

analysis. All food matrices must be validated for the method, and the use of stable isotope internal standards added to the samples before extraction enables accurate quantitation against a solvent standard. Results could be improved with a sample cleanup that removes more interferences without removing the analytes.

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OR4

Cd and Pb time trends 2008-2018 in bivalve mollusks from the Adriatic Sea, FAO 37.2.1 area

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Summary: *During the 2008-2018 timeframe, georeferenced samples from Mytilus galloprovincialis (N = 415) and Chamelea gallina (N=1557) were analysed by ICP-MS, under strict QA/QC for heavy metals. Apart seasonal variation, no time-trends in Pb and Cd contamination were assessed both in farmed and wild mollusks from classified Marche coastal waters.*

Keywords: *ten years monitoring results, Cadmium and Lead, bivalve mollusks*

Introduction

Bivalve mollusks farming represents a relevant seafood commercial activity in the Adriatic sea. The breeding follows strictly food safety EU 854/2004 Regulation, that prescribes to check the compliance with Pb, Cd, and Hg maximum level routinely (once a year) in mollusks farmed/collected in authorized waters. Here we report the database of Pb and Cd in *Mytilus galloprovincialis* and *Chamelea gallina* collected along Marche coast during the last ten years (2008-2018). The results obtained achieved under QA/QC by the officially accredited lab of IZSUM – Ancona, have been studied using different statistical approaches, in order to assess time-trends in the occurrence of the aforesaid heavy metals.

Experimental

In the period 2008-2018, mussels (*M. galloprovincialis*) and clams (*C. gallina)* samples were collected along the Marche coast from official authorities in the sites reported in Figure 1 and submitted for official food safety checks. All the samples were analysed by ICP-MS under QA/QC to measure Pb, Cd, Hg, As, V, Ni and Cr levels. The analytical method is accredited and routinely used by the laboratory: it foresees a microwave digestion of the samples using HNO_3 (6 mL), HF (50 μL) and ICP-MS measure of the analytes using a matrix matched calibration curve. The Pb and Cd concentrations determined in mussels and clams were studied by a time series approach to understand if a time trend could be found. A time series variable consists in a combination of signal - a collection of systematic, predictable components - and noise, an unpredictable, random component (series = signal + noise or series = fit + residual). A time series data analysis is divided into three components: trend, cycle and seasonal. The ten years sampling period was subdivided in 44 equally-spaced sub-periods of three months - January - March, April - June, July - September, October - December, to intercept a possible variability induced by anthropogenic influences.

Results and discussion

Hg was not included in the statistical analysis because its concentrations fall always below the limit of quantification (0.010 mg/kg) .

The main statistical descriptors of Pb and Cd and other determined heavy metals in mussels and clams are reported in Table 1. The time sequence plot in Figure 2 displays the time series data in sequential order, together with the results of applying the following two smoothers, 5RSSH resistant smoother and the simple moving average of 3 terms, respectively to adjusted Pb concentrations.

The time-series refer to Pb and Cd only, spanning from 2008 to 2018 (Figure 2), allowing a sound assessment of time-trends.

On average, both the elements show the same temporal trend that can be approximated by a linear model. They show seasonal peaks but none of them appear to influence a systematic upper- or down-trend (P-value for these tests was ≥0.05). The regular monitoring of concentration of the other heavy metals will allow in the future to evaluate their time-trends, as soon as a suitable timeframe will be reached. To conclude, even if seasonal variation are present, these are not a factor to determine time-trends in Cd and Pb concentration [1, 2]. Anyway, the study of the Pb, Cd and Hg ten years database may give the control authority the tool to optimize future bivalve mollusks monitoring plans.

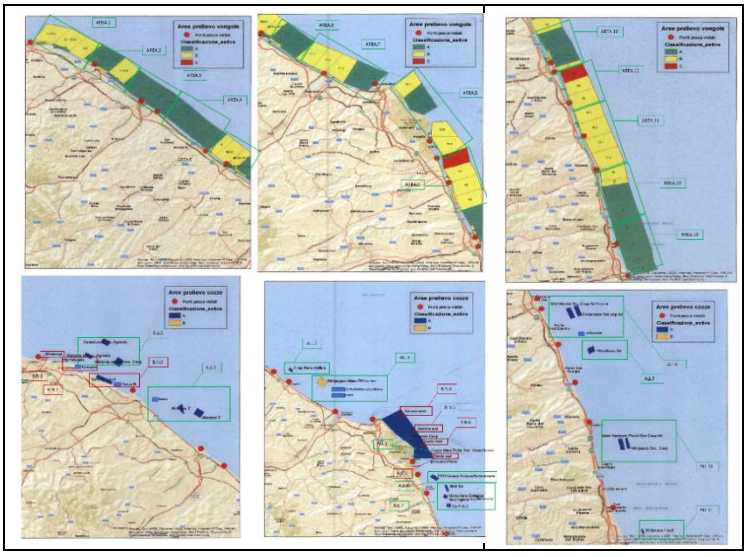

Fig. 1. *sampling areas of mussels (above) and clams (below) in the Adriatic Sea of Marche – FAO 37.2.1*

Table 1. *Statistical descriptors of heavy metals concentration in bivalve mollusks mg/kg*

	Pb	Cd	V	Ni	Cr*	As*
Mussels						
count	344	343	73	113	84	105
min	0.059	0.023	0.063	0.105	0.065	2.19
average	0.185	0.158	0.489	0.478	0.268	3.5
median	0.165	0.15	0.461	0.479	0.249	3.34
max	0.616	0.849	1.38	1.14	0.632	7.01
Clams						
count	1458	1457	645	742	1163	41
min	0.013	0.023	0.072	0.35	0.086	1.62
average	0.097	0.078	0.318	0.802	0.675	2.41
median	0.086	0.078	0.286	0.769	0.354	2.35
max	0.362	0.178	1.11	6.15	10.4	3.75

** indicates no speciation.*

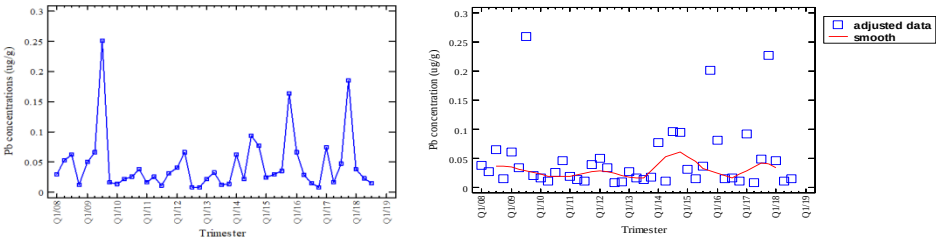

Fig. 2. *Raw (left) and smoothed (right) time trends in Pb concentration in mussels collected from 2008 to 2019*

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Acknowledgements

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OR5

Searching for bioactive nutraceutical and functional food ingredients in *Vitis vinifera* leaves

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Summary: *In this work we performed an untargeted metabolomics analysis of Vitis vinifera cv. 'Pinot noir' leaves, analyzed their fatty acid content and estimated their antioxidative capacity, with the purpose of investigating its nutritional and medicinal value.*

Keywords: *Grapevine; FT-ICR-MS; metabolomics*

Introduction

Over the past few decades, consumers have been increasingly aware of the importance of food composition in human health. The demand for enriched foods and nutraceuticals has brought suppliers' attention to the reuse of agro-industrial wastes, rich in healthy plant ingredients. One example is the grapevine industry, in which leaves are a by-product that has been overlooked as a potential source of bioactive ingredients. Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops worldwide with over 7.5 Mha of global area under vines (data from Organisation of Vine and Wine). The majority of the crop production is used in wine making and commercialization of fresh and dried grapes for consumption. Grapevine leaves are already included in the human diet in several countries of the Mediterranean Basin. With an increasing demand for grapevine leaves, top European grapevine producing countries like Italy, are now considering these products as important for their economy [1]. Having in mind the growing potential of grapevine leaves as a food product, it is important to have the most complete knowledge on their metabolic composition.

We developed an efficient metabolite extraction protocol for grapevine leaves compatible with direct infusion- Fourier transform ion cyclotron mass spectrometry (FTICR-MS, [2]). The detected compounds included several highly relevant compounds for human health, with potential nutraceutical interest [3]. Moreover, both antioxidant potential and fatty acid content of these leaves were determined [3].

Experimental

V. vinifera 'Pinot noir' leaves were collected at the Portuguese Ampelographic Grapevine Collection (CAN, international code PRT051), at INIAV-Dois Portos. Metabolite extraction was performed following a previously developed method [2]. The different obtained fractions were analysed by direct infusion on a 7T-

FT-ICR mass spectrometer (Brüker Daltonics), operating in both, positive (ESI+) and negative (ESI−) electrospray ionization modes. Leucine enkephalin (YGGFL, Sigma Aldrich) was used as internal standard and added to all samples. Spectra analysis, compound identification and annotation were also performed as previously described [3]. MassTRIX database [4] was used to obtain compound formulas.
Fatty acids' methyl esters (FAME) were prepared by direct trans-methylation and quantified by gas chromatography [1]. Lipid nutritional quality was assessed through the determination of atherogenicity (IA) and thrombogenicity (IT) indexes [3].
Total antioxidant capacity was analysed by measuring the Trolox equivalent antioxidant capacity (TEAC) [3, 5, 6].

Results

In this work, the metabolic composition of *V. vinifera* 'Pinot noir' was investigated to assess their potential as a source of nutra-pharmaceutical compounds. Through an untargeted metabolomics analysis by DI-FT-ICR MS, nearly 1000 metabolic entities (unique masses) were identified. From these unique masses detected, chemical formulas were assigned and a total of 857 different formulas were obtained, after combining the data from ESI+ and ESI−.
The annotation of these formulas revealed that within the major metabolic classes, lipids was the most prevalent, with fatty acids (FAs) the most represented lipids' secondary class. Being lipids such an important fraction of *V. vinifera* 'Pinot noir' leaves, the fatty acid content was quantified.
Alpha-linolenic acid (C18:3) was the most abundant FA in leaves, followed by linoleic acid (C18:2) and palmitic acid (16:0). Concerning the lipid nutritional quality, 'Pinot noir' leaves have low IA and IT levels, being therefore ideal to include in a healthy diet.
Besides the presence of numerous compounds with diverse nutritional and pharmacological properties in the metabolic profile of 'Pinot noir' leaves, we identified several polyphenols and phenolic compounds with known antioxidant activities. Hence, the antioxidant activity in 'Pinot noir' leaves was measured revealing a very high antioxidant capacity.

Conclusions

There has been a growing attention to food supply and regional self-sufficiency, food security, and food waste reduction. Grapevine leaves are a valuable grapevine by-product from the vine industry, either for consumption, or as a source of nutraceutical compounds, as they contain a wide variety of compounds with interesting properties in the context of the human health.

Acknowledgments

We acknowledge projects UID/MULTI/00612/2013, PEst-OE/QUI/UI0612/2013, PEst-OE/BIA/UI4046/2014, PTDC/BAA-MOL/28675/2017, IF/00819/2015, CEECIND/02246/2017 and grant SFRH/BD/116900/2016 from Fundação para a Ciência e Tecnologia (Portugal); the Portuguese Mass Spectrometry Network (LISBOA-01-0145-FEDER-022125); and the Project EU_FT-ICR_MS, funded by the Europe and Union's Horizon 2020 research and innovation programme under grant agreement nr. 731077.

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OR6

High-resolution mass spectrometry metabolomics of different *Glera* grape clones used to produce D.O.C.G. Prosecco sparkling wine

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Keywords: *Prosecco, clones, QTOF*

Glera is the main grape variety used to produce Prosecco sparkling wine, one of the Italian wines most known all over the world. It is grown mainly in the northeast of Italy, traditionally in the hills of the province Treviso province. Recently, the Conegliano Valdobbiadene hills, where is produced Prosecco D.O.C.G. (label guaranteeing the quality and origin of a wine) were recognized as UNESCO World Heritage Site.
Currently are available 14 commercial Glera clonal selection that differ in vigor, yield, bunch size and compactness, composition of grape must and, as a consequence, the wine quality.
A current research aims to the characterization of 5 Glera clones selected by CREA-VE for their interesting agronomic characters by using a targeted method of grape metabolomic performed by high-resolution mass spectrometry (HRMS) previously developed [1]. The clones studied are ISV-VA 4, ISV-VA 6, ISV-VA 7, ISV-ESAV 14, and ISV-ESAV 19. Grapes were collected in 2017 and 2018 from vines grafted onto the same rootstock and grown in the same experimental vineyard.
Tentative of differentiation among the clones mainly based on study of QTOF profiles of the polyphenols and terpene glycosides are here presented.

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OR7

An investigation on the reactivity of wine polyphenols towards SO_2

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Summary: *Sulfonation of natural polyphenols is a poorly investigated reaction in flavanol-containing foods including wine. However, it deserves our attention, given that SO_2 is one of the most widely used preservatives in food processing. This study aim to shed light on this particular reaction, its kinetics and mechanism.*

Keywords: *Flavanols, Sulfonation, Kinetics*

Introduction

Flavan-3-ols are a complex family of ubiquitary plant flavonoids, naturally abundant in grapes and red wines. They are strongly involved in the final quality of red wine, influencing its ageing potential, astringency, stability and color [1] as well as the nutritional value. The importance of polyphenols and in particular of flavanols dwells behind the formation of condensed tannins which are polymers consisting of flavanols subunits linked by C-C bonds. Condensed tannins in wine derive primarily from seeds and skin of grapes during winemaking, so red wines contain a larger amount than white wines, and they can also be the result of oenological practices[2].

Sulfur dioxide is the most widely used preservative in winemaking as well as in food conservation in general. Thanks to its antimicrobial action and antioxidant effect, SO_2 is able to protect wine from various unwanted reactions and it is commonly considered as an indispensable additive. At the same time, SO_2 and sulfites are among food allergens, since they may cause breathing difficulties, sneezing, hives, migraine ecc. [3].

Condensed tannins can eventually react with SO_2 producing additives leading to sulfonated products. Sulfonation is basically the addition of a sulfonic acidic group to an organic compound, and it is a widespread industrial process used in a diverse range of products [4].

This reaction is also known to occur in wines ($3<pH<4$) , involving several metabolites such as polyphenols and indoles. In particular studies have been conducted on the reactivity of B type procyanidins with sodium metabisulfite, and proved that the reaction leads to the formation of 4-sulfonates of epicatechin and its polymers [5]. More recently, sulfonated dimeric and monomeric flavanols were detected in red wines and were suggested as promising tentative markers of sub-optimal wine storage [6]

Experimental and Discussion

The current study is aiming to shed light on sulfonation reaction of catechin, epicatechin and their procyanidin dimeric analogues by preparation, purification and structural elucidation of the corresponding sulfonated end-products. Moreover, the overall kinetics parameters of this process have been evaluated

through temperature (and pH) dependence of the rate of these processes. We arrived at this process characterization by exploiting from one side the sensitivity and linearity of the UPLC-PDA-MS system and from the other side the powerful capability of NMR spectroscopy for the structural identification of the end products.

All the sulfonated products were synthesized starting from the commercially available pure starting materials adding $Na_2S_2O_5$ 50eq. as the source of HSO_3^- in 5% EtOH/H_2O.

The outcome of the work led to the evaluation and understanding of the different kinetics parameters involved in the sulfonation of catechin versus epicatechin (Figure 1) as well as a monomeric versus oligomeric flavanols (Figure 2); the pH-dependence of these parameters has been also measured (Figure 3).

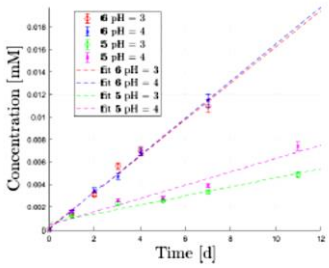

Fig. 1. *Comparison between catechin and epicatechin reactivity regarding sulfonation products, respectively catechin sulfonate (5) and epicatechin sulfonate (6) at pH= 3,4 and T=60°C.*

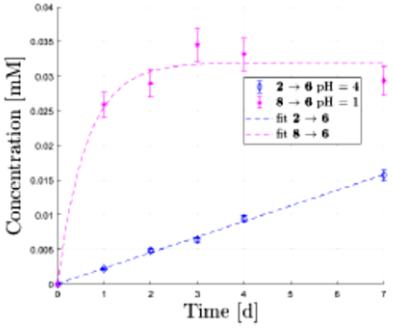

Fig. 2. *Rates of appearance of the sulfonation product using epicatechin (blue) or its dimer (pink) as starting materials.*

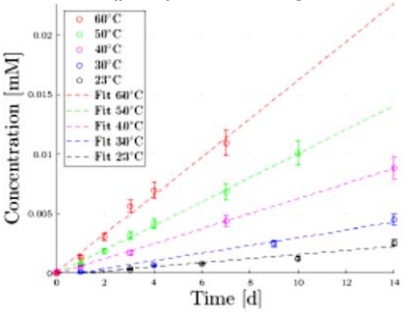

Fig. 3. *Kinetics of epicatechin sulfonation by HSO_3^- in time dependency at different temperatures.*

Conclusions

Sulfites are by far the most important class of exogenous additives used in wine-making. It has been proved that they do not only possess antiseptic properties but they also react with polyphenols present in wines. Sulfonation is a complex and slow process, not easy to investigate in particular due to the different behavior with respect to the two considered monomers, catechin and epicatechin and the experimental variability due to the many parameters that we need to take into consideration. However, we succeeded to i) follow the kinetics of the process, ii) to evaluate their kinetics parameters such as Gibbs free energy of activation, iii) to assign 3D-structures to the sulfonated end-products and iv) to propose a reasonable reaction mechanism. The results of this study on low molecular flavanols provides key information needed to model in the future also the more general topic of the sulfonation of polymeric procyanidins.

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OR8

Volatile profiles of Italian monocultivar EVOOs during 12 months of storage: different trends associated to polyphenols and fatty acids content

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Summary: *In the present study we investigated the variation in volatile organic compounds of a large number of monocultivar extravirgin olive oils after 6 and 12 months of storage at 15 °C. The initial polyphenol profile or the composition in fatty acids of the EVOOs is partially responsible for the different evolution observed among the samples during storage.*

Keywords: *Monocultivar extravirgin olive oils, volatile organic compounds, polyphenols*

Introduction

Aroma is one of the most important and the first to be perceived attribute of high-quality extravirgin olive oil (EVOO). Volatile organic compounds (VOCs) derived from lipoxygenase (LOX) pathway are considered the most important and impacting for olive oil aroma. In a previous study we quantified such compounds in 130 monocultivar EVOOs from 67 genotypes belonging to a Tuscany germoplasm collection [1]. During the storage of EVOOs, the VOCs responsible for the pleasant flavour became less dominant and compounds responsible of negative sensory attributes may arise. The main chemical alteration of EVOO is due to oxidation that is influenced by storage conditions, such as temperature, light exposure, oxygen availability, and also by oil composition [2]. Even at optimal storage condition, auto-oxidation of fatty acids takes place further contributing to the modification of EVOO composition. For these reasons the aroma profile in EVOO is not stables but changes from the production until the consumption. The present study investigated the fate of VOCs in 60 monocultivar EVOOs after 6 and 12 months of storage and evaluated the possible influence on it of the initial polyphenol availability and the fatty acids compositions.

Experimental

Sixty monocultivar EVOOs were stored at 15 °C in the dark until 12 months. Details about olives and oil production can be found in [1]. VOCs were measured by SPME/GC-MS before storage (1) and after 6 (2) and 12 (3) months as reported in [1].

At T0, compositions in fatty acids and in polyphenols were determined as well using official methods [3].

Results

The different VOCs evolved differently during the storage. (E)-2-hexenal, the most abundant compound, initially present in the EVOOs, significantly decrease after 6 months while in the successive months an inversion in this trend was observed (Fig. 1, left). Similar behaviour was observed for (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol and (Z)-3-hexenyl acetate. Differently, hexanal, the second most abundant compound initially present in the EVOOs, did not change significantly after 6 months while increased at 12 months of storage (Fig. 1, right). In order to investigate the possible influence on VOCs evolution of the initial composition in polyphenols and fatty acids, a PCA was computed using these composition data. Four classes were identified that correspond to the 4 quadrants defined by the first two plotted scores where the total polyphenols vary along the 1st component while oleic/linoleic vary along the 2nd component. Main features of the 4 classes are reported in table 1.

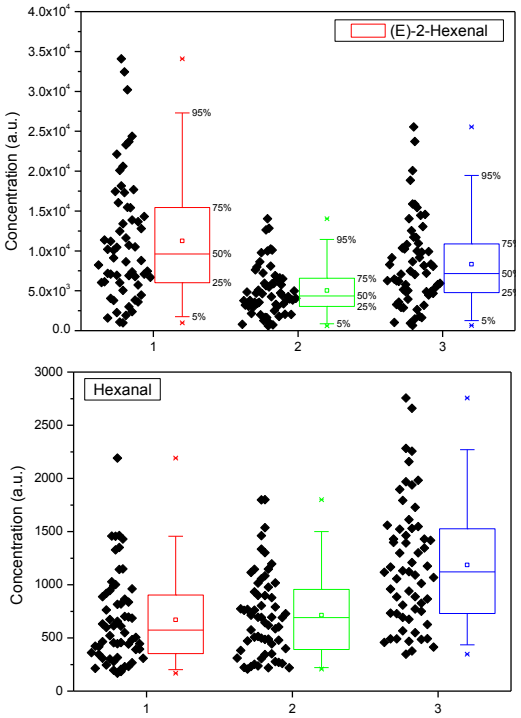

Fig. 1. *Boxplot of the concentrations of (E)-hexenal and hexanal at production time (1) and after 6 (2) and 12 (3) months of storage at 15°C in the dark.*

Table 1. *Class of EVOOs identified based on the PCA of chemical composition data*

Class	Number of EVOO	Total Polyphenols	oleic/linoleic ratio
1	17	High	High
2	16	Low	High
3	15	High	Low
4	12	Low	Low

The largest variations, significantly influenced by the initial EVOO composition, were observed after the first 6 months of storage. Several VOCs show different magnitude of variation according the identified class. In figure 2 is reported the example of (Z)-1,3-pentadiene that after 6 months of storage shows significant ($p<0.001$) higher depletion in group 1.

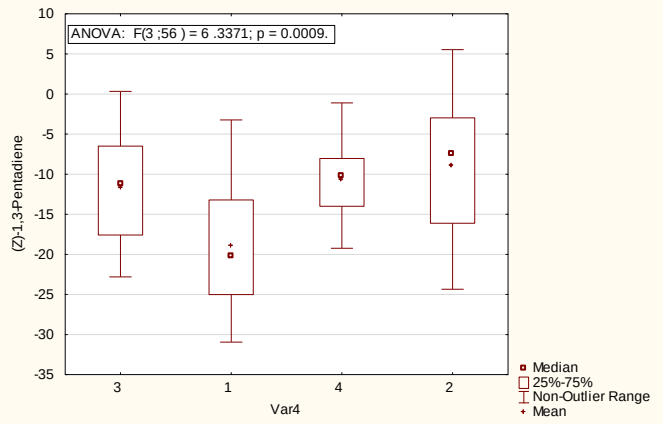

Fig. 2. *Boxplot of the variation in concentrations of (Z)-1,3-pentadiene after 6 months of storage within the four classes reported in Table 1.*

Conclusions

We investigated the VOCs in a large number of monocultivar EVOOs during storage, in controlled conditions, showing that their evolution may differ among the oils partly influenced by the initial composition of the samples itself.

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OR9

Metabolomics as a tool for the optimization of agronomical parameters of Ribolla Gialla for the production of sparkling wine

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Summary: *Despite the increasing area of cultivation with Ribolla Gialla, there is little information about the effects of agronomic parameters used in the vineyard on the quality of monovarietal sparkling wines. Using the metabolomic approach during wine production can therefore help to improve chemical and sensorial characteristics of the aforementioned wines*

Keywords: *Metabolomics; Ribolla Gialla; sparkling wine*

Introduction Ribolla Gialla is an indigenous and an old white grape variety mostly cultivated today in North- Eastern Italy (in the Friuli Venezia Giulia region), in Slovenia and on the Ionian Islands in Greece, where it is known as Rebula and Robola, respectively [1]. Due to its limited area of cultivation throughout the history, the winemakers often blended Ribolla Gialla with other local varieties in the winemaking process, but nowadays the growing area with Ribolla Gialla is increasing, as well as it potential for the production of quality monovarietal sparkling wine. However, the study of geographical features of the vineyards (especially hills versus flat areas) and how the agronomic procedures affects the ripening of the grapes is still needed, in order to obtain the best ratio between quality and quantity of grapes. For instance, cluster thinning is a commonly adopted viticultural technique for selective removal of excessive clusters and allows calibrated vine productivity with increased accumulation of metabolites in the fruit [2]. Previously, the authors have been dealing mainly with the genetic diversity of Ribolla Gialla grapes [1], and not so much on the metabolomic profile of the wines [3]. For this purpose, the multitargeted approach was adopted in this study, focusing on the aromatic characteristics of the sparkling wines obtained from Ribolla grapes, including their volatile organic compound (VOC) content, lipids, and the metabolites of aromatic amino acids. All of these compounds are important because they may be linked with positive or negative aspects of wine quality.
The aim of the present work was therefore to investigate the effect of cluster thinning in two different vineyard sites, and how these factors affect the overall metabolomic profile at all stages of the sparkling wine production.

Materials and methods
The viticultural trial was carried out during the 2017 growing season in two commercial vineyards in different DOC (*Denominiazione di Origine Controllata*) districts (Corno di Rosazzo and Casarsa della Delizia) in the Friuli Venezia Giulia region in North-East Italy. The vines were divided into two treatments in

each vineyard site: no thinning (CT-0), and 20% thinning (CT-20). Both treatments were performed in the hilly district of Corno di Rosazzo (CR), as well as in the flat district of Casarsa della Delizia (CD). At the optimal phenological and technological maturation, the grapes were harvested, crushed and pressed, and all the musts obtained were immediately inoculated with selected *Saccharomyces cerevisiae* yeast. The sparkling wines were produced by the Charmat method, where the base wine is left to undergo a second alcoholic fermentation in sealed autoclaves.
By using SPME-GC-MS/MS, and UHPLCMS/ MS methods, the content of volatile compounds [4], the metabolites of aromatic amino acids [5], and the lipids [6] were determined in grapes, as well as in the base wines and the sparkling wines.

Preliminary results
The results of principal component analysis (PCA) on Figure 1, shows clear differences concerning the total VOC composition of the sparkling wines obtained from hilly and flat vineyards. Moreover, wines from both locations demonstrated also a distinction concerning the performed viticultural trials, therefore, there are four different groups on PCA plot, each representing its very vineyard site and the percentage of the cluster thinning, respectively. A total number of fifty-nine volatiles were tentatively identified in the wines, including acids, alcohols, esters, C6 compounds, diols, terpenes, norisoprenoids, and carbonyls. Among those, esters were the most representative class of volatile compounds found in Ribolla Gialla sparkling wines, and they generally confer floral, fresh and fruity notes (rose, banana, pear, green apple) to the wines [7], which are generally recognized as typical in Ribolla wines.

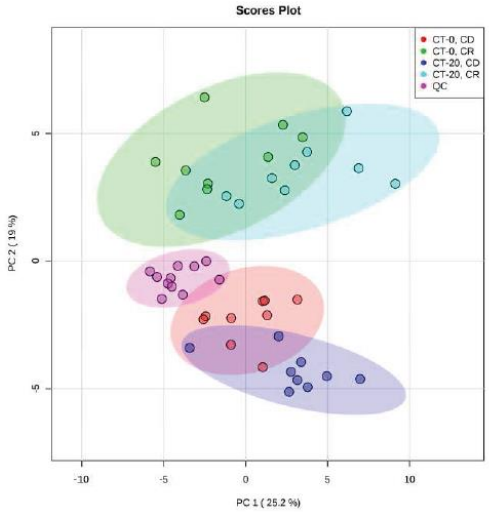

Fig. 1. *PCA plot of volatile compounds detected in sparkling wines*

The second group of metabolites analyzed was the aromatic amino acids and its catabolites. There were nineteen compounds detected, among which also the essential amino acid tryptophan (TRP) and indole-3-acetic acid (IAA), that

are considered to be potential precursors of 2- aminocetophenone (2-AAP), which is an aroma compound that causes an atypical ageing offflavour in *Vitis vinifera* wines. The off-flavour is often described with aroma descriptors such as “acacia blossom”, “furniture polish”, “wet wool”, or “fusel alcohol” [8]. Despite the fact, that 2- AAP was not present in base wine samples there are some evident differences between both vineyard sites in amount of 2-AAP precursors, as shown in Table 1.

Table 1. *Concentration with standard deviation (both expressed in mg L-1) of aromatic amino acids metabolites in Ribolla Gialla base wines*

Samples	TRP	IAA
CT-0, CR	1.13 ± 0.18	0.08 ± 0.01
CT-20, CR	1.09 ± 0.42	0.10 ± 0.02
CT-0, CD	0.17 ± 0.14	0.05 ± 0.01
CT-20, CD	0.21 ± 0.18	0.06 ± 0.01

Lastly, fatty acids (FA) originate from the firm tissues of the grapes, but the greatest amount is formed during alcoholic fermentation. Therefore, fatty acids may be present in wine in free or bound form as ethyl esters. Twenty-nine lipid compounds were found in the samples of the grapes according to UHPLC-MS/MS method performed. Most of the compounds found were saturated long-chain fatty acids (LCFAs), although the mid-chain fatty acids (MCFAs) and their esters have a major influence on the organoleptic properties of wine [9]. Ribolla Gialla showed a higher average concentration of saturated FA in clustered grapes from hilly vineyard site (3.34 mg L-1), compared to the unsaturated FA (1.66 mg L-1), which may lead to higher foam height in the later production of sparkling wines, and represents one of the key quality features of sparkling wines in general.

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OR10

Mass spectrometry for free polyphenols in untreated samples of beer

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Summary: *A selective and sensitive HPLC-PDA-ESI-MS/MS method for the determination of fourteen polyphenols among the most widespread ones in food and beverages was developed operating in the Selected Ion Recording mode, in a single HPLC run, and applied for detection and quantification of free polyphenols in untreated samples of craft beer.*

Keywords: *untreated beer samples; polyphenols; Selected Ion Recording (SIR) technique*

Introduction

A high proportion of dietary antioxidants intake occurs via commonly consumed beverages, coffee[1,2] being the main contributor, followed by wine, beer and tea. Phenolic acids are natural antioxidants mainly contained in beer, coming from malted barley, other raw or malted grains and hop, so that a wide choice of different beer types is available and needy of being characterized in terms of phenolic profile and content. Most of analytical methods reported in literature to characterize beers need sample clean-up and/or hydrolysis procedure to free phenolic compounds from their bond forms.[3] In this study extracted PDA chromatograms and Selected Ion Recording technique were used as combined tools to unequivocally detect free polyphenols for profile and content characterization of beer as such.

Experimental

Chromatographic separation of a solution containing fourteen standards (STDs), belonging to different phenolic classes, i.e. benzoic acids, hydroxicinnamic acids and flavonols, was optimized by using a High Performance Liquid Chromatography separation module coupled to a PhotoDiodeArray detector and linked to a tandem mass spectrometry system with an ElectroSpray Ionization (ESI) source.[4] ESI source parameters were optimized by infusion experiments on the STDs solution to obtain a good molecular peak for all the STDs. Spectrometric data were aquired with the Selected Ion Recording (SIR) technique, selecting the $[M-H]^-$ m/z monoisotopic value for each standard.

Calibration curves were calculated analysing the SIR peak area against the STD nominal concentration.

Craft beer samples were degassed, filtered and diluted with the mobile before injection for analysis.

Results

A good separation of 14 phenolics among benzoic acids, hydroxycinnamic

acids and flavonols, was obtained by a single HPLC run, as well evidenced in Fig. 1 where the PDA chromatograms extracted
at the phenolic class characteristic wavelenght are shown. The highly selective and sensitive SIR technique allowed the unequivocal detection of free phenols, the strategy resulting suitable for quantitative analysis without any samples cleanup or hydrolysis procedures (Fig. 1, insets).

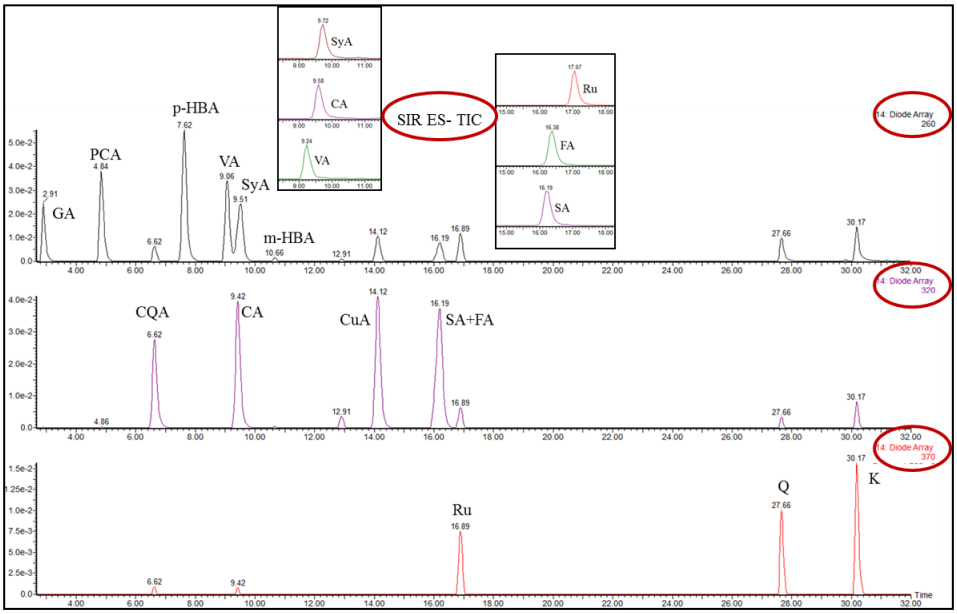

Fig. 1. *PDA extracted chromatograms at characteristic wavelenght: λ_{ex}=260 nm for benzoic acids, λ_{ex}=320 nm for hydroxycinnamic acids and λ_{ex}=370 nm for flavonols. Selective SIR mode in the insets.*

Conclusions

The choice of developing a selective and simple at the same time method arose from the need of fast analysis of complex matrices as food and beverages, useful for screening, production process monitoring, selected analyte of interest detection/quantification and other. Molecules belonging to different class of polyphenols were chosen to develop a single chromatographic method for compounds commonly co-present in natural matrices. In particular, standard generally present in beer were chosen to apply the method to craft beers. In general, the combined use of a PDA detector with a SIR technique offered a strategic tool for fast, selective and sensitive analysis of complex samples simply filtered and diluted with mobile phase before analysis.

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PL2

Towards on-site food analysis by (trans)portable mass spectrometry

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Nowadays we are enabled to measure several health-related parameters using our smartphone and -watches. Similar principles will be used in the future by both consumers as well as food inspectors to check food for unwanted contaminants. The first methods that will become available for on-site inspection will be based on bioaffinity tests targeting only a single or a few contaminants and will not be multi-analyte and provide simplified yes/no or, at best, semi-quantitative screening results. According to legislation, as a follow-up suspect samples still need to be transferred to a laboratory where skilled technicians will perform mass spectrometry (MS) based confirmatory analysis. We envisage that the next-generation methods for on-site analysis will be more advanced and even high-end laboratory equipment, like mass spectrometers, will be applied for field analysis. Currently, there are very few MS instruments available that can be used on-site for food analysis. The majority of these MS instruments are transportable and not truly portable, making on-site application limited and not flexible. It is realistic to envisage that truly portable MS, already successfully applied for warfare agents and in forensic applications, can also be used in food and feed control.

In recent years, we have invested in research which should lead to effective on-site MS applications for food quality and safety control. To that end we mainly focussed on the elimination of tedious sample clean-up and studied simplified sample introduction methods as an alternative to cumbersome LC/MS methods. Several ambient ionization methods were critically evaluated using a set of selected food contaminants as representatives for the entire field. In this presentation we will provide an update of our most recent results. From the alternatives tested, Coated Blade Spray (CBS) turned out to be a highly promising option for liquid samples and extracts.

Meanwhile, following an extensive evaluation of available portable MS instruments, we recently purchased a portable GC/MS that is fully self-sustaining in the field, containing a battery, small gas cylinder, computer, GPS, and a WiFi connection to our institute via the cloud. The MS is operated under high-vacuum and nevertheless operational in 10 min after switching the system on. The ionization is based on electron ionization and the mass analyzer is a robust quadrupole. Different sample inlet options are available, e.g. membrane inlet MS (MIMS), thermal desorption and split/splitless injection. This truly portable MS is currently evaluated for various food applications. Depending on the application, an inlet method is chosen. For example, MIMS for chemical incidents (volatiles), thermal desorption for food authenticity-related questions

and splitless injection for pesticide analysis. Initial results of the evaluation of this MS system for food safety related applications will be presented and discussed as well.

OR11

Authenticity of honey: is DART-MS an effective screening tool?

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Summary: *DART-MS was explored as alternative methodologies for food authenticity issues. Twenty honey samples, of different geographical origin, were fingerprinted by means of a DART ion source coupled with a single-quadrupole mass spectrometer. Chemometric processing allow to clearly discriminate the two groups of sampels (i.e. Italian and Non-Italian).*

Keywords: *Honey Authenticity, DART-MS, chemometrics*

Introduction

In terms of authenticity, honey is among the most challenging food products, being addition of non-permitted substances (i.e. syrups or sugars), bees overfeeding, mislabelling and abuse of veterinary drugs, among the main adulteration practices. Nowadays, melissopalynology still remains the reference method for many of the authenticity issues in honey, such as floral and geographical origin, undeclared blending and/or filtration, etc. However, such approach is highly time-demanding and strongly conditioned by the analyst skills. ThereforeTherefore, lot of effort has been focused on developing alternative analytical methods for authentication purposes. In particular, fast and reliable screening techniques are highly claimed for effective management of a fast-paced global food network. Since ambient mass spectrometry meets the requirements in terms of speed and ease of use, substantial literature has been reported so far about the potential and limitations of Direct Analysis in Real Time-Mass Spectrometry (DART-MS) in food analysis [1].

Experimental

In a previous work, 10 Italian and 10 non-Italian Acacia honeys were analysed by means of several techniques (namely, NIR, NMR, ICP-MS, and untargeted LC-HRMS) in order to evaluate their capability in discriminating the honeys' geographical provenance. Under a data fusion approach, results coming from different platforms were merged, leading to a 100% correct classification rate. Although extremely powerful, the approach was however, time- and labour intensive. Therefore, with the aim of exploring alternative methodologies, the ability of direct MS to discriminate the same set of honey samples in terms of geographical origin, was challenged.

The same sample set was fingerprinted by means of a DART ion source (IonSense, Saugus, USA) coupled to a QDa single-quadrupole mass detector (Waters Corp., Manchester, UK). The sample preparation was minimal, with a total analysis time of 5 minutes. Data pretreatment was aimed to improve the overall data quality, overcoming pitfalls in data reproducibility (Fig 1).

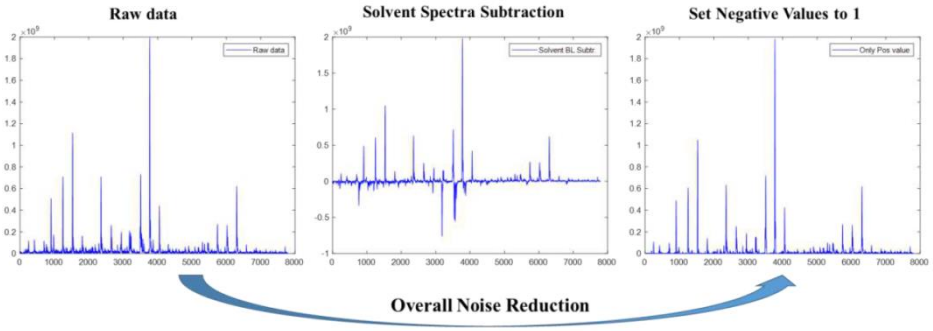

Fig. 1. *Blank solvent spectra subtraction process.*

Results

Multivariate data analysis was carried out and partial least squares discriminant analysis (PLSDA) was chosen as classification algorithm, reaching a 100% correct sample discrimination. Furthermore, similarities were found between the PLS-DA scores plot obtained with the merged data of the previous work and the DART-QDa data (Fig. 2).

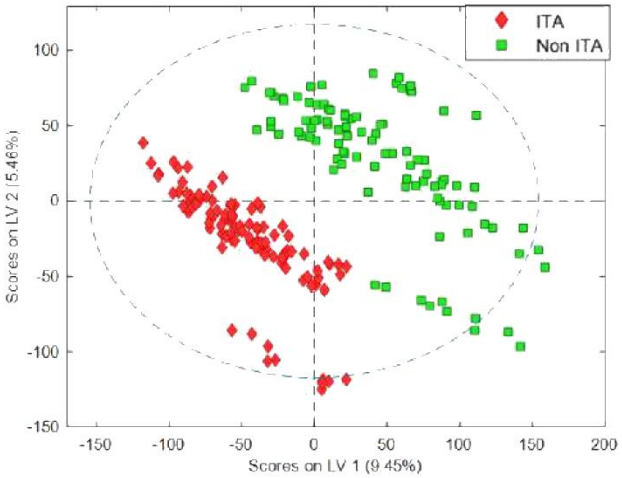

Fig. 2. *PLS-DA scores plot of DART-QDa data.*

Conclusions

The results of the present work proved the potential of DART-MS as rapid screening platform for honey authenticity.

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OR12

UniSpray™ a novel atmospheric pressure ionization source for LC-MS/MS for routine analysis

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Keywords: *Ionization source, Unispray, residual analysis*

In mass spectrometry, the type and design of ionization source play a key role on the performance of a given instruments. It will be showed comparative results between ESI and US source in two common routine analysis: pesticide residues and micotoxins in different commodities.

KN1

Proton transfer reaction mass spectrometry and sensory techniques to investigate biotransformations of hop derived compounds in beer

Patrick Silcock

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

Summary: *Harnessing yeast biotransformations may provide a pathway to create beers with greater flavor diversity. Use of proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS) and sensory analysis helped to unlock how yeasts modulate flavour and how the magnitude and kinetics of these biotransformations can differ markedly between yeast strains.*

Keywords: *fermentation, flavor development*

Introduction

Beer drinkers and brewers are increasingly looking for bolder flavours and greater diversity in beer. To achieve these distinctive flavours, brewers are increasingly interested in taking advantage of yeast driven biotransformations. Yeasts are responsible for the generation of higher alcohols and esters that form the main flavour profile of beer, and are also able to modify terpene and polyfunctional thiol precursors found in hops to generate additional flavour compounds. The role of yeast in modulating the concentrations of compounds contributing to hop flavour is gaining widespread attention in industry and academia. However, there is an incomplete understanding of both ester and hop-derived yeast induced biotransformations and how these impact beer flavour. A better understanding of the nature of yeast-induced bio-transformation may enable brewers to select yeast strains to accentuate specific flavors in the beers they produce.

Experimental

Two studies were carried out using PTR-Tof-MS. Study 1 used two yeast strains (White Labs WLP 028 Scottish ale and WLP001 California Ale) x two hop varieties (Motueka and Nelson Sauvin). Study 2 involved six yeast strains (Fermentis WB-06, US-05, S-33, S-189, S-23, and W-34/70) x 1 hop variety (Motueka). A further study (study 3) produced 12 beers for sensory analysis using 12 yeast strains (Fermentis WB-06, US-05, S-23, W-34/70 and BE-256; Wyeast Wy1272; Anchor VIN 13 Wine Yeast Hybrid and Exotic SPH Wine Yeast; White Labs WLP 001 and WLP730 [Chardonnay White Wine]; and two yeast strains from the UoO yeast collection: OTA 29 and OTA 79). All beers in Study 3 were using the same standard wort late hopped with one hop variety (Motueka)

Wort preparation and fermentation

For study 1 and 2, wort (10.0 °P, ~20 International bitterness units, IBU) was prepared using malt extract (120 g/L), Waimea bittering hops (0.24 g/L), boiled for 30 min then cooled to 90 °C, aroma hops (5.0 g/L) added and held for 5 min at 90 °C. The wort was aerated (filtered air) for 10 min and propagated yeast

added (about $1.0x10^7$ yeast cells/mL). Each of the treatments (6 x 3 mL) were transferred into separate headspace vials (20 mL) which were capped and placed into the PTR-ToF-MS auto sampler (Gerstel, Mulheim am Main, Germany) where the fermentations were carried out at 15, 20 or 25 °C. During fermentation, the headspace of each vial was sampled every 6 h for four days with the first set of measurements conducted within 2 h of inoculation.
For study 3, wort (10.0 °P, ~20 IBU) was prepared in a 2000 L mash tun using malted barley (NZ ale malt), Simcoe hops for bittering and Motueka hops added at 5g/L post boil at 90°C for 5 min. Wort was oxygenated inline (20°C) using O_2 and yeast pitched at 1 x 10^7 cells/mL. Fermentation (10L) was carried out for 7 – 9 days at 20°C followed by conditioning at 4°C for 9 d.

PTR-ToF-MS
PTR-ToF-MS 8000 (Ionicon Analytik GmbH, Innsbruck, Austria; drift voltage, 520 V; drift temperature, 110 °C; E/N of ~130 Td; coupled with an autosampler) was used to measure the sample headspace, which was carried out for 1 min at a flow of 120 mL/min (3 min break between samples). During sampling, the headspace in the vial was replaced with N_2. Ar was directly injected into the drift tube at a flowrate of 1.2 mL/min to dilute the samples (1:2) prior to measurement [1]. Data was extracted and pre-processed (including dead time correction, calibration, peak detection and peak extraction) [2], converted to parts per billion by volume [3] and the mean of 20 spectra for each measurement were calculated.

Sensory sorting task
Beers (12 x 40 mL at 10°C) were assessed in triplicate (1 replicate in 1 session) with all beers presented simultaneously in a balanced, randomised order. Beers were tasted from left to right, formed into groups (minimum of 2 and maximum of 11 groups permitted) and descriptors that characterised each group were recorded from each panellist. Data was collated and analysed by multiple correspondence analysis.

Results
Headspace sampling under dynamic conditions allowed the time of volatile generation to be observed during fermentation. Ethanol and carbon dioxide release provided a valuable estimation of fermentation vigor and time to completion. Time to maximum CO_2 release was dependent upon yeast strain, yeast inoculation and fermentation temperature.
PTR-ToF-MS measurements allowed ester generation to be matched to CO_2 release, and thus fermentation rate. It was observed that the highest rate of ethyl hexanoate production occurred up to 12 h after the maximum rate of CO_2 release. This observation was interesting as the time of ester generation during fermentation has been associated with the period of most vigorous yeast growth [4]. Temperature influenced ester generation in a yeast strain specific manner, where time to maximum ester concentration decreased as temperature increased, although the yeast strain effect seemed more complex that simply being related to differences in fermentation vigor.
Generally, the contribution from hops to the volatile composition was greatest at the start of fermentation, indicating that though biotransformed compounds make important contributions to hop flavor, hops directly contribute to the volatile composition of beer. Differences in the release rates of the hop-derived

compounds during fermentation in beers produced using different yeast and fermentation conditions allowed insights into terpene-related flavor generation pathways. Sensory analysis confirmed that the differences measured during fermentation resulted in perceivable differences between the beers originating from the same starting wort.

Conclusions

PTR-ToF-MS enabled real-time data to produced, thereby providing insight into the generation and release kinetics of volatiles important in beer flavor. The use of an autosampler allowed multiple variables to be simultaneously examined and replicated to provide robust results. The combination of PTR-ToF-MS and sensory analysis allowed insights into the bio-transformations of terpene-related compounds that were perceivable to the panelists. The results demonstrated that the amount and range of compounds produced by bio-transformations is heavily yeast-strain dependent.

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OR13

VOC profiling of raw and cooked gilthead sea bream (*Sparus aurata*) of different geographical origin by PTR-ToF-MS

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Summary: *Fish volatile compounds are related to quality and freshness of fish. In this study, proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS) equipped with a multipurpose head-space automated sampler was successfully applied for investigation of VOC profiles of raw and cooked gilthead sea bream (Sparus aurata) from different geographical origins and farming methods.*

Keywords: *fish, aroma, direct injection*

Introduction

Consumers mainly evaluate fish freshness and quality by its aroma [1]. Volatile organic compounds (VOCs) released from fish depend not only on freshness of the product, but also dietary treatment and some other factors of fish husbandry [2]. The evaluation of aroma profiles of wild and reared gilthead sea bream (Sparus aurata) is typically studied by GC-MS [2-4]. Proton Transfer Reaction-Mass Spectrometry with Time-of-Flight analyser (PTR-ToF-MS) is a valuable alternative for the analysis of fish samples in a rapid and non-invasive way. Coupling PTR-ToF-MS to a multipurpose head-space automated sampler gives the possibility to analyse raw and cooked fish in an automated way with a precise control of experimental conditions and increase the analytical throughput

Experimental Methods

Fresh wild and reared gilthead sea bream (*Sparus aurata*) were selected for studying their VOCs profiles by a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) coupled to a multipurpose head-space automated sampler (Gerstel GmbH, Mulheim am Ruhr, Germany). Reared fish was originated from two different farms: one in the Adriatic Sea and the other in the Tyrrhenian Sea. 3 g of the dorsal part of fish fillet of five fishes of each origin were sampled in triplicate and put in 20 mL glass vials at 10°C. Prior to measurements samples were stored at -80°C. Before analysis the samples were thawed at 4°C, then each vial was incubated at 25°C for 25 min and measured for 1 min. Cooking was simulated by incubating each vial at 70°C for 25 min and cooling down at 25°C for 25 min. Measurements follow as for the raw samples. To increase sensitivity, an ion funnel was operated at the end of the drift tube[ref].

Data processing of PTR-ToF-MS spectra consisted of dead time correction, external calibration and peak extraction [5] followed by descriptive statistics and multivariate analysis.

Results and discussion

Two hundred fifty nine out of 429 mass peaks were selected for the further analysis eliminating mass peak of impurities, isotopes, and water clusters. Many of them could be tentatively identified based on sum formula, isotopic pattern and literature. Principal component analysis (PCA) was performed on the selected mass peak of all samples showed the clear separation between raw and cooked fish. The aroma profile of cooked fish was more intense than the cooked one. From the score plot of PCA of raw fish samples, a clear separation between three types of fish samples was observed. According to the PCA score plot of cooked fish the differences between raw and reared fish remained but the differences between two farming systems became less evident.

We have indication that all fish samples were equally fresh because no differences are observed for trimethylamine and S-containing compounds and for lipid oxidation aldehydes and alcohols [2]. The wild fish samples were richer in butanal, butanone, butanol, acetone, methanol and some other compounds. The concentration of these compounds augmented significantly with cooking.

The reared fish from Adriatic Sea was richer in monoterpene compounds from essential oils [3] and aromatic compounds which are usually associated with the metabolism of carotenoids or thermal degradation of sugars and amino acids [3]. For these compounds a negligible effect of cooking was observed.

In this experiment, for the first time PTR-ToF-MS coupled to a multipurpose headspace automated sampler was applied for a rapid and non-invasive screening of aroma profile of raw and cooked gilthead sea bream. The methods could demonstrate the freshness of all samples and could identify several markers associated to cooking, rearing system and season.

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KN2

High performing VOC phenomics to improve the horticultural production chain

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Summary: *The instrumental characterization of volatile organic compounds is essential to have a precise and reproducible estimation of food aroma and, therefore, of the overall product quality. This contribution aims at supporting the development of high-performing VOC phenotyping, based on PTR-MS technology, suitable to address the aroma complexity of agro-food products in different situations.*

Keywords: *PTR-ToF-MS; Chemical Ionization Mass Spectrometry; fruit aroma*

The current crucial mission of agro-food industry is to guarantee food safety and, at the same time, improve perceived food quality and fulfil consumer expectations. To address this issue a broad and objective quality detection of food products is needed. Examples of chief quality traits for the agro-food industry are the development of volatile organic compounds (VOCs) associated with the shelf life and taste quality of fruit and vegetables [1]. However, the so called "phenotyping bottleneck", caused by the absence of high-throughput and non-invasive methodologies, impedes an effective evaluation and prediction of food VOCs.

The extreme complexity of food VOC aroma, both families of compounds and concentration ranges, is a challenging issue for any existing analytical technology. The rapid development of mass spectrometry (MS) application in metabolomic studies had a significant impact in the field of VOC analysis. The progress of MS techniques is mostly focused on instrumental improvements of mass resolution, mass accuracy, sensitivity, and enhanced reproducibility. Direct injection mass spectrometric techniques, such as PTR-ToF-MS (Proton Transfer Reaction – Time of Flight- Mass Spectrometry) and SIFT-MS (Selected Ion Flow Tube – Mass Spectrometry), have opened new possibilities for food aroma analysis by decreasing the time needed for sample preparation and analysis, and by providing the possibility of non-destructive, real time and high-throughput volatilome analysis. These achievements enhanced the relevance of VOC assessment into the horticultural modelling for instance to better predict the product shelf-life or to estimate the final quality of a processed food based on the raw material.

This contribution aims at reviewing several prototypical analytical approaches, based on chemical ionization mass spectrometry, suitable to address the aroma complexity of agro-food products in different situations: i) non-destructive VOC assessment; ii) high-throughput automated headspace analysis; iii) dynamic destructive analysis; iv) real-time process monitoring (Table 1).

Tailored pre- and post-harvest studies confirmed the potentials of PTR-ToF-MS application into the whole agro-food production chain, from breeding to

consumers. These studies allowed to estimate the interaction between genetic variability, ripening stages and storage condition on the perceived quality of several fruit species (i.e apple, strawberry, and blueberry) [2-4]. Another important attainment was the development of putative VOC biomarkers linked with fruit spoilage caused, for instance, by the occurrence of postharvest disorders (i.e. superficial scald or anoxic stress) or by fresh-cut processed deterioration [5].

Table 1. *Different analytical approaches based on chemical ionization mass spectrometry and their possible application in the AgriFood production chain*

Analytical approach	AgroFood application
Non-destructive headspace analysis	- Effect of different packaging material on F&V quality - Predict the end of the quality shelf life of F&V based on aroma analysis - Predict F&V storage disorder based on some VOC biomarker - Develop smart labels sensitive to some VOCs - Host-parasite interaction - Plant/fruit VOC signaling - Food fraud
High-throughput automated headspace analysis	- Screening of F&V germplasm and population - GWAS and QTL mapping - Screening of microorganisms VOC production - Complex experimental design with lot of biological replicates
Dynamic destructive Analysis	- Nose-space analysis to predict consumer perception during food consumption - VOC release during artificial chewing
Real-time process monitoring	- Modification of aroma during cooking/roasting - Modification of aroma during fermentation processes - Modification of aroma during pasteurization - Modification of aroma of fresh cut F&V

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OR14

Volatilome fingerprinting and gene expression profiling of fresh produce: a multi-trait approach to identify markers of food quality

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Summary: *Fruit quality is a key driver for consumers. It is important to optimize product quality throughout the supply chain. Here, the volatilome of one peach and one nectarine was analysed. In parallel, differentially expressed genes associated with VOC metabolism were identified. These are useful tools for objective quality assessment.*

Keywords: *GCxGC, Peach, markers*

Introduction

Fruit quality has become an important characteristic for the consumer, with numerous traits including sweetness, colour, aroma, acidity and firmness being evaluated. To meet consumer expectations, the fruit industry needs to guarantee product quality throughout all stages of the supply chain.

Numerous factors can affect the quality of fruit, including geographical origins, post-harvest storage and supply chain processing (such as washing, drying, and trimming). Volatilome fingerprinting combined with gene expression profiling can provide evidence for these how these factors affect quality.

Peach fruits are characterized by a rapid deterioration at room temperature meaning that cold storage is widely used to delay post-harvest ripening of the fruit and extend its commercial life [1]. However, chilling can affect production of flavour- and health-related metabolites [2, 3]. It is therefore of considerable scientific and economic interest to improve our knowledge of the mechanisms by which fruit respond to cold stress.

The FRUITY project aims to use innovative technologies to characterise Calabrian peach quality and measure its resilience to chilling. The main objective is to develop objective quality tests to optimize internal and sensorial quality of fresh fruits throughout the entire supply chain, especially when long

distance transport and chilled storage are required.

Experimental:
The case study is represented by peach (*Prunus persica* (L.) Batsch) fruits. In particular, we used three different cultivars of peaches and three different cultivars of nectarines, grown under the same environmental and agricultural conditions on a Calabrian farm, sampled at two time points – immediately after commercial harvest (D0) and after storage at low temperature (1°C) for 7 days (D7). The analysis of the peach fruit volatilome was carried out using thermal desorption (TD) coupled with comprehensive two-dimensional gas chromatography (GC×GC) combined with time-of-flight mass spectrometry (TOF-MS). Gene expression experiments were performed by real time PCR on genes associated with volatile organic compound (VOC) metabolism.

Results:
TD-GCxGC-TOF MS was used in this study to provide high-sensitivity sampling and enhanced separation of wide-ranging VOCs, allowing a comprehensive chemical signature to be obtained for each of the peach cultivars. Improved chromatographic resolution is provided by coupling two columns of different selectivity, so that compounds with similar retention times in the first dimension can be separated in the second dimension based on a different chemical property. Thus for example, the more polar components (such as benzaldehyde, acetophenone, ethyl hexanol and linalool) are well-resolved from the less polar terpenes and n-alkanes, providing cleaner spectra and more confident identification. This study also investigated the use of a novel ion source designed to provide both hard (70 eV) and soft (10-20 eV) electron ionisation simultaneously. The ability of soft EI to provide enhanced molecular ions whilst retaining structurally-significant fragment ions provided greater orthogonality between the mass spectra of isomeric compounds, greatly simplifying compound identification and reducing reliance on retention indices.
A total of 115 VOCs were identified for the six peach/nectarine cultivars studied through identification using the NIST-2017 database. These included nine acetate esters and 25 non-acetate esters, 15 alkanes, 14 terpenes, 14 ketones, eleven cycloalkanes, six alcohols, five lactones, five aldehydes, four aromatic compounds, four alkenes, and one alkyne. Terpenes were the most abundant family in the VOC profiles. Canonical Analysis of Principal coordinates (or CAP) on VOCs profiles showed discrimination across cultivars and post-harvest treatments. Combining sensory evaluation with the VOC profiles showed the same trend reported by CAP analysis.
The Random Forest algorithm was used to find the smallest set of predictor VOCs to discriminate amongst cultivars or treatments. In the classification of cultivars using normalised relative abundance of the VOCs, the discriminant features ranked by random forest, based on mean decrease in accuracy included: Camphene (C16), cis-3-Hexenyl isovalerate (C107), Octanoic acid, methyl ester (C76), α-Pinene (C24), Cyclooctane, butyl- (C25), Acetic acid, hexyl ester (C39), Caryophyllene (C145), and n-Amyl isovalerate (C82). Furthermore the predictor features for cold treatment ranked by random forest were: Indene (C55), α-Pinene (C24), α-Phellandrene (C34), α-Terpineol (C101), Linalool (C69), Octanoic acid, ethyl ester (C98) and Camphene (C16). In agreement with previous studies [1 2], the levels of linalool significantly decreased with storage time under cold storage.

Furthermore, correlation between the expression profile of flavour-related genes and specific related VOCs was shown.

Conclusion

Overall the combination of sensory evaluation, VOC profiles and gene expression could help the producer to understand which traits/aroma are most relevant for consumer perception and perhaps assist in the export of locally produced cultivars to unexplored markets. These analysis methods could also help breeders to understand which traits/aroma are most relevant to consumer appreciation. Furthermore, understanding of metabolic and genetic changes occurring in fruit VOC patterns post-harvest could contribute to providing a suite of simple diagnostic checks to monitor fruit quality throughout the supply chain.

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OR15

Coupling *in vivo* nose-space and sensory methods to investigate flavor release and perception

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Summary: *We present case studies to demonstrate how the coupling of dynamic sensory methods and real-time in-vivo flavor compound analysis can deepen our understanding of flavor perception and support product development. The methodology allows to understand inter-individual differences in flavor release/perception and highlights chemical and physiological effects of product/consumer and product/carrier interaction.*

Keywords: *nose space analysis, PTR-MS, flavor perception, flavor release.*

Introduction

Among the different human senses, flavor perception is one of the most complex since it involves smell, taste and chemesthesis. A complete disentanglement of the complex process of flavor perception is far from being achieved [1] but it has been accepted that its key drivers are the volatile organic compounds (VOCs) reaching the olfactory receptors [2].

Proton Transfer Reaction Mass Spectrometry provides a unique tool to monitor in real time VOCs ensuing from the retro-nasal pathway during *in vivo* food consumption [3]. Due to analysis rapidity and high sensitivity, the technique, together with other direct injection mass spectrometry techniques, has become a reference tool for the investigation of the phenomena related to flavor perception and release [1]. In the last years, the technique has been applied to different food matrixes [4-6] demonstrating its optimum coupling with dynamic sensory methods as Time Intensity (TI) and Temporal Dominance of Sensation (TDS). This short review describes several studies which coupled PTR-MS with dynamic sensory methods to better understand flavor perception and release phenomena in a variety of foods (chewing gum and mayonnaise).

The first two studies investigated the influence of ingredient formulation and aroma concentration in chewing gums on aroma release and perception . The third study explored the effect of physiological parameters (oral cavity volume, salivary flow and papillary count), gender and ethnicity on flavor release and perception. The fourth study investigated the interplay of mayonnaise properties (fat content, viscosity) and carrier properties (hardness of bread and potatoes) and its impact on aroma release and perception.

Experimental

In vivo nose-space analysis with two different PTR-MS machines (PTR-ToF-MS and PTR-QiTOF-MS, Ionicon Analytik, Austria) was applied. For the chewing gum studies sensory evaluation on flavor intensity and sweetness was performed by applying a discontinuous TI method at different time intervals. In the first two studies different gum formulations and aroma concentrations were

tested by 10-12 trained panelists in replicate. For the third study a total of 30 panelists divided between Caucasian-European and Asian-Chinese evaluated the same type of chewing gum in triplicates. For the mayonnaise study dynamic perception of lemon intensity through TI and *in vivo* aroma release of two lemon aroma compounds (*limonene, citral*) were assessed by 14 trained subjects in triplicate for three mayonnaises consumed alone and in combination with bread and potato of different hardness.

Results

In the first and second chewing gum study, significant differences in total aroma release ($p < 0.05$) were observed between different chewing gum formulations. In particular, it was observed that these differences were compound dependent and that PTR-MS signals are associated to a high variability reflecting panelists' physiological differences. In-nose aroma concentration was proportional to the initial aroma concentration in the chewing gum.

In the third study, ethnicity was found to have a significant effect on both in nose-space concentration and sensory perception. For different mass peaks associated to mint flavor compounds, Chinese panelists exhibited higher levels than European ones ($p< 0.05$) generally after 90 seconds of consumption and after the gum was removed from the mouth (Fig 1). The same trend was found in sensory perception both for flavor and sweetness. No significant differences were found between panelists of different gender. The product characteristics were also confirmed to have an effect on both flavor perception and release.

In the fourth study, fat content and viscosity influenced dynamic release patterns of limonene and citral. Addition of carrier foods (bread, potato) decreased lemon perception intensity of all mayonnaises while, in some cases, it increased the in-nose concentrations. Harder bread decreases lemon intensity to a larger extent than softer bread, whereas only small effects of potato hardness on lemon intensity were observed.

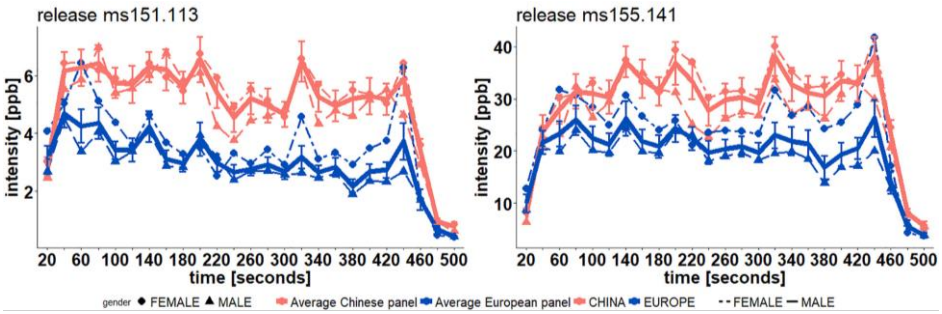

Fig. 1. *Average release during time for all some of the relevant mass peak: (A) m/z = 151.113 ($C_{10}H_{14}OH^+$) Menthofuran. (B) 155.141($C_{10}H_{18}OH^+$) 1–8 Cineole /Menthone . Distinction between Chinese and European panelists are shown based on colour while distinction based on sex is indicated by different line types and symbols [7].*

Conclusions

Real time mass spectrometry analysis by PTR-MS can detect and monitor *in-vivo* VOCs release during food consumption and can optimally support

dynamic sensory methods providing an accurate time-resolved quantification of the sensory stimulus.

Our results indicate a multimodal effect of aroma release on both flavor and sweetness intensity in chewing gum. The first two studies indicated a relevant impact of chewing gum characteristics (i.e aroma concentration and gum base composition) on flavor release. The third study highlighted that individual differences due to physiological, biochemical and physicochemical phenomena have a relevant effect in aroma release and thus in sensory flavor perception. Finally, the last study showed that both dynamic perception and *in vivo* aroma release of condiments are affected by condiment properties in a complex interplay with carrier food type and properties. The proposed *in-vivo* methods allow the dissecting of their complex interaction also in the case of real food.

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OR16

Usefulness of HS-SPME-GC-MS quantification of volatile compounds for quality control of virgin olive oil

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Summary: *A HS-SPME-GC-MS method for quantification of 73 VOCs in virgin olive oil was validated and used for building reliable and robust chemometric approaches for supporting the panel test in virgin olive oil classification and for authentication of the geographical origin of virgin olive oils from the main worldwide producing countries*

Keywords: *panel test, authentication, internal standard*

Introduction

Extra Virgin Olive Oil (EVOO) is considered as the highest quality product among edible oils mainly thanks to its pleasant taste and smell and the high content of bioactive phenolic compounds. In this context, economic frauds regarding false claim of geographical origin [1] and commercial category [2] cannot be fully avoid to date. Moreover, virgin olive oil classification is based, among other, on its sensory assessment carried out by a panel of trained experts, according to International Olive Council (IOC) trade standards and European legislation [3]. Over the last years, the possibility of using reliable chemical data related to the composition of the volatile fraction of virgin olive oils, has been recognized as more and more crucial. The use of HS-SPME-GC-MS analysis for quantification of volatile organic compounds (VOCs) has gained great attention in the last years and quantification of 29 VOCs by this technique has been validated [4].

In this study, carried out over many years of work, we aimed at developing chemometric approaches for the quality control of virgin olive oil. First we optimized and validated the HS-SPME-GC-MS quantification of virgin olive oil VOCs by using several internal standards. Then, we used the validated method for analyzing more than 1000 oil samples; the obtained set of data has been statistically treated for proposing reliable and robust approaches to support the panel test in virgin olive oil classification and to authenticate the geographical origin of virgin olive oils from the main worldwide producing countries.

Experimental

A refined olive oil free from VOCs was used for preparing internal and external standard solution, used for both method validation and samples analysis. More than 1000 virgin olive oil samples from different geographical origin (Italy, Spain, Greece, Tunisia, Portugal) and category (EVOO, VOO, LVOO) were collected in the Carapelli laboratory.

Method optimization and validation was carried out using a Trace CG-MS

Thermo Fisher Scientific, equipped with a ZB-FFAP capillary column (Zebron) 30 m × 0.25 mm ID, 0.25 µm DF. Method was then applied to virgin olive oils using a 6890N GC system equipped with a MS detector, model 5975 by Agilent, equipped with a HP-Innowax capillary column 50 m × 0.2 mm ID, 0.4 µm DF. In both cases, analysis of VOCs was carried out weighting 4.3 g of sample and 0.1 g of internal standard solution into a 20 ml screw cap vial fitted with a PTFE/silicone septa. A SPME fiber 50/30 *µm* DVB/CAR/PDMS was exposed for 20 min in the vial headspace under orbital shaking at 400 rpm after equilibration for 5 minutes at 45°C, then the adsorbed VOCs were desorbed in the injection port of the GC system. Mass detector worked in scan mode within the range of 30-350 Th, 1500 Th/s at ionization energy of 70 eV. Each VOC was quantified using a calibration curve in which the area ratio (ratio between areas of that VOC and the selected ISTD) was plotted versus the amount ratio.
Suitable statistical tools were employed for data analysis, aiming at building the chemometric approaches above mentioned: Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), Analysis of Variance (ANOVA) and t-test

Results and Conclusion

In the first step of the work [5], quantification of 73 VOCs was optimized and validated using 11 internal standard and selecting the more suitable one for each of the quantified VOCs. This approach allowed overcoming several issue previously reported as critical when HS-SPME-GC-MS is used for quantification purpose [6], resulting in a good linearity in ranges of calibration wider than those previously reported in studies performed only using a single internal standard. The validated method was then applied for quantifying the 73 VOCs in virgin olive oils selected for building chemometric approaches aimed to:

- Supporting the panel test in virgin olive oil classification (1223 samples)
- Authentication of the geographical origin of virgin olive oils from the main worldwide producing countries (1217 samples).

The main results can be summarized as follow:
Four chemometric approaches for supporting the panel test in virgin olive oil classification have been proposed and externally validated with a set of independent samples. The PCA-LDA model gave the best results, when the classification was compared with the results of the panel test (Table 1). The *t*-test-DSV model allowed strongly simplifying the analytical work, in that it gave a very good predictive performance only using quantitative data of 10 VOCs. During development of the proposed approaches, it also emerged that, from the qualitative point of view, octane, heptanal, pent-1-en-3-ol, Z-3-hexenal, nonanal and 4-ethylphenol must be considered the VOCs more suitable for discriminating different classes of virgin olive oils during their classification.
Three chemometric approaches for authentication of the geographical origin of virgin olive oils from the main worldwide producing countries have been proposed. The best results (Table 2) were obtained by the ANOVA-LDA model, only using 25 VOCs, selected using ANOVA. For some origins, the proposed model showed a prediction capability higher than 97%
In conclusion, quantitative data obtained by HS-SPME-GC-MS using several internal standard are suitable for building reliable and robust chemometric approaches for virgin olive oil quality control, thus allowing protecting consumers and producers from incorrect claiming of geographic origin and

commercial classification.

Table 1. *Comparison of the classification of Samples obtained during external validation of the four proposed models*

model	non-classified (%)	among the classified samples (%)	
		correct classification (wrong defect)	misclassified
1. PCA–LDA	5.3	83.5 (12.0)	16.5
2. *t* test–LDA	4.7	79.7 (10.1)	20.3
3. *t* test–DSV	8.0	80.1 (13.8)	19.9
4. chemical indices	8.7	77.0 (5.5)	23.0

Table 2. *Comparison of the classification of Samples according to their geographical origin obtained during external validation of the three proposed models*

	Model	Classified samples (%)	Correctly classified samples (%)
1	**PCA-LDA (FAs + VOCs)**	97.2	86.3
2	**LDA (VOCs from LOX)**	85.5 (86.6)	82.6 (77.1)
3	**ANOVA-LDA (25 VOCs)**	91.4 (94.5)	88.1 (87.3)

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OR17

Stable isotopes and elemental profiles for botanic and geographic characterisation of gum arabic

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Summary: *Gum arabic is obtained as dried exudate from Acacia seyal or A. senegal plant species. In Sudan, the Kordofan region is well known for producing the highest quality gum arabic, known as Kordofan gum. 45 commercial gums were analysed using ICPMS and IRMS for botanic and geographic characterisation.*

Keywords: *Acacia, ICPMS, IRMS*

Introduction

Gum arabic (GA), probably the oldest and best known of all natural gums, is a gummy exudation which naturally flows through cuts made in tree trunks and branches of the *L. Acacia senegal* and other Acacia species from the sub-Saharan region of Africa. Among the different African productions, *A. senegal* gum produced in the Kordofan region is commercially regarded as one of the best for its organoleptic and technical quality. This complex mixture of macromolecules, mainly polysaccharides and glycoproteins, is widely used as edible ingredient in several food and industrial applications [1]. Its use in winemaking is regulated by OIV and only *A. senegal* and *A. seyal* gums are recognised as approved sources, recommending a maximum dosage in wine of 300 mg/L [2]. Considering its extensive consumption and the steadily increasing global demand, any analytical approach suitable for assessing the GA origin is definitely welcomed by public authorities in order to contrast food frauds and enforce food safety.

This study aims to investigate if the different botanical origins (*Acacia senegal vs A. seyal*), and the specific climatic or geological characteristics of the production areas (Kordofan region *vs* other Sub-Saharan areas) could induce characteristic isotopic and elemental profiles.

Experimental

45 commercial gums were analysed using ICPMS and IRMS.

The $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ ratios were measured in one run (around 0.5 mg) using an isotope ratio mass spectrometer (IRMS) (Isoprime, Isoprime Ltd., UK) following total combustion in an elemental analyser (VARIO CUBE, Elementar Analysensysteme GmbH, Germany). The $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios were measured in one run (around 0.5 mg) using an IRMS (Finnigan DELTA XP, Thermo Scientific) coupled with a pyrolyser (Finnigan TC/EA, high temperature conversion elemental analyser, Thermo Scientific). Fifty-six macro-, micro- and trace elements (Li, Be, B, Na, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Y, Mo, Pd, Ag, Cd, In, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Er, Tm, Yb, Re, Ir, Pt, Au, Hg, Tl,

Pb, Bi, Th and U) were quantified using an inductively coupled plasma mass spectrometer (ICP-MS; Agilent 7500ce Agilent Technologies, Tokyo, Japan), equipped with a reaction/collision cell in order to remove the principal polyatomic interferences.

Results

A Principal Component Analysis (PCA) that globally used isotope and elemental profiles clearly distinguished the 2 different botanical origins. Furthermore, a second PCA using the percent of C and N, Cs, Mg and Na made it possible, inside the *A. senegal* group, to discriminate the Kordofan ones.

Conclusions

This study demonstrated that a combination of multi-element analysis and stable isotope ratio analysis could provide a non-conventional approach for tracing the botanical origin of gum arabic. Furthermore, the evaluation of C%, N%, Cs, Mg and Na made it possible to discriminate the geographical origin among *A. senegal* samples, recognising in particular the higher quality gum, the Kordofan. This provides a new approach for the identification of the authenticity of the origin of gum arabic.

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PL3

Novel insights in the occurrence and bioavailability of endocrine active food components

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Summary: *Humans are continuously exposed to xenoestrogens out of different sources, including native food constituents, processing-related compounds to contaminants. The development of novel multimethods enables improved exposure assessment of endocrine active xenobiotics.*

Keywords: *Xenoestrogens, chemical mixtures, exposomics*

We are constantly exposed to a variety of bioactive molecules out of different sources including those mimicking endogenous estrogens. These compounds are collectively referred to as xenoestrogens and hold the potential to affect the delicate hormonal balance of the human body. Prominent sources of bioactive molecules with estrogenic potential are, among others, soy-based products, linseeds and beer, containing so called phytoestrogens out of different structural classes. But not only native food constituents might add to the “estrogenic load”, also several potential food contaminants are known to possess estrogenic properties. Among these are synthetic compounds such as several pesticides, plasticizers, personal care product additives and other industrial chemicals. However, also fungal spoilage might result in contamination with estrogenic compounds, so called mycoestrogens, with the *Fusarium* toxins zearalenone and its reductive metabolite α-zearalenol as the most prominent representatives. Recent studies demonstrated that mycoestrogens might also be formed by *Alternaria* species, so called black mold (1). So far, the content of *Alternaria* toxins in food and feed are neither monitored nor regulated. We developed and validated a multitoxin method for monitoring of *Alternaria* toxins in different food matrices (2). A respective food survey detected, among others, mycoestrogens formed by *Alternaria* spp. in samples of tomato sauce, sun flower oil and wheat flour (3), but also in infant formula and breast milk samples. These results underline the potential exposure of humans to xenoestrogens out of different sources over the whole lifetime, depending on the respective life style and diet pattern. Human biomonitoring opens a new perspective to achieve a more comprehensive exposure assessment. Thus, to monitor exposure and investigate potential health implications, comprehensive analytical methods covering all major xenoestrogen classes are urgently needed. We developed a LC-MS/MS method for the simultaneous determination of multiple classes of endogenous as well as exogenous estrogens in human urine, serum and breast milk. In total, 75 analytes were included, whereof a majority was success-fully in-house validated in the three matrices (4). The results illustrate the importance of multi-class exposure assessment in the context of the exposome paradigm. Specifically, they highlight the need for estimating total estrogenic burden rather than single analyte or chemical class measurements and its potential impact in endocrine

disruption and hormone related diseases including cancers.

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OR18

Exploring extra-terrestrial acceptability of interplanetary food with the aid of mass spectrometry

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Summary: *With current space exploration sights set on a manned mission to Mars, adequate calorific and nutritional uptake by astronauts on the minimum 21 month expedition is vital. Current evidence from the International Space Station indicates insufficient consumption to maintain muscle mass. This talk explores factors potentially influencing astronaut eating behaviour.*

Keywords: *space food, flavour perception, environment*

Introduction

The first small steps for a man, but giant leap for mankind were made by Neil Armstrong half a decade ago, when in 1969 he made history as the first person to step foot on the surface of the moon. The arduous *Apollo 11* mission was a feat of science, engineering and endurance, but in terms of sustenance, the eight day round-trip is brief in comparison to the planned manned mission to Mars, which will require a journey time of at least 18 months, with an additional minimum three month sojourn on Mars' surface.

Empirical data on astronaut food consumption aboard the *International Space Station*, ISS (Fig. 1), indicate that calorific intake is lower than required to maintain muscle mass, thereby posing a risk to the health of crew members. There is concern that an under-consumption of food and loss of body weight may put the success of longer-term space exploration at risk.

The *European Space Agency*, ESA, has invited a panel of experts to explore the factors that might affect the food choices made by astronauts on board the *ISS* in order to further our understanding of flavour perception and astronaut wellbeing in space. Such knowledge will be used to adapt and optimise food rations that will ultimately aid in ensuring health on the planned lengthy space expeditions of the future.

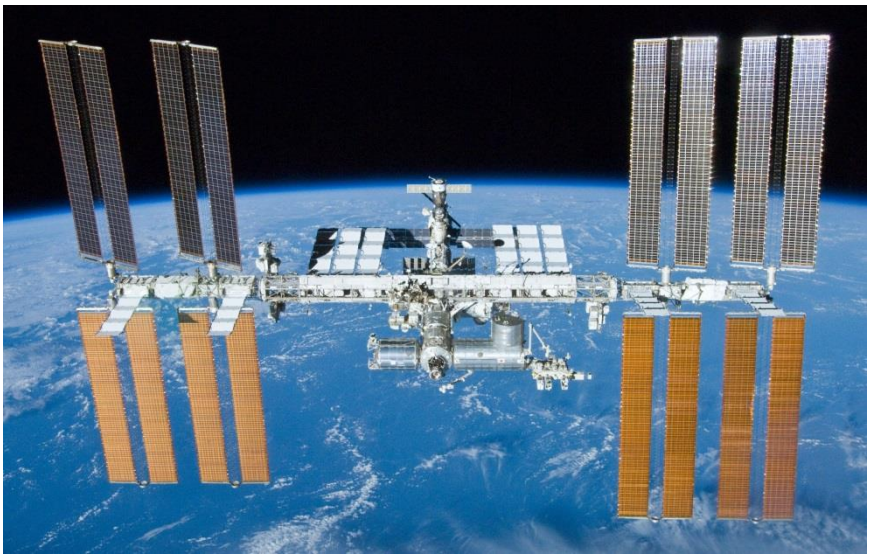

Fig. 1. *The International Space Station as it orbits Earth. Image © NASA*

The role of mass spectrometry

Mass spectrometry, MS, has played a critical role in food research over the years, providing insights into diverse aspects ranging from food constituents, stability and shelf-life, and flavour release and perception, to name just a handful. Equally, MS is employed in many other life science disciplines to explore and understand our environment.

The ESA expert panel has identified several considerations that could potentially affect the choices made by astronauts for food consumption; these are listed in Table 1.

The scientific literature reporting on MS-based data on these parameters will provide crucial information in ascertaining the potential importance of each aspect, ranging from the environmental conditions on board the spacecraft (e.g., air quality[1,2,3], water quality[1,4,5]), to flavour release in microgravity, and perception. Other factors such as noise[6] or psychological wellbeing[7] might also play a role in influencing what, when and how much astronauts choose to consume. Any knowledge gaps might be further explored using MS and other techniques in either terrestrial or ISS-based experiments.

Table 1. *Overview of factors potentially influencing food consumption by astronauts*

Factor	**Modulator**
Sensory properties of space food	Thermo-stabilised vs. dehydrated food; texture; shelf-life
Water quality	Use in rehydrating food; contamination
Air quality	Higher CO_2 levels; ambient (odorous) volatile levels
Noise	Background noise; psychological effects
Odour sensitivity in space	Physiological effects; odour receptor effects

Conclusion

This talk will present the challenges faced in sustaining a manned crew to Mars, outline the factors that potentially influence astronaut food consumption, and focus on existing data provided by mass spectrometry that brings us further in our understanding and ultimately optimisation of calorific and nutritional uptake by astronauts that will ensure their physical and mental wellbeing on extended space exploration endeavours, such as a 21-month mission to Mars.

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OR19

Robust and integrated, analytical workflows delivering improved sensitivity and compliance in routine food testing labs

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Summary: *Challenging analyses in food safety often require laboratories to invest a lot of effort in developing and optimizing robust methods which are fully compliant with regulatory requirements. An alternative approach is for the instrument manufacturer to develop and validate off-the-shelf integrated compliant sample to result analytical workflow.*

Keywords: *Pesticides, dioxins, compliance*

Introduction

Regulators continue to set the permitted maximum level/limits of undesired chemical substances in food products at ever decreasing values in order to protect consumers. The determination of substances at low levels can often present severe analytical challenges, even to experienced analysts working in well-equipped laboratories. To enable food testing laboratories to meet more stringent regulated levels, a new range of dedicated analytical workflows have been developed for pesticides and dioxin analysis. These workflows include all necessary components, starting from sample extraction and clean up, chromatographic separation, detection and reporting. Inclusion of system suitability check standard solutions, consumables, method parameters, software and comprehensive user guidelines enable fast implementation and delivery of ongoing optimum performance.

Each of these workflows is based on an extended validation undertaken with different kind of sample commodities and for long-term analysis of hundreds of samples, to test the robustness and suitability for routine analysis. Validation of the method performance criteria, including sensitivity, precision, accuracy and calibration was carried out using multiple different analytical systems, located in different laboratories in different countries. The objective of the extensive validation was to test that implementation of the method could be deployed with ease in any laboratory across the world. This presentation highlights the performance and results obtained from the extended validation of three new analytical workflows.

The Anionic Pesticides workflow

The Anionic Pesticides Explorer, is an ion-chromatography triple quadrupole mass spectrometry based workflow for the robust, sensitive, reliable, multi-analyte determination of polar anionic pesticides and metabolites at low µg/kg levels in a wide variety of sample types. This method consolidates the determination of polar anionic compounds into a single analysis, increasing productivity and reducing costs. This workflow has been developed and validated for grape, wheat flour, leek and baby food. The results are fully compliant with the EU SANTE 11813/2017 Guidance document on analytical

quality control and method validation procedures for pesticide residues and analysis in food and feeds. Figure 1 shows a block diagram of the system and Figure 2 a total ion chromatogram of 15 anionic pesticides and metabolites. The method enables the quantitation and identification of anionic pesticides and metabolites at low µg/kg levels with good excellent accuracy and precision, even in complex matrices such as wheat.

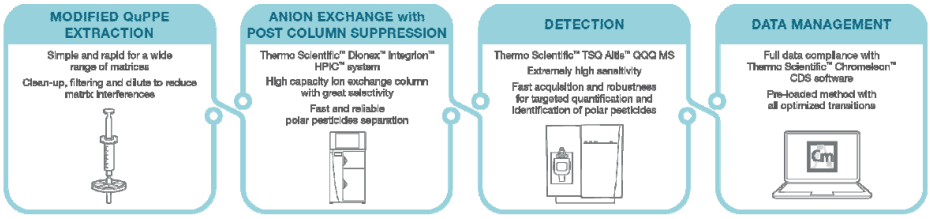

Fig. 1. *Block diagram of Anionic Pesticides Explorer workflow*

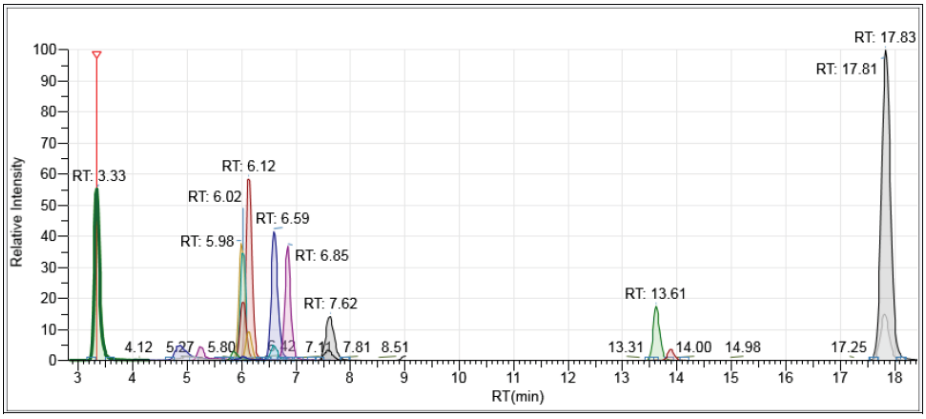

Fig. 2. *Total Ion Chromatogram of 15 anionic pesticides and metabolites: Bialphos, Chlorate, Cyanuric acid, Ethephon (HEPA), Fosetyl-aluminium (Phosphonic acid), glyphosate (N-acetyl glyphosate, AMPA, N-acetyl AMPA), glufosinate, (N-acetyl glufosinate , MPPA), and perchlorate (contaminant)*

The Pesticides Explorer II workflow
The pesticide Explorer II, is a is robust, fast, easy to use, LC-MS/MS multi-class pesticide residue workflow validated for three representative commodities (wine, olive oil and baby food) and delivers the sensitivity, accuracy, and precision required to meet the EU SANTE 11813/2017 guidance criteria. The workflow uses QECHERS acetonitrile extraction and includes a compound data base of over 700 compounds with optimized SRMs along with all MS method parameters and conditions for chromatographic separation with a run time of 15 minutes. For proof of concept the method has been fully validated for the quantitation and identification of 250 representative pesticides at low µg/kg levels.

The Dioxin Analyzer The Dioxin Analyzer is an end-to-end workflow based on a high sensitivity triple quadrupole GC-MS/MS system with an advanced

ionisation source. It is designed to deliver robust and sensitive quantitation of polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzo furans (PCDF), and dioxin-like polychlorinated biphenyls (dl-PCBs) in food and feed samples in compliance with EU regulation 589/2014. In total 29 different food and feed samples were prepared, extracted and cleaned up to validate the performance of this workflow. A single software, Chromeleon CDS, was used to acquire, process and automatically report data for the analysis of dioxins. This includes the ability to perform isotope dilution quantification, with reporting elements for ion ratio, response factor, internal standard recovery, real-time updates of WHO-PCDD/F-TEQ and WHO-PCB-TEQ values during processing as well as the option to flag exceedance of maximum limits.

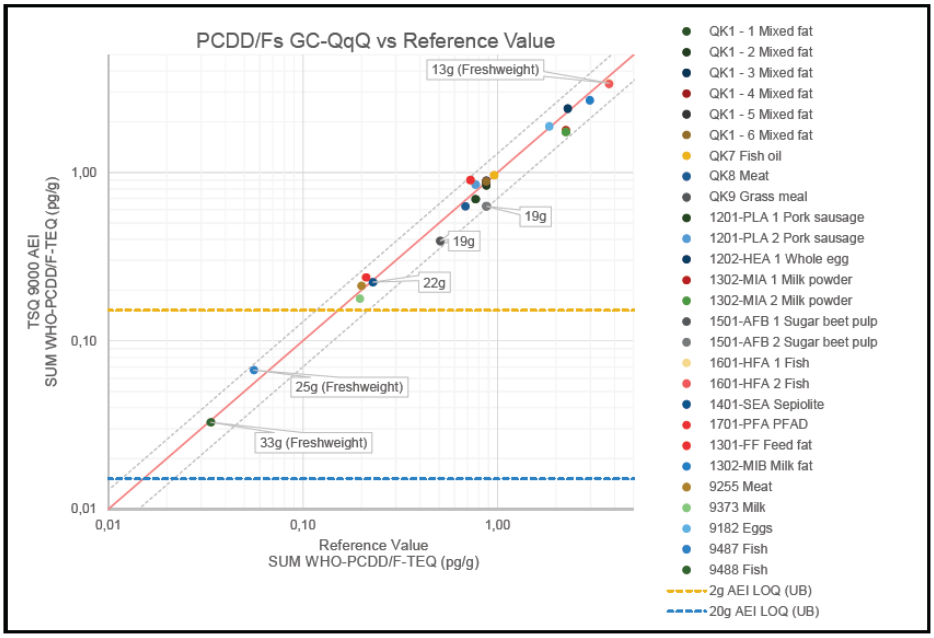

Fig. 3. *Comparison of results obtained using the GC-MS/MS and the EURL reference values. The center red line represents 100% agreement with the reference value and the upper and lower greyed lines represent a ±30% deviation from this value. Unless specified, sample intake weight was 2 g; amount scales are logarithmic to aid comparison*

OR20

Phospholipids profiling in San Andreas strawberries by using hydrophilic liquid chromatography coupled to ESI tandem mass spectrometry

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***Summary**: The characterization of phospholipids (PL) in fresh strawberries by applying hydrophilic liquid interaction liquid chromatography coupled with electrospray ionization mass spectrometry (HILIC-ESI-MS) and tandem mass analyses (MS/MS) is described. The regiochemistry of major PL classes has been fully established here.*

***Keywords:** strawberry, phospholipids, LC-MS*

Introduction

Strawberry fruits are consumed and beloved all over the world due to their taste, aroma, soft texture, nutritional and curative values [1]. They are abundant in bioactive compounds with antioxidant properties, mainly including phenolic compounds, especially anthocyanins [2] being the largest subgroup of phenolic compounds [3]. Anthocyanins group has been found to have many biological benefits, such as anti-inflammatory and anti-carcinogenic activity. In addition, antioxidant capacity of strawberries can participate in the prevention of cancer, cardiovascular and other chronic diseases [2]. Many studies have been addressed to determine these bioactive compounds of strawberries and a few researches are focused on the characterization of lipid group especially concentrating on fatty acids [4]. However, there are no precise information about total polar lipids and phospholipids. In literature one study describing the main PL classes in these fruits has been found [5]; here the presence of major PL namely phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidyl-glycerol (PG), acidic phosphatidic (PA), phosphatidylethanolamine (PE) was ascertained being the PC the major phospholipid group on strawberry [5]. No detailed information on their characterization in terms of bound fatty acids can be retrieved. The purpose of this research project is to analyze and to quantify major phospholipids in fresh strawberries by using hydrophilic interaction liquid chromatography (HILIC) coupled with high resolution/accuracy mass spectrometry. San Andreas strawberry (*Fragaria x ananassa* cv. San Andreas) was taken as a model sample to characterize the phospholipids, because it has exceptional appearance and flavor, their disease resistance ability and low chill requirement, enough for warmer regions.

Experimental

Total lipids of strawberry were extracted by applying the Bligh-Dyer protocol. To 1 g of strawberries previously washed and made as a pulp, 800 µL of water and 3 mL of $CH_3OH/CHCl_3$ (2:1 v/v) were added and mixed. After mixing the sample, 1 mL of water and 1 mL of chloroform were added to solution and vortexed again. Separation of organic phase centrifugation was necessary for

15 minutes at 3000 x g. The organic phase was collected and dried under nitrogen. Resuspension of lipid extract was done with 100 µL of CH_3OH and analysed using HILIC-ESI-MS. Chromatographic separation was performed by using narrow-bore column (150×2.1 mm ID, 2.7 µm particle size) equipped with security guard cartridge (5×2.1 mm ID) Ascentis Express HILIC (Supelco, Bellefonte, PA, USA). The adjusted binary elution program, based on water and 2.5 mmol l^{-1} (solvent A) and acetonitrile (solvent B), both containing 0.1% (v/v) of formic acid, was adopted: 0-5 min, linear gradient from 97 to 88% solvent B; 5-10 min, isocratic at 88% solvent B; 10-11 min, linear gradient from 88 to 81% solvent B; 11-20 min, linear gradient from 81 to 70% solvent B; 20-22 min, linear gradient from 70 to 50% solvent B; 22-28 isocratic at 50% solvent B; 28-30 min, return to the initial composition, followed by a 5 min equilibration time. Flow rate was 0.3 mL $min^{-1}$and MS analyses were carried out either on Q-Exactive (Q-Orbitrap) or VelosPro (Linear ion trap) spectrometer in positive and negative ion mode.

Results

HILIC-ESI-FTMS total ion current chromatogram obtained from various strawberries extracts showed several peaks corresponding to various phospholipid classes. Applying the gradient elution program described in the experimental section, the separation of each lipid class was achieved in approximately in 20 min. The elution order, reflecting the increasing polarity, is PA, PG, PI, PE, lysoPE (LPE), PC and LPC. As expected, since LPE and LPC have only one acyl chain with the other acyl chain replaced by a hydroxyl group in sn-1 or sn-2 position, they elute later respect to their intact forms.

The characterization of the lipid species detected under each band was carried out. Besides the accurate m/z value we utilized the all ion fragmentation (AIF) signal acquisition approach, provided by high resolution/accuracy Orbitrap instrumentation, that helped in the recognition of the main PL classes thanks to the generation of specific class-related product ions without the necessity to isolate and fragment certain precursor ions. By exploiting the high collisional energies provided by the HCD cell, the informative fragment ions generated from the head group were used to identify the lipid class of interest.

Tandem mass spectrometry at lower energy regime (CID, collisional induced dissociation) was used to confirm fatty acid chains and their position on the glycerol. This approach permitted the detailed structural characterization of more than 200 PL species.

Conclusions

HILIC-ESI(-)-LIT-MS/MS spectra in negative ion mode of deprotonated molecules allowed to unambiguously establish the regiochemical of fatty acyl chains because the differences in intensity of the signals related to their losses. By this approach more than 200 phospholipids have been characterized. More selected internal standards were added, and it was possible to find out that PC were the most abundant species followed by PE, LPC, PA, PI, PG and LPE.

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PL4

Applications of MS-based metabolomics to investigate the host:microbiome co-metabolic processing of food components

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Summary: *A cross-over blind human trial was designed, where healthy volunteers were given 250 ml of cloudy apple juice or the same apple juice enriched with an apple extract enriched in apple polyphenols. The first aim of the study was to efficiently identify the metabolic products of various classes of apple polyphenols using an untargeted metabolomic approach and to follow their dose dependent appearance in plasma and excretion in urine after different intake. The second aim was to investigate the relations between microbiota and metabolites resulting statistically significant after enriched juice intake. In conclusion, we have identified specific blood and urine metabolic biomarkers of apple polyphenol intake and identified putative associations with specific genera of faecal bacteria, associations which now need confirmation in specifically designed mechanistic studies.*

Keywords: *mass spectrometry, polyphenols, microbiota, bioavailability, nutrikinetics*

Introduction

The polyphenols are widely distributed in higher plants, and enter the human diet through fruits, vegetables, nuts and plant-derived beverages. After ingestion, they appear in the circulatory system mainly as phase I and II metabolites. However, substantial quantities of the parent compounds and their metabolites pass into the colon where they are further metabolized by the local microbiota, giving rise principally to low-molecular-weight phenolic acids and other congeners, that can be absorbed to varying extents into the circulation, distributed to organs, and ultimately cleared via renal elimination. This lecture aims to discuss how the investigation of the nutritional fate of the polyphenols via untargeted metabolomics combined with metagenomics can improve our understanding of their bioavailability.

Metabolomic data-sharing

Untargeted data in mzXML format and metadata are available for download from the MetaboLights repository http://www.ebi.ac.uk/metabolights/, with the persistent unique public identifier MTBLS473.
Meta-data of the study are also available in the Dash-in Web based federated analyses website (http://www.enpadasi.eu/deliverables_final_pdf/D3.2.1.pdf). The persistent unique public identifiers to find this data-set is https://dashin.eu/interventionstudies/AGER-MELO

Experimental and Discussion

The AGER-MELO study was a cross-over, blind human trial, where 12 healthy volunteers consumed 250 mL of cloudy apple juice (CAJ), Crispy Pink apple

variety, or 250 mL of the same juice enriched with 750 mg of an apple polyphenol extract (PAJ). Plasma and blood were collected at time 0, 1, 2, 3 and 5 h. Urine was collected at time 0 and 0–2, 2–5, 5–8, and 8–24 h after juice consumption. Faecal samples were collected once from each individual during the study for 16S rRNA gene profiling.
Plasma sample were extracted with 96 well plate Sirocco (Waters) and diluted 1:2, while urine samples were filtered with 96 well plate with PVDF filter and diluted 1:4. Samples were analysed by Fourier Transform LTQ FT Orbitrap mass spectrometer (Thermo Fisher) interfaced to a Dionex HPLC system. MS operated under HR data-dependent-acquisition (DDA) in positive and negative ionization modes. Using barcoded 454 amplicon pyrosequencing of the 16S bacterial ribosomal RNA gene (V3-V5) of 12 faecal samples, the gut microbiota profiles were analyzed. A number of 138,280 reads were processed using the MICCA pipeline to assign operational taxonomic units (OTUs) by clustering the sequences with a threshold of 97%.
This study allowed to efficiently identify the metabolic products of apple polyphenols using an untargeted metabolomic approach, and to follow their nutri-kinetic in plasma and urine after different intake.
The observation of coherent trends for both plasma concentration and urine excretion profiles for the same metabolic pathways provide experimental evidence of the presence of two main nutrikinetics patterns.
A fraction of the native polyphenols is quickly absorbed and metabolized in the upper gut with little or no contribution from the human colonic microbiota and is rapidly excreted in urine. This group of catabolites is characterized by an early absorption peak in plasma, which is followed by a rapid decrease (within five hours) in their concentration and a fast appearance in urine. A second group of catabolites do not reach their maximum concentrations in plasma within the five hours after juice ingestion and they are characterized by a delayed appearance in urine, suggesting a prolonged metabolism along the gut with a likely involvement of the gut microbiota.
In total, as many as 110 metabolites were significantly elevated following intake of PAJ, with large inter-individual variations. The comparison of the average area under the curve of circulating metabolites in plasma and in urine of volunteers consuming either the CAJ or the PAJ demonstrated a stable metabotype, suggesting that an increase in polyphenol concentration in fruit does not limit their bioavailability upon ingestion.
We have confirmed the strong involvement of the intestinal microbiota in the systems-level metabolism of complex plant polyphenols ingested with food and speculated that the late appearance of several small phenolic catabolites of apple polyphenols in blood and urine can be significantly correlated to the relative abundance of different, phylogenetically distantly related bacterial genera.
Paving the way to the design of multi-omics experiment aimed to investigate the relations between microbiota and metabolites.

Conclusions

This work takes a small initial step in linking systems level metabolic processing of dietary polyphenols with microbiome architecture, a necessary move away from limited taxonomic descriptions or measurement of metabolic potential and towards improved understanding of microbiome metabolic function and nutrikinetics.

OR21

Untargeted metabolomics approach and antimicrobial assays to characterize chemical profiles of different Satureja montana L. essential oils

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Summary: *Untargeted fingerprints of four different Satureja montana L. essential oils (SEOs) were investigated by ESI/APCI FT-ICR MS, enabling to identify (semi-)polar metabolites. The antimicrobial activity (MIC, MBC) of SEOs and the efficacy of therapeutic nanoproduct (Oil in Water Nanoemulsions) is reported to correlate the chemical composition to the biological activity.*

Keywords: *Metabolomics, FT-ICR, Satureja*

Introduction

Satureja montana L., commonly known as mountain savory, is a perennial small herb which spontaneously grows and matures in sunny, rocky and arid regions of the Middle East and Mediterranean Europe, as well as in West Asia, North Africa and South America. It has been considered nowadays one of the richest plant in the Lamiaceae family for the large amount of biologically active phytochemicals. Indeed, its essential oil (SEO) owns remarkable antiseptic, antioxidant and antifungal properties[1]. Additionally, it is often used in Mediterranean cooking and, more recently, as antibacterial agent in food packaging. During the last years, all these properties of the SEO have spurred the chemical characterization of the volatile fraction. Interestingly, both growth and environmental conditions strongly influence the metabolic expression and thus the oil composition. Here, an untargeted metabolomics approach of four different SEOs has been performed for the first time on the polar fraction by means of Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry (MS) coupled to an electrospray ionization (ESI). The characterization of the less polar fraction has been performed exploiting an atmospheric pressure chemical ionization (APCI) source[2]. Minimum inhibitory (MIC) and minimal bactericidal concentrations (MBC) have been also evaluated to find a correlation between chemical composition and antimicrobial activity. Finally, stable nanoemulsions (NEs), useful drug delivery systems, containing the different SEOs, have been prepared and characterized.

Experimental

The present study has been conducted on three wild SEOs (SEO1, 2 and 3), all obtained by hydro distillation from the plant aerial parts differing for harvesting conditions, and on a reference sample (SEOT), supplied from a commercial source. For the MS analyses, 37 µl of each oil were filtered and diluted in methanol. The high resolution MS experiments have been carried out on a Bruker BioApex 4.7 T. Both the ESI and the APCI experiments were performed in either positive and negative polarity mode. Raw mass data have been acquired and annotations have been achieved with a mass deviation of 3ppm from the theoretical mass. Further confirmation of the peaks attribution has been obtained by collision induced dissociation (CID) assays. Several visualization tools for the metabolomics data (van Krevelen plot, Venn diagrams) have been used to compare and characterize the samples. The antimicrobial activity have been evaluated by determining MIC and MBC values against ten clinical strains of either Gram positive and Gram negative bacteria, cultivated in Tryptic Soy Agar (TSA) for 24 h at 37°C. MIC values have been obtained by the broth microdilution method by serial twofold dilution, while MBC values were achieved by sub-culturing 10 µL from each SEO with no visible growth in 180 µL of fresh Muller Hinton Broth. Finally, the NEs have been obtained with different amounts of Tween20 and Tween80 in different ratios. These formulations have been characterized by measuring the Droplet size distribution and the ζ-potential by dynamic light scattering.

Results

The untargeted metabolomics approach presented here allowed to gain relevant information about polar and semi-polar components of four SEOs.

An example of the high resolution mass spectra, recorded in ESI+ in the *m/z* 90-450 mass range, is reported for SEO3 (figure 1). An overlapping of the MS signals recorded for the four SEO samples has allowed to identify unique and common features. Overall, terpenes, terpenoids, fatty acids and their derivatives were found in all SEOs.

In ESI-, only 20 common metabolites were found, corresponding mainly to terpenoids and fatty acids, thus revealing the high variability in the metabolic expression of the polar component.

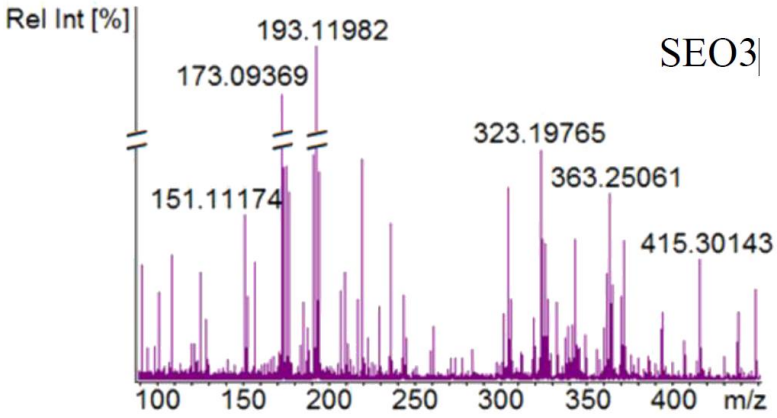

Fig. 1. *SEO3 ESI+-FT-ICR mass spectrum in the m/z 90-450 mass range*

The most relevant components recorded using either ionization sources are reported in Table 1.

Table 1. *Example of terpenes and terpenoids revealed in SEOs by means of ESI and APCI source*

Compound	m/z	ppm
$[Santene+H]^+$	123.11708	2.0
$[Cymene+H]^+$	135.11717	2.7
$[Carvacrol-H]^-$	149.09620	0.7
$[Carveol+H]^+$	153.12774	2.3
$[Linalool\ oxide+Na]^+$	193.11984	-0.3
$[Linolenic\ Acid+Na]^+$	301.21425	1.5

Based on the annotated molecular formulas, the hydrogen/carbon (H/C) and the oxygen/carbon (O/C) ratio were also extrapolated to build the van Krevelen diagrams. This visualization tool allows to classify the expressed metabolites in well-defined regions, as reported for SEO1 in figure 2.

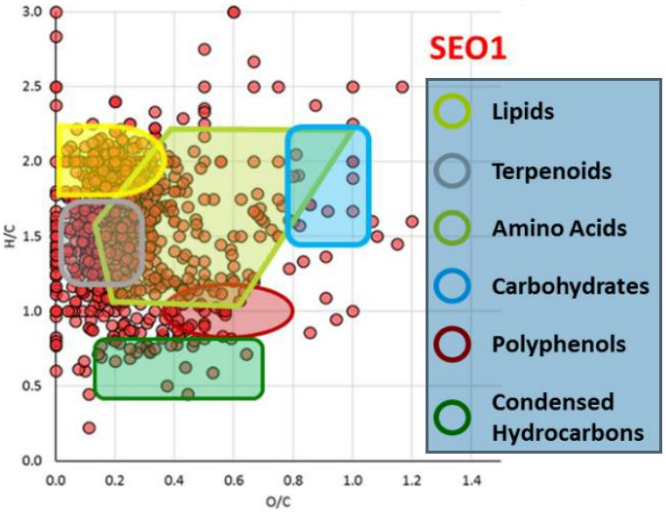

Fig. 2*: SEO3 van Krevelen diagram. Lipids and teprenoids are the main expressed classes*

All the sampled SEOs have shown significant antimicrobial activity. These results confirm the biological activity against all the testes strains, especially against the Gram – bacteria. In only one case, an additional antibiofilm activity has been found, alone or in combination with the antibiotic gentamicin[3]. Slight fluctuations in the MIC and MBC values have been observed, related to SEO composition. NEs with different SEO/surfactants ratios have been prepared in order to highlight their physical-chemical characteristics. The mean droplet size was found in the range of 55-120 nm and the dimensions seem to be correlated with the surfactant amount. In particular, a decrease of the dimensions has been found by increasing the surfactant content[4]. The obtained polydispersity index (lower than 0.3) has indicated that all formulations are characterized by homogeneous population.

Conclusions

This work confirms how the determination of chemical profiles of different SEOs can be related to the antimicrobial activity. Nanocarriers (NEs) containing the present SEOs have been prepared as potential innovative drug delivery systems.

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OR22

Comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry featuring tandem ionization: adding an extra-dimension to hazelnuts (*Corylus avellana l.*) primary metabolome fingerprinting

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Summary: *Targeted and Untargeted (UT) Fingerprinting of hazelnut primary metabolome by comprehensive bi-dimensional GC coupled with BenchTOF-MS with simultaneous tandem acquisition at different ionization energies to reach an additional informative level about complex food matrices.*

Keywords: *Corylus avellana, Tandem ionization, GC×GC*

Introduction

This study focuses on hazelnuts (*Corylus avellana* L.) primary metabolome (i.e., amino acids, mono and disaccharides, low molecular weight acids and amines) and its characteristic chemical fingerprint in fruits of different geographical origins, pedoclimatic condition and processed by different postharvesting practices. The complexity of the primary metabolome is captured by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry with a system enabling to work with variable ionization energies across a single analytical run. The primary metabolome includes several non-volatile precursors of (key)-aroma compounds and potent odorants [1, 2] that may be used to predict hazelnuts flavour potential. During industrial roasting, non-volatile precursors, mainly amino acids and reducing sugars, react within the Maillard reaction framework to produce a complex array of volatiles including those responsible of the characteristic hazelnuts aroma profile.

Experimental

The hazelnut samples studied are characterized by different variables regarding the cultivar (e.g., Tonda Gentile Trilobata and Anakliuri), the geographical origin (Italy and Georgia) and shelf life (from time 0 to 12 months). Moreover, different post-harvest practices and storage conditions are also considered. Sample preparation consists of a selective extraction of primary metabolites with polar solvent mixtures, followed by selective derivatization (oximation-sylilation) to obtain volatile and semi-volatile derivatives amenable to comprehensive two-dimensional gas chromatographic separation and mass spectrometric detection (GC×GC-TOFMS). GC×GC is run with a apolar × semi-polar column combination and TOF MS detection is by a system featuring Tandem Ionization. Two electron energies are explored: 70 and 12 eV and tandem data is acquired across the analytical run with a 50Hz acquisition frequency per channel in a mass range between 40 to 550 *m/z*. Untargeted/targeted fingerprinting (*U*T fingerprinting) work-flow is carried out

combining template matching strategies [3] on the 2D-patterns of derivatized primary metabolites. To correlate non-volatile precursors distribution with key-aroma compounds developed after roasting, hazelnuts are roasted in a lab-scale under controlled conditions and volatiles sampled by Head Space-Solid Phase Microextraction (HSSPME) before GC-MS profiling.

Results

The hazelnuts primary metabolome 2D-pattern, illustrated in the color plot of Figure 1, accounts for about 500 2D-peak-regions, most of them identified by matching linear retention index data with MS spectra signatures at 70 eV. A dedicated methodology is proposed for sugars, primary metabolites playing a relevant role as aroma precursors. A long 1D column (60 m x 0.25 mm ID) enable better separation of isomers, and their relative “multiplets”, and gives more confident identification. Low ionization energy (12 eV) produces spectra with complementary information power for most of the informative analytes compared to those at 70 eV thanks to lower fragmentation rate and higher abundance of fragments with high m/z ratios. The primary metabolome fingerprints collected from the different samples under study, is explored by the UT fingerprinting approach; 2D-UT peaks normalized responses, when observed by hierarchical clustering (HC) based on Euclidean distances, enables a clear clustering of samples based on the key-variables represented within the sample set (e.g., cultivar, geographical origin, drying and roasting condition, storage time). A closer look to key-aroma compounds precursors enables to define statistically relevant differences in metabolite patterns, suggesting strong influence of geographical origin and of the predominant cultivar on the hazelnut flavor potential.

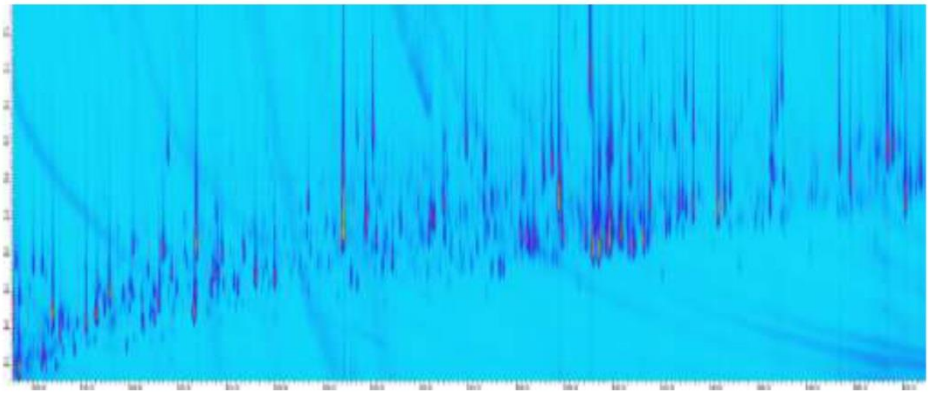

Fig. 1. *2D-GC chromatogram of primary metabolome of Tonda Gentile Trilobata, harvested in 2018 in Georgia*

To validate the role of primary metabolome as predictor of hazelnut flavour potential, odorants developed by lab-scale roasting are profiled by HS-SPME-GC-MS and they responses correlated to known non-volatile precursors. The Pearson correlation matrix, based on the dataset composed by both targeted volatiles and primary metabolites, is explored to highlight stronger correlations. Among the otters, those with a great relevance are between isoleucine and 2-methylbutanal, leucine and 3-methylbutanal, alkyl-pyrazines and proline, furfural and aldo- and chetoesoses reducing sugars.

Conclusions

The analytical approach here proposed enables the accurate and informative chemical fingerprinting of hazelnuts based on their primary metabolites signatures. In a single analytical run, tandem GC×GC data are acquired enabling a more confident identification of analytes – due to the complementary nature of hard- and soft- ionization spectra - and a good coverage of the non-volatile precursors for key-aroma compounds in hazelnuts. By combining data from primary metabolite distribution and volatiles, produced after lab-scale model roasting, several positive correlations (statistically relevant) between precursors and Maillard reaction products are established posing solid foundation to a rational strategy for hazelnut quality assessment. Robustness of the proposed approach is under validation over a wider sampling design covering different harvest years and storage conditions

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OR23

Polar pesticides method: a new and unique solution for both food complex matrix and environmental samples

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Keywords: *Polar Pesticides, No Ion Suppression, No Derivatization*

Recent increase in public concern regarding the presence of Glyphosate has significantly increased the requirement to analyze it and its metabolites in food, feed and the environment, so has accelerated the need for a more efficient and robust analytical method. The extraction and chromatography of these compounds is well described in the EURL-QUPPE method, but the separation is not robust in practice, so system and method maintenance are intensive. Several different HPLC or HILIC based methods have failed to address the issues of reproducibility and sensitivity, so FMOC derivatization prior to analysis is often still employed for Glyphosate, AMPA and Glufosinate. Although possible to automate, this procedure is still time consuming or expensive, and is not applicable to the other polar pesticides of interest.

Ion chromatography has been shown to be beneficial for separation, but the need for a suppressor is detrimental to MS analysis and the inefficiencies of changing inlet systems on a heavily used mass spectrometer makes it impractical in a busy lab performing primarily reverse-phase LC. So, the final method, presented here, makes use of an IC column in a method-switching reverse phase (RP) system with MS amenable mobile phases. Such conditions configure Glyphosate ideally for MS detection with good retention and separation of the other analytes and matrix interferences. The method meets the DG-SANTE1requirements of reproducibility (<20%) and recovery (80-110%), and the LOD of the method is below 0.01 mg/kg for Food samples. Excellent long-term stability and robustness were achieved throughout the validation of this method for food samples extracted by the QUPPE procedure.

Where environmental samples require testing, the regulatory limits are much lower and interference from matrix more problematic in traditional analyses with a short retention time, so derivatization is often the only option. However, since Glyphosate is well retained in this new method, the potential to further develop it for direct large-volume injection was investigated. By modifying the gradient conditions and optimizing the injection parameters, a second method specific to environmental water samples has been developed. Although the large volume injection (LVI) is more susceptible to changes in pH (for example, due to evaporation of mobile phase) robustness has been shown to be similarly good, and allows detection of the same suite of analytes with a LOD of <0.02 ng/l.

The methods were found to be considerably more robust and sensitive than other approaches described in various publications and have achieved the target limits of detection required to meet existing and proposed future regulations.

OR24

Determination of the polyphenolic fraction of food real-world samples by comprehensive two-dimensional liquid chromatography coupled to photodiode array and mass spectrometry detection

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Summary: *Food analysis is the discipline dealing with the development, study and application of analytical procedures for the characterization of the properties of foods and their constituents. Comprehensive two-dimensional liquid chromatography (LC×LC) with reversed phase conditions in both dimensions, coupled to mass spectrometry, was successfully employed for the determination of the polyphenolic fraction of selected food samples.*

Keywords: *Food analysis; polyphenols; comprehensive two-dimensional liquid chromatography; mass spectrometry.*

Introduction

Food chemistry is continuously involved in the assessment of quality and authenticity, with a special focus on the characterization of molecules with a possible beneficial effect (nutraceuticals) or a toxic effect on human health. In this context analytical methods should be capable to allow the determination of the main components of food and natural products samples, but can also be selective and sensitive enough to determine minor components.

Comprehensive two-dimensional liquid chromatography (LC×LC) is a powerful alternative to conventional one-dimensional liquid chromatography (1D-LC) for the analysis of complex samples. The technique involves the coupling of two or more independent or nearly independent separation steps, increasing significantly the separation power of the one-dimensional liquid chromatography counterpart.

Over a decade, several LC×LC methodologies using different column sets and different instrumental set-ups, often in combination with mass spectrometry, have been developed and successfully employed for the characterization of different chemical classes in food products.

In this contribution different applications of LC×LC coupled to photodiode array and mass spectrometry detection for the characterization of the bioactive components in food samples will be presented.

Experimental

LC×LC analyses were performed on a Nexera-e liquid chromatograph (Shimadzu, Kyoto, Japan), consisting of a CBM-20A controller, a Nexera Mikros pump, two LC-30AD dual-plunger parallel-flow pumps, a DGU-20A_5R degasser, a CTO-20AC column oven, a SIL-30AC autosampler, an SPD-M30A photo diode array (PDA) detector (1.0 μL detector flow cell volume). The two dimensions were connected by means of two high speed/high pressure two-position, six-ports switching valves with micro-electric actuator (model FCV-32 AH, 1.034 bar; Shimadzu, Kyoto, Japan), placed inside the column oven and equipped with two identical stainless steel loops. The Nexera-e liquid chromatograph was hyphenated to an LCMS-8050 mass spectrometer, through an ESI source (Shimadzu, Kyoto, Japan).

1D-LC separations were performed on an Ascentis Express C18 column (Merck Life Science, Merck KGaA, Darmstadt, Germany; 150×4.6 mm I.D., 2.7 μm d_p). LC×LC separations were conducted by using either a first dimension (^{1}D) Ascentis Express Cyano (ES-CN) or Ascentis Express RP-Amide columns whereas in the second dimension (^{2}D) either an Ascentis Express C18 or RP-Amide columns, all from Merck Life Science, Merck KGaA, Darmstadt, Germany.

Mobile phases employed consisted of: A) 0.1% formic acid in water (pH 3), (B) 0.1% formic acid in ACN, under gradient mode. PDA range was from 200 to 440.

LCMS-8050 detection was achieved through ESI-MS in negative ionization mode. Mass spectral range in full scan mode: *m/z* 100-1200; event time: 0.5 (1D-LC analyses), 0.2 sec (LC×LC analyses); nebulizing gas (N_2) flow: 3 L min^{-1}; drying gas (N_2) flow: 15 L min^{-1}; heating gas flow (air): 10 L min^{-1} same; heat block temperature: 400 ^oC; desolvation line (DL) temperature: 250 ^oC; interface temperature: 300 ^oC; interface voltage 3.50 kV; detector voltage: 1.80 kV.

Results

The knowledge in the polyphenolic profile of food real-world samples can be useful to discriminate the geographic origin and/or preserve authenticity. In many cases, the chemical composition of such samples can be rather complex with a considerable number of overlapping compounds, despite a careful optimization of the chromatographic conditions. To improve peak separation and resolution of overlapped components, an RP-LC×RP-LC system coupled to PDA and ESI-MS was investigated. At first, each dimension was optimized independently. Usually, ^{1}D separations are run under suboptimal flow conditions in order to decrease the amount of the eluate transferred from the ^{1}D. Interestingly, a micro LC pump was employed being compatible with the micro-bore column (1.0 mm I.D.) Also, the optimization of individual ^{2}D separation conditions is critical for achieving effective RP-LC×RP-LC separations and ^{2}D analysis time has to be kept as short as possible in order to get the maximum number of ^{2}D analyses per ^{1}D peak. The performance of full-in-fraction, segmented and shifted secondary gradients were tuned according to the nature of the samples under investigation. Further, the selectivity and sensitivity of the multiple reaction monitoring operation made “target” analyte quantification more robust, as demonstrated for the determination of selected food polyphenols.

Conclusions

In this contribution, different comprehensive two-dimensional liquid chromatography methods coupled to photo diode array and mass spectrometry detection were exploited for determination of the polyphenolic fraction of various food real-world samples. The employment of different gradient strategies resulted in a significant increase in the separation space available, allowing to resolve several co-elution issues thus leading to the detection of a higher number of compounds with respect the conventional one-dimensional LC analysis. The use of a micro LC pump in the ^{1}D of the LC×LC was capable to deliver reproducible flow rates down to the microliter range.

Acknowledgments

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PL5

Protein oxidation and glycation compounds in food: targeting individual structures by MS techniques

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Oxidation and glycation ("Maillard reaction") are the most important deterioration reactions of food proteins [1,2]. The Maillard reaction is responsible for the aroma, taste, and appearance of thermally processed food. Just as protein oxidation, it may induce structural and functional changes in food proteins. Recent studies suggest that dietary glycation compounds present in heated foods, often denoted as "advanced glycation end products" or "AGEs", may pose a nutritional risk as "glycotoxins" due to a possible link to chronic inflammation and other metabolic diseases. The specific expression of an "AGE content", either in foods or biological samples, is not scientifically justified without giving structural details. For a reliable quantification of individual glycation compounds, state-of-the art methods such as LC-MS or GC-MS and thoroughly characterized reference materials must be used. The analysis of malt, beer, honey, and pasta products by HPLC-MS/MS and correlations with the processing conditions will be presented. These data will show the limited applicability of ELISA-based sum methods that are often used in (food) glycation research.

Protein oxidation is sometimes assessed by measuring "protein carbonylation" through reaction with 2,4-dinitrophenylhydrazine (DNPH), however, this method cannot give a reliable picture on protein oxidation in food, because structures such as methionine sulfoxide and oxidation products of tyrosine and tryptophan do not react. Methionine oxidation was analysed in milk products by HILIC-HPLC-MS/MS [3]. Milk proteins were isolated and hydrolysed enzymatically in the absence of oxygen. An isotopically modified methionine probe was added in order to record artificial methionine oxidation during hydrolysis. In UHT and evaporated milks, up to 8% of methionine was oxidized, whereas up to 33% of methionine was oxidized in milk drinks containing added cocoa or coffee components. The concentrations of methionine sulfoxide are far above the concentrations of protein carbonyls measured in milk products by the DNPH method [4].

We conclude that sum methods based on the (immuno)reactivity of more or less characteristic structures of protein oxidation and glycation may have been helpful in gaining first insights into these reactions. However, in order to avoid misinterpretations and learn about the chemical background of the reactions in food, chromatography coupled to mass spectrometry is the method of choice.

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OR25

Phosphopeptide profile of kefir as affected by the production technology

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Summary: *In the present work, a comprehensive characterization of caseino-phosphopeptides (CPPs) was performed in kefir obtained with kefir grains, using preliminary enrichment on hydroxyapatite followed by dephosphorylation and analysis by LC-ESI-QTOF-MS/MS. The influence of kefir production technology, namely, temperature, pH and 2-step fermentation on the phosphopeptide profile was then studied.*

Keywords: *kefir, phosphopeptides, LC-ESI-QTOF-MS/MS*

Introduction

Among bioactive peptides in kefir, caseino-phosphopeptides (CPPs) are casein-derived phosphorylated peptides able to bind and solubilize minerals [1], exerting several beneficial effects, such as enhancement of minerals absorption in the gastrointestinal tract [2], tooth enamel remineralization in the oral cavity and buffering of plaque pH [3]. CPPs are originated from milk caseins by: i) digestive enzymes ii) enzymes of microorganisms iii) endogenous enzymes of milk. Their low concentration, compared to normal peptides who compete for ionization when co-eluting with phosphorylated ones from the chromatographic column, can strongly impair their detection by LC-MS/MS analysis. To overcome this drawback, several approaches have been proposed in the literature for the selective enrichment of phosphopeptides including the use of Hydroxyapatite (HA)-based affinity chromatography [4]. A systematic characterization of phosphopeptides from kefir obtained with kefir grains, focused on the incidence of production technology on their abundance in the product was the main goal of the present study.

Experimental

Kefir grains were activated by inoculation of the semi-skimmed cow's milk with 18 g/L of grains and incubation at 25°C for 24 h, in accordance with the manufacturer's information. Grains were then separated from the milk with sterile sieve and added (2%, w/w) to a new 150 mL aliquot of semi-skimmed cow's milk. Kefir was manufactured in triplicate under the following conditions: i) control, at 25°C, final pH 4.7 (K25) and ii) at 18°C, final pH 4.7 (K18), to investigate the influence of temperature, iii) at 18°C, final pH 4.0, to assess the

influence of pH (K18a), and iv) at 25°C, final pH 4.7, using a back-slopping approach (grains from a 25°C fermentation were left at 4°C for 5 days and used for a second fermentation), (K25bs), to ascertain the influence of the repeated use of grains. After fermentation, samples were left to mature at 4°C for 24 h before evaluation.
Extraction and dephosphorylation of phosphopeptides in kefir samples was carried on as reported in literature [5].
The HPLC-MS system consisted of an HPLC 1200 Series system (Agilent Technologies, Palo Alto, CA) equipped with a Zorbax SB-C18 column (2.1 × 100 mm i.d., 1.8 µm packing; Agilent). The mobile phase was a mixture of 0.1% formic acid aqueous solution and acetonitrile and the separation was conducted, at a flow rate of 0.2 mL/min, by applying a gradient elution. The HPLC device was connected online to a MicroTOF-Q II quadrupole-Time of Flight mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with an electrospray ionization source (ESI). The instrument was operated in positive ion mode. First, an MS *full-scan* acquisition was performed to obtain preliminary information on the predominant m/z ratios observed during the LC elution. Afterwards, the mass spectrometer was operated in *data-dependent mode* to automatically switch between MS and MS/MS acquisitions, with the latter scheduled for the 3 most abundant ions detected in MS spectra.
Phosphopeptides were tentatively identified by using MS/MS data as input for searches against the UniProtKB database based on the MS-Tag software included in the Protein Prospector portal. In the present case, searches were focused on all bovine milk proteins for which phosphorylation is reported in the Uniprot KB database, namely, α_{S1}- (accession number: P02662), α_{S2}- (P02663), □- (P02666), and κ-casein (P02668), serum albumin (P02769), GlyCAM-1 (P80195) and osteopontin (P31096). No enzyme restriction was set for the *in silico* cleavage of protein sequences.
Finally, the peak area retrieved from the Extracted Ion Current (EIC) chromatogram relevant to each peptide (0.2 m/z units wide window) was used as a parameter to compare the abundance of a specific peptide in different kefir samples.

Results

Seventy-three phosphopeptides sequences, with lengths ranging from 10 to 43 amino acids, all including from three to five serine residues in their sequences were identified in kefir products. For identification purposes, peptides characterized by a mass accuracy better than 15 ppm and by a Matched Intensity (i.e. the ratio between the sum of intensities of explained signals and the sum of intensities of all signals in the MS/MS spectrum) greater than 40%, with all the predominant signals accounted for by product ions of the y and b series, were considered. Most of phosphopeptides arised from the 1-40 region of β-casein (79%) and almost all (71 out of 73) showed the “SerP-SerP-SerP-Glu-Glu” motif involved in mineral binding. Cleavage sites E5-L6/L3-E4, N7-V8, N27-K28, K28-K29, E14-S15, T24-R25, E31-K32, F33-Q34, L6-N7, P9-G10, E4-E5/E36-E37, listed in order of descending frequency, were observed more than five times, indicating a preference of the acting enzymes.
To investigate the influence of temperature and pH and of the repeated use of grains on the phosphopeptide profiles of kefir, samples obtained under different conditions were compared.

K25 and K18 phosphopeptide profiles were very similar, though a lower response was observed for most phosphopeptides in sample K18, with the exceptions of some peptides with molecular weights larger than 2000, thus suggesting that the lower temperature slowed down the proteolytic activity in the K18 sample.
The phosphopeptide chromatographic profile obtained for the K18 sample showed a higher response for retention times higher than 21 min with respect to the K18a sample. In this interval most of peptides with high molecular weights identified during this study, all including the sequence motif "SerP-SerP-SerP-Glu-Glu", were generally eluted. Smaller peptides predominated in K18a. Consequently, a lower level of proteolysis could be inferred for sample K18, compared to the K18a, obtained under more acidic conditions and for a longer fermentation time, i.e., conditions enabling a more extended proteolysis by bacteria.
Finally, the influence of the repeated use of grains was investigated. The phosphopeptide chromatographic profiles of samples K25 and K25bs were significantly different. Sample K25 showed a prevalence of peaks in the range 19-24 min, whereas sample K25a showed higher peaks in the range 24-28 min. K25bs contained longer peptides compared to K25, thus suggesting a lower proteolytic activity in K25bs, with a poor reproducibility in the kefir phosphopeptide profile occurring when grains are reused.

Conclusions

A comprehensive characterization of the phosphopeptides profiles in kefir samples obtained under different production conditions could be achieved by an analytical method based on preliminary enrichment on hydroxyapatite, subsequent dephosphorylation and final analysis based on LC-ESI-QTOF-MS/MS. Seventy-three phosphopeptides sequences were identified; most of them arised from β-casein (79%) and almost all (71 out of 73) showed the "SerP-SerP-SerP-Glu-Glu" motif involved in mineral binding. Moreover, the proteolytic activity, and thus the final profile of phosphopeptides, could be modulated through appropriate modifications of the production technology.

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OR26

HPLC-MS/MS method for fast and comprehensive quantification of sesame lignans

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Summary: *A fast HPLC-MS/MS method for the simultaneous detection of 10 major sesame lignans was developed to allow a more comprehensive profiling going beyond the main lignans sesamin and sesamolin.*

Keywords: *Sesame, lignans, HPLC-MS/MS*

Introduction

Sesame has been known for various beneficial effects on human health since ancient times and research is still ongoing. Many of the favorable properties of sesame could be attributed to lignans, of which especially the sesame seeds are very rich[1], but lignans can be found in most plant parts.[2] Lignans are widespread plant secondary metabolites[3-5] originating from two linked phenylpropanoide units.[6] Different types of lingna exist, while in sesame, bisepoxylignans are predominant (Scheme 1). The biological activities of lignans have been attracting interest for decades and numerous studies deal with it.[4,7-9] Some lignans show antitumor activity, others serve as antioxidants.

Sesamin and sesamolin are dominant lignans in sesame oil[10] and many studies about the lignans of sesame focus on sesamin and sesamolin. However, the health-prompting qualities of sesame certainly result from a combination of active substances and cannot be reduced to two lignans. Thus, when it comes to the breeding of sesame and selection of varieties for cultivation, a more comprehensive profiling of sesame lignans should be performed.

Sesame lignans are UV-absorbing and therefore, UV-detection is frequently employed and also handy. However, LC-MS/MS is much less prone to interferences than LC-UV and thus, extensive sample preparation and shorter chromatographic runs can be used. None of published methods for the detection of sesame lignans covering more analytes than sesamin and sesamolin used LC-MS/MS. We therefore closed this gap, introducing a fast LC-MS/MS method for the quantification of sesamol, sesamin, pinoresinol and pinoresinol-di-glycoside, piperitol, sesamolin, sesaminol, sesamolinol and di- and tri-glycosides of sesaminol.

Experimental

A Phenomenex Synergi Hydro RP column, 50x2 mm with 2.5 µm particle size was employed. A linear gradient from 5 to 98% methanol in 10 minutes was run, 98% MeOH were hold for 1.5 minutes and the column was equilibrated for 4.5 minutes resulting in in total run time of 16 minutes. An Agilent 1290 Infinity II LC system was coupled to an Agilent 6460 triple quadrupole. The MS/MS transitions and retention times are given in Table 1.

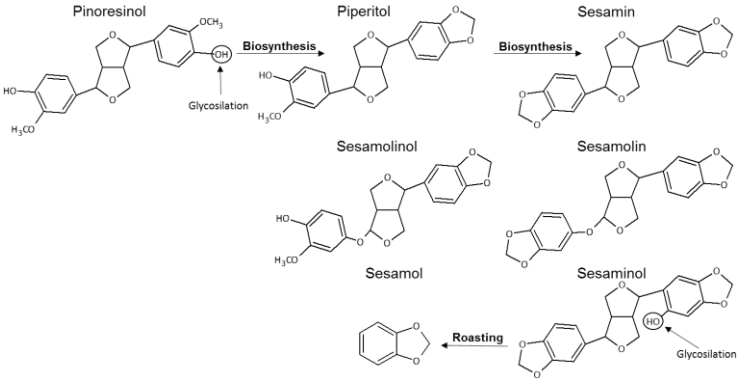

Fig. 1. *Major sesame lignans.*

Table 1. *Method detail for the monitored sesame lignans.*

Lignane	Retention time [min]	Ionization mode	Parent ion	Collision Energy [V]	Product ions
Sesamol	4.9	Positive	139.0	10 17	81.0 108.8
Sesamin	9.2	Positive	337.0	19 19	135.0 289.0
Pinoresinol	6.6	Negative	357.1	10 10	151.0 135.9
Pinoresinol-di-glycoside	3.4	Positive	341.2	15 5	137.0 323.2
Piperitol	8.2	Positive	327.1	15 15	243.0 257.1
Sesamolin	9.5	Positive	233.0	10 10	215.0 187.1
Sesaminol	8.6	Negative	369.1	17 17	219.0 340.0
Sesaminol - di-glycoside	7.2	Positive	353.1	10 10	185.2 335.2
Sesaminol - tri-glycoside	6.5	Positive	353.1	10 10	185.2 335.2

Conclusions

A new, fast LC-MS/MS method to quantify 10 sesame lignans was developed. The method was used for the analysis of lignan content in seeds, root and hairy roots of 25 accessions of sesame.

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OR27

Characterization of Italian authentic saffron by using stable isotope ratio analysis

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Summary: *$\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$, $\delta^{2}H$ and $\delta^{18}O$ of 46 Italian samples of bulk authentic saffron have been analysed to check the possibility to discriminate between three Italian macroareas. Moreover, 9 samples of commercial saffron have been taken into account to check differences between their isotopic values and the rest of the dataset.*

Keywords: *saffron, isotope, isotope ratio mass spectrometry*

Saffron derives from the dried stigmas of *Crocus sativus L*. To produce a kilo of saffron it is necessary to pick up about 150.000 flowers, which entails around 500 hours of work. Because of its high cost and the great demanding production, saffron has often been subject of adulterations [1,2,3]. In the present work, for the first time, $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$, $\delta^{2}H$ and $\delta^{18}O$ of 46 samples of bulk saffron coming from different regions of Italy have been analysed. The aim of this work was to carry out a geographical characterization to check the possibility to differentiate between three Italian macroareas: the North (considering Veneto, Piedmont and Liguria), the South (considering Abruzzo, Basilicata, Molise, Sardinia and Sicily) and the central part of Italy (considering Tuscany and Umbria). Moreover, also 9 samples of commercial saffron was considered in the present work.

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OR29

Development of a new analytical method for 30 bioactive compounds quantification in Spent Coffee Ground

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Summary: *The purpose is to develop a method for 30 bioactive compounds quantification in Spent Coffee Ground. The analysis was performed using an UHPLC-MS/MS working in Dynamic-MRM mode. Different extracts were evaluated and will be tested to assess their biological activities in the perspective of their use as nutraceuticals.*

Keywords: *Spent Coffee Ground, HPLC-MS/MS, bioactive compound*

Introduction

Spent Coffee Ground (SCG) is the byproduct mainly produced from the soluble coffee industry. Each year, it has been estimated that around six million tonnes of SCG are generated and then disposed of in landfill [1]. Hence, the research of an innovative reuse of this organic waste is becoming a serious issue of general interest in a world with ever-growing needs to reduce waste and to move toward more sustainable practise of waste processing [2]. The purpose of this project was to develop a method for the quantification of 30 bioactive compounds (caffeine, chlorogenic acids, and other important coffee polyphenols, such as flavonoids and phenolic acids) in Spent Coffee Ground and to evaluate, in term of amount of bioactive compounds, different extraction processes.

Experimental

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 30 bioactive compounds quantitation. The acquisition was performed in Dynamic “multiple reaction monitoring” (Dynamic-MRM) mode. The separation was achieved using a Kinetex PFP analytical column (100 x 2.10 mm i.d., 2.6 µm) using a binary gradient of water and methanol both with 0.1% of formic acid. SCG was extracted using different solvents and different procedures such as ultrasound-assisted extraction and magnetic stirrer.

Results

The method was sensitive (LOQs for all compounds were in the range 1-100 µg L^{-1}), linear (R^2 for all analytes was equal to or higher than 0.9907), accurate and robust. The acquisition was performed in Dynamic-MRM mode because this permitted to monitor several compounds in one single analysis without affecting sensitivity, since each transition was monitored at a specific time, according to retention time of the analyte. In this manner, the mass analyser was able to scan the different transitions with an adequate cycle/second. The

best extraction procedures, in term of amount of bioactive compounds, were the ultrasound-assisted extractions using hydroalcoholic solvents: ethanol: water (70:30) (1308.39 mg kg^{-1}), ethanol:water (50:50) (1287.84 mg kg^{-1}) and ethanol:water (30:70) (1259.06 mg kg^{-1}).

Conclusions

Spent Coffee Ground is revealed to be a good source of bioactive compounds; the most promising extracts will be tested to evaluate their prebiotic and antimicrobial activities and for safety properties, in the perspective of their reuse as nutraceuticals.

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OR30

Polyphenols in coffee by-products extracts: determination and cytoprotection in an undifferentiated neuroblastoma cell line

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***Summary**: The cytoprotective effect of polyphenols extracted from two coffee by-products (silverskin coffee and spent coffee) in SH-SY5Y cells jointly two treatments (pre-treatment and simultaneous) and two mycotoxins (α-ZOL and BEA) were studied. Five extraction procedures were performed in both coffee by-products and polyphenols determination was carried out through Q-TOF LC/MS.*

***Keywords:** SH-SY5Y cells, coffee by-products, Q-TOF LC/MS*

Introduction

Two coffee by-products: silverskin coffee and spent coffee, have demonstrated to have high amount of polyphenols depending on the coffee variety. Silverskin coffee (SC) is a thin tegument of the outer layer of the two beans present in the coffee cherry [1] which it is possible to collect in the roasting process. Spent coffee grounds arise as waste products through the production of instant coffee and coffee brewing; the unutilized portion of the coffee bean left after brewing [2]. The presence of mycotoxins in coffee is associated to the contamination by filamentous fungi at various stages as harvesting, preparation, transportation, or storage, and in fermentation and drying, especially where the water activity is lowers [3].

Polyphenols have strong evidence of their beneficial impact on brain function during aging; in fact, caffeine has protective effects against Alzheimer´s Disease and Parkinson´s Disease and potential mechanisms to protect against blood-brain barrier leakage [4, 5].

Considering that coffee is an excellent substrate for the growth of mycotoxins and, that polyphenols exert protective effect as demonstrated in *in vitro* assays, in this study determination of polyphenols of coffee samples and effect on SH-SY5Y cells exposed to two mycotoxins (α-ZOL (50 µM) or BEA) were studied and three exposure strategies with coffee extracts were assayed: direct treatment, simultaneous treatment and pre-treatment.

Experimental

1 gr of silverskin coffee and 10 gr of spent coffee were weighted and five different types of extraction procedure were carried out: Boiling water (50 ml of water and 2h in bain-Marie); MeOH:H_2O (70:30) (50 mL); EtOH:H_2O (70:30) (50 mL); H_2O:MeOH (50:50) (50 mL); MeOH (50 mL). All extracts except boiling water procedure were sonicated for 2h, centrifuged and evaporated to dryness to be redisolved and filtered in 1 or 2 mL of MeOH for silverskin coffee and spent coffee, respectively.

Q-TOF LC/MS: Agilent 1200 Infinity LC system with a mobile phase of 0.1 % formic acid in water and acetonitrile was used. An Agilent Infinity lab Poroshell 120 EC-C18 (3 x 100 mm, 2.7 µm) column was used with 15 min gradient. The MS was Agilent 6540 UHD Accurate Mass Q-TOF LC/MS.
SH-SY5Y cells were seeded in 96-well culture plates at 2 × 10^4 cells/well and allowed to adhere for 24 h before extracts and mycotoxin additions. Times assayed were 24 and 48h. Direct treatment consisted in exposing cells to pure extract and 1:2 dilutions. Simultaneous treatment: extract at fixed dilution (1:4) of the coffee by-product extract and α-ZOL (50 µM) or BEA (2.5 µM) at dilutions 1:2. Finally for pre-treatment procedure, cells were exposed as in direct treatment and after 24h cells were exposed to α-ZOL or BEA at dilutions 1:2.

Results

The total content of polyphenols was investigated using the Folin-Ciocalteau assay in order to have a quantitative overview and to compare the five extraction procedures followed (Fig. 1). Afterwards, 15 different polyphenols were identified by Q-TOF LC/MS and a high amount of chlorogenic acid was detected in spent coffee extract (615 mg/g) while salicilic acid (53 mg/g) in silverskin coffee extract.

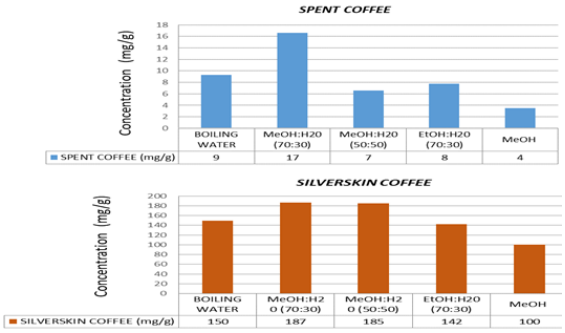

Fig. 1. *Total content of polyphenols present in different extracts in coffee by-products.*

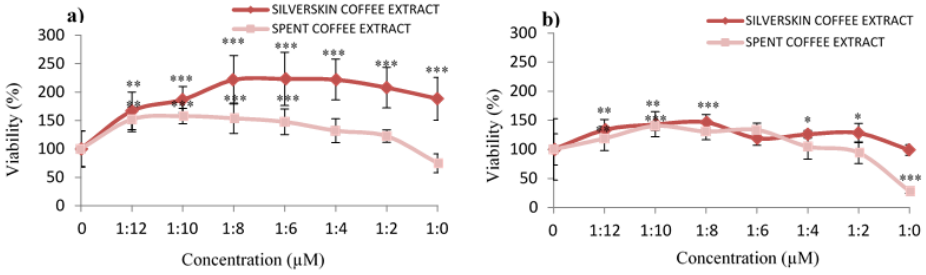

Fig. 2. *Cytotoxicity of boiling water extract for silverskin coffee and spent coffee on SH-SY5Y cells after 24h (a) and 48h (b) of exposure by MTT assay. Serial coffee extracts were 1:2 dilutions from 1:0 to 1:12.*

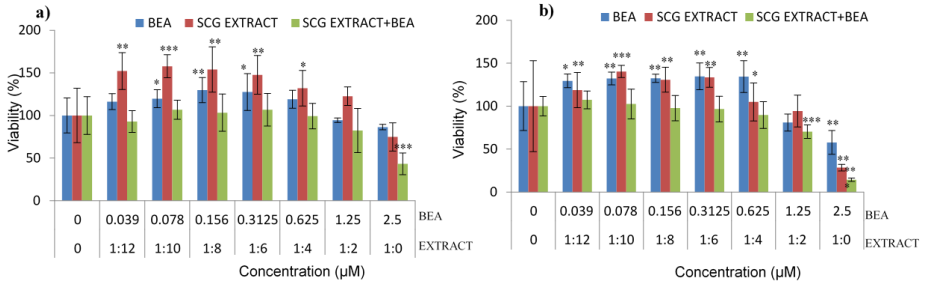

Fig. 3. *Cytoprotection obtained with SH-SY5Y cells after pre-treatment with boiling water extract of spent coffe during 24h with BEA (50 μM) during 24h(a) and 48h (b) by MTT assay.*

Conclusions

SH-SY5Y cells on direct treatment were sensitive and the highest cell viability was obtained by extracts that were found with high concentrations of total polyphenols.

In the pre-treatment assay the spent coffee was more sensitive to BEA than to α-ZOL, showing no cytoprotective effect and generating a viability below the IC_{50} at 24 and 48h; in silverskin, both in the pre-treatment and in the simultaneous assays, greater sensitivity was found towards α-ZOL. Considering the high viability provided for silverskin coffee extracts obtained these extracts can present benefits as natural and sustainable food ingredient.

Silverskin coffee generates greater viability when exposed, in pre-treatment and simultaneous treatment, with the BEA mycotoxin, so values of IC_{50} are not obtained; spent coffee, on the other hand, shows a greater viability with the mycotoxin α-ZOL with even more protective activity than the extract tested alone.

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OR31

Extraction of espresso coffee by changing particle size distribution and evaluation of bioactive compounds through HPLC-VWD and HS-SPME/GC-MS

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Summary: *The aim of this research project is to optimize the extraction of espresso coffee by decreasing the amount of ground coffee from 14 to 12 g at different particle size distributions, and by applying various heights of perforated discs (4, 5, 6 and 7 mm) and three different filter baskets (A, B, C). The analysis was performed using an HPLC-VWD working in gradient mode. Volatiles were analyzed by HS-SPME/GC-MS.*

Keywords: *Espresso Coffee, particle size distribution, bioactive compounds*

Introduction

Espresso machines nowadays maintain constant the extraction process of espresso coffee (EC) by guaranteeing stable brewing temperature and pressure. However, it is difficult to grind roasted coffee beans in homogeneous way, with particles of the same shape and size. Possible variations in particle size is indeed another variable that can influence the extraction.

This research aims to optimize the extraction process of EC by decreasing the amount of ground coffee per cup (grinding finer ground coffee and using different filter baskets), while keeping the same quality of the beverage.

Experimental

Three types of filter baskets and various heights of perforated discs (4, 5, 6 and 7 mm) were used to extract EC. These extractions were then assessed through a sensory evaluation process. Quantitative and qualitative analyses on caffeine, trigonelline and chlorogenic acids were carried out with HPLC-VWD [1], whilst volatile compounds were scanned with HS-SPME/GC-MS [2]. Caffeine and trigonelline analysis with a Gemini C18 110A analytical column (250 x 3 mm I.D., 5 µm, Phenomenex, Cheshire, U.K.), using a binary gradient of water with 0.3% of formic acid and methanol; and chlorogenic acids analysis with polar-PR analytical column, using a binary gradient of 0.1% of formic acid in water and 0.1% of formic acid in methanol were conducted by HPLC-VWD. Divinylbenzene – carboxen - polydimethylsiloxane (DVB-CAR-PDMS) Stable Flex fiber of 50/30 µm Supelco, USA) was used for the HS-SPME.

Results

The effect of particle sizes on EC extraction in different filters has been studied. When the size of particles (200-500 µm) and the amount of ground coffee (14-12 g) were kept constant, a significant alteration on bioactive compounds amount was highlighted by using different filter baskets. Filter basket A, compared to baskets B and C, allowed a gradual stable rise of all biocomponents in most cases and samples. For instance, caffeine in Arabica

(14 g) was 73.20 mg/l at 500-1000 µm and 85.66 mg/l at 200-300 µm. Almost all bioactive compounds at 12 grams and 500-1000 µm were in fact nearly 50% lower than at 14 grams, while those obtained at 200-300 µm were lower only 20%. Extracting with smaller particles escalates the quantity of bioactive compounds and develops descriptive notes of EC.

Conclusions

The comparison between different extractions of EC with three different filters while reducing the amount of ground coffee from 14 grams to 12 grams provides results about the influence of different particle sizes of grinded coffee, and various heights of perforated discs in the extraction process. Moreover, the changes in the structure of the perforated disc can help generate almost equal concentration of bioactive compounds when the mass of ground coffee in the basket is only 12 g. The outcomes will trigger and support further studies on different extraction processes, to develop more sustainable and economically affordable coffee of high quality.

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Poster Communications

P1

Organic (PBDEs, NDL-PCBs) and Inorganic (Pb, Cd, Hg, As, Ni, Mn) contaminants in hunted wild boar from Central Italy

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Summary: *Wild boar* (*Sus scrofa*) *is an omnivore species often used as environmental pollution sentinel. It is hunted and consumed as food, therefore may expose population to relevant levels of contaminants. This study investigate the presence of some organic (NDL-PCBs and PBDEs) and inorganic (Cd, Hg, Pb, As, Ni, Mn, Cr, Co, Zn and Cu) pollutants in wild boars caught in Central Italy*

Keywords: *PBDEs, PCBs, Heavy metals, wild boar, contamination sentinel*

Introduction

Polychlorobiphenyls (PCBs) and Polybrominated biphenyl ethers (PBDEs) are persistent chlorinated organic contaminants of anthropic origin. These substances are persistent, lipophilic and tend to bioaccumulate and biomagnify. Heavy metals are ubiquitous in soil, water and air. Their transfer to the food chain is an important environmental issue that could represent a risk to human health. Cd, Hg, Pb and As are among the most dangerous heavy metals. Some other metals, such as Mn, Ni, Cr, Co, Zn and Cu are microelements with biochemical and physiological functions.

Wild mammals, often, may be used as biological indicators of environmental pollution. Among mammals, wild boar (*Sus scrofa*) is commonly used as bioindicator because it is omnivore, widely distributed and easily found during the annual legal hunting season. Moreover, is a game specie and the presence of contaminants in muscle and organs can pose serious threat to human health. In this study the presence of NDL-PCBs, PBDEs, Cd, Hg, Pb, As, Ni, Mn, Cr, Co, Zn and Cu in muscle, liver and lung of wild boars from Central Italy was investigated, to assess environmental pollution and possible detrimental effects on animal and human health.

Experimental

Wild boar samples came from different hunting districts situated in Umbria and Marche region (Central Italy) (Figure 1). Muscle, liver and lung of 106 wild boars (44 from Macerata and 62 from Umbria), hunted between November 2017 and January 2018, were collected. Gender, weight and age were determinate and the samples grouped by tissue (muscle, liver and lung), sex and weight. As a result, 31 pools of wild boar for each matrix were homogenised, giving 93 laboratory samples. 6PCBs-NDL and 15PBDEs were analyzed in GC-MS/MS using the routine procedure in isotopic dilution [1,2]. Pb, Cd, As, Ni, Mn Cr, Co, Zn and Cu were determinate with ICP-MS after

microwave digestion with HNO3 (6 mL) and HF (50 □L), while Hg was analysed by Cold Vapor Atomic Absorption spectroscopy.

Fig. 1. *Wild boar sampling site (hunting districts) in Marche and Umbria region*

Results and discussion

While heavy metals were analysed in all the 93 laboratory samples, PCBs and PBDEs only in 63, because of lack of sample. Cr, Co, Zn and Cu were determinate only in wild boars hunted in Umbria, while Cd, Hg, Pb, As, Ni, Mn in all the samples. The obtained results don't differ significantly among districts. The levels of NDL-PCB were below the limits set from European Commission regulation 1881/2006 in muscle and liver, for all samples. The content of Σ6PCB *upper bound* in muscle tissue was the highest and ranged from 2.0 to 29 ng/g fat (mean value 11 ng/g fat, equal to 1.0 ng/g wet/weight). In liver and lung the mean value was 0.70 ng/g and 0.16 ng/g respectively. No correlation was observed between PCB levels and sex or weight of the board. Also in case of PBDEs, muscle was more contaminated than liver and lung, where only traces of BDE-47 were detected in few samples. In muscle instead BDE-47, 99, 100 were measured in almost all the 21 pool analysed and the □15PBDE *lower bound* ranged from 32 to 3044 pg/g with a mean value of 311 pg/g. Again, no correlations were observed with age and weight. Only few data are available in the literature for PBDEs in wild boar, but the levels measured in the present study seem higher than those reported from Zacs et al. in muscle of Latvian wild boar [3]. As far heavy metals are concerned, the European Commission regulation 1881/2006 set MRLs in meat only for Cd and Pb. The average Cd (N=31) level in muscle (0.027 mg/Kg) was below the maximum limit (0.050 mg/Kg), although five of the 31 samples analysed were above (concentration between 0.053 and 0.103 mg/Kg). Cd mean concentration in liver was 0.15 mg/Kg and no sample exceeds the MRL (0.50 mg/Kg). The mean Pb value (N=31) was 3.5 mg/Kg in muscle and 0.09 mg/Kg in liver. All the livers were below the MRL (0.50 mg/Kg liver; 0.100 mg/Kg muscle) while 19 of the 31 muscle exceeded Pb maximum limit (ranged from 0.16 to 84 mg/Kg). The high Pb level in muscle may be a consequence of Pb bullet fragmentation [5].

Average value of 0.021, 0.0.31 and 0.086 mg/Kg were measured for Hg in lung, muscle and liver respectively, while As was always below the limit of detection. Ni, Mn, Cr, Zn, Co and Cu microelements were measured in different concentration. In agreement with Amici et al. [5], Zn and Cu were the once with higher levels in all the tissue analysed. Higher levels were measured in liver, where the mean concentration reaches 33 mg/kg for Zn and 5.4 mg/Kg for Cu. All the microelements have physiological function which could turn into

intoxication when the levels are too high.

Table 1. *PCBs, PBDEs and Chemical elements concentration (Mean value, min, max) in wild boars*

		Σ6PCB u.b.	Σ15PBDE l.b.	Pb	Cd	Hg	As	Ni	Mn	Cr	Co	Zn	Cu
		ng/g fat	pg/g					mg/Kg					
MUSCLE	Mean	10.9	311	3.48	0.03	0.03	<0.02	0.23	0.73	0.34	<0.020	31	3.0
	min	*2.0*	*32*	*0.015*	*<0.005*	*<0.01*		*<0.10*	*<0.05*	*<0.10*		*13*	*2.3*
	max	*28*	*3044*	*84*	*0.10*	*0.08*		*1.01*	*2.1*	*0.91*		*40*	*5.3*
	n	21	21	31	31	31	31	31	31	24	24	24	24
		ng/g w.w.	pg/g					mg/Kg					
LIVER	Mean	0.7	38	0.09	0.15	0.09	<0.02	0.17	2.05	0.17	0.04	33	5.4
	min	*0.14*	*<10*	*0.014*	*0.02*	*<0.01*		*<0.10*	*1.1*	*<0.10*	*<0.02*	*19*	*2.8*
	max	*2.15*	*145*	*1.16*	*0.47*	*0.36*		*0.30*	*4.2*	*0.174*	*0.06*	*99*	*17*
	n	21	16	31	31	31	31	31	31	24	24	24	24
LUNG	Mean	0.16	107	0.46	0.04	0.02	<0.02	0.20	0.99	0.12	0.01	17	1.4
	min	*0.14*	*<10*	*<0.010*	*<0.005*	*<0.01*		*<0.10*	*<0.50*	*<0.10*	*<0.02*	*10*	*0.8*
	max	*0.32*	*181*	*0.57*	*0.082*	*0.05*		*0.36*	*1.4*	*0.13*	*0.021*	*32*	*4.2*
	n	18	10	28	28	28	28	28	28	21	21	21	21

Conclusion

The results reported are surely very preliminary but interesting. Scarce are the data available in the literature for PBDEs and PCBs in wild fauna. The concentrations of Cd and Pb in muscle found in the present study may arise food safety issues. The problem of Pb contamination due to bullets fragmentation received, recently, attention in the literature and the possible impact on human health should not be underestimated.

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P2

Extraction and characterization of bioactive compounds from agro-industrial by-products for spray packaging applications to extend the shelf-life of highly perishable foods

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Summary: *The aim of this study is the extraction and characterization of bioactive compounds from agro-alimentary by-products in order to realize a new biopolymeric solution characterized by biodegradability and compostability, to be used as an innovative, active and edible packaging in a form of film or spray.*

Keywords: *circular economy, active edible packaging, by-products*

Introduction

The possible reintegration of agro-industrial by-products within the production chain is in line with the Circular Economy model [1], which at industrial level aims to achieve a significant goal, defined as "Zero Waste", consisting in the complete elimination of waste and total exploitation of productive resources. Scientific research has the objective of promoting the use of products that are currently considered unusable, through methods that allow the recovery of bioactive molecules, to reinsert them into the production cycle, giving rise to new products that regain economic value. "Closing the loop" has become the key word for building a resource-efficient society. The main goal of this innovative trend is to move towards environmental sustainability.
The common technological processing of raw materials, such as artichokes, asparagus, cardoons, onions, generates abundant amount of waste. still containing bioactive substances that can represent useful molecules for multiple purposes. Recently, scientific research is working to study its composition, through a qualitative and quantitative analytical characterization, in order to develop extraction protocols that allow to recover these bioactive molecules characterized by antioxidant and antibacterial properties [2,3]. In this work, we aimed at promoting their use to obtain new products that could provide an additional economic value in packaging field.

Experimental

Extracts of agro- industrial by-products derived from artichoke, thistle, onion and asparagus were obtained using different solvent such as water or ethanol, by microvawe assisted extraction. The bioactive compounds were characterized by HPLC-DAD-ESI-MS and HPAEC-PAD. Besides, total polyphenol content was evaluated by Folin-Ciocalteau assay, and the ability to inhibit oxidation was measured by Oxitest.

The obtained extracts were used as ingredient to produce a polisaccharidic-based solution that can be used for production of active and edible thin films or spray [4].

Results

Chromatographic techniques allowed the identification and quantitation of many antioxidant compounds in the extracts, as quercitin, quercetrin, caffeic acid, chlorogenic acid, gallic acid. Antioxidant properties, higlhy correlated with total phenolic content were assessed using a vegetable oil as model.

Furthermore, the presence of oligosaccarides with prebiotic properties was confirmed.

The solution, combined with opportune selected ingredients, once dried, forms a thin and transparent coating having antioxidant and antibacterial properties, and acts as active packaging that can protect food products and enhances their shelf-life. For example an increment of 120% in the shelf-life of fresh berries, and of 100% of fresh meat (hamburger) was achieved.

The films also show good mechanical properties and high resistance.

Besides, the same solution could be proposed as a biodegradable, edible and active compostable spray-packaging, or can be used for the oil impermeabilisation of cellulose-based food contact materials.

Conclusion

The active solution, used as a spray, or converted in films, represents an innovative material characterized by being biodegradable, and suitable to realise active and edible food packaging containing natural antioxidant agents and with prebiotic features. It has been shown to be successfully employed to prolong shlef-life of vegetables and meat.

Many applications also in cosmetic and nutraceutical fields can be proposed.

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P3

Characterization of new putative polyphenols in post-harvest withered grapes (*V. vinifera L.*) by high-resolution mass spectrometry

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Keywords: *polyphenols, grape, withering*

Grape withering is used for production of a number of high-quality reinforced and sweet Italian wines, e.g., Amarone di Valpolicella, Recioto, Raboso Passito. Parallel to water loss and of concentration of sugars in the berry, dehydration induces a decrease of some polyphenols, such as anthocyanins, flavanols and procyanidins, glycoside flavonols and quercetin glucuronide and increase of some others, such as *trans*-resveratrol, taxifolin, quercetin, some methoxylated flavanones, acylated anthocyanins [1-3]. In general, these compounds are characterized by biological properties and influence the organoleptic and nutraceutical properties of grapes and wines [4]. In the present study, a tentative of identification of new polyphenols isobaric to some previously identified in Raboso Piave and Corvina withered grapes by UHPLC/QTOF MS was performed. Ten putative polyphenols not found in grape before or just proposed in supplemental material of previous studies on grape withering, but not confirmed [5,6], were characterized by HR-MS/MS. They belong to the chemical classes of flavonols and flavanonols, flavanones and flavones, and peonidin-*O*-pentoside. The compounds identified enlarge the panorama of grape nutraceuticals and can be useful in the study of polyphenolic biosynthetic pathways.

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P4

Determination of co-eluted isomers in wine samples by application of MS/MS deconvolution analysis

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Summary: *Two organic acids isomers, 3-isopropylmalic acid (3-IPMA) and 2-isopropylmalic acid (2-IPMA), were distinguished and quantified in wine extract samples without any chromatographic separation. For this purpose the sample MS/MS data were processed with a linear equations deconvolution analysis (LEDA) mathematical tool.*

Keywords: *organic acids, tandem mass spectrometry, isomers resolution, LEDA, ERMS*

Introduction

Wine is defined as an alcoholic beverage, which is produced by fermentation of fresh grapes or must and the organic acids of beverages are important in several respects. All organic acids have this quality to some degree, but some have their own characteristic flavour, taste or aroma. For example, citric acid has a fresh acid flavour different from that of malic acid, while succinic acid has an unusual salty, bitter taste in addition to its sourness [1]. Wine is also rich in phenolic compounds that are important components with health promoting properties and they also affect organoleptic characteristics, such as colour, astringency and aroma [2]. Recently, Ginjom et al. identify and quantify the individual phenolic compounds present in wine at different wine-making stages from crushing through to bottled and aged wine, produced in different Queensland (Australia) wineries [3]. Interestingly, they were not able to identify a compound (named U1) with a low molecular mass (m/z at 175) and relatively large eluting peak. In a recent paper, our research group have identified in wines two isomeric compounds, corresponding to U1, with molecular weight of 176 Da by using two LC-MS systems, i.e. ion trap (LC-IT) and LC-Q-Orbitrap. The two isomers were organic acids, i.e. 3-isopropylmalic acid (3-IPMA) never identify in wines, and 2-isopropylmalic acid (2-IPMA), never quantify in wines [4]. In this study, the quantification of these two compounds was performed by using LC-IT in ten among red and white wine samples and the average concentrations were determined at 1.78 mg L^{-1} (range 0.56-4.13 mg L^{-1}) and 23.0 mg L^{-1} (range 6.7-41.6 mg L^{-1}) of 3-IPMA and 2-IPMA, respectively. The LC-IT method used MS/MS analyses at 0.9 V excitation amplitude by extracting the product ions m/z 73 and m/z 115 for 3-IPMA and 2-IPMA respectively. Thus, the two compounds were separated by using extract ion chromatogram

(EIC) of the two characteristics m/z ions. However, the different fragmentation yield of their product ions affects on the quantitation sensitivity. Therefore, to ensure the similar sensitivity between two isomers, their chromatographic separation is needed with increasing the analysis time. In order to ensure the requested specificity of the method, a different approach, based on a series of energy resolved MS/MS experiments, was carried out. By this approach a clear differentiation among all the isomers was obtained but, to emphasize such differences, it was necessary to develop a mathematical algorithm that distinguishes the MS/MS spectra of the isomers. This algorithm (LEDA) consists in the application of matrix of linear regression equations to different experimental data. In our case, the experimental data used were the abundance ratios of product vs. precursor ions selected during the MS/MS method set-up [5-7]. In this way, it was possible to resolve the MS/MS spectra assigning the correct signal to the isomer also for chromatographically unresolved peaks.

Experimental

The LC-MS/MS analysis was carried out using a Varian 1200L triple quadrupole system (Palo Alto, CA, USA) equipped by two Prostar 210 pumps, a Prostar 410 autosampler and an Elettrospray Source (ESI) operating in positive ions. The product ion scan spectra were acquired in the m/z range from 50 to 650, scan time 600 ms; argon was used as collisional gas and the collision energy (CE) was increased stepwise in the range 5 to 40 V. The obtained energy resolved tandem mass spectrometry (ERMS) spectra were employed to study the fragmentation of molecular species of studied isomers and build their breakdown curves. The chromatographic parameters employed to analyse the samples were tuned to minimize the run time. The column used was a Pursuit XRs C18 30 mm length, 2 mm internal diameter and 3 □m particle size, at constant flow of 0.25 mL min^{-1}, employing a binary mobile phases elution gradient by 10 min. total run time. The solvents used were 10 mM formic acid in water solution (solvent A) and 10 mM formic acid in acetonitrile (solvent B). The LEDA post-processing mathematical tool was used to guarantee the identification of the isomer present in analyzed samples without their chromatographic separation. The algorithm is based on the consideration that each MS/MS spectrum might be represented as the sum of contribution of each isomer present in the unresolved chromatographic peak. In order to obtain reliable data, the relative abundances of the different product ions were calculated with respect to the reference ion abundance, so that possible misleading results due to compound-dependent different product ion yields are avoided. For this purpose, the available signal of the precursor ion was acquired as reference ion (Ri) which allowed us to obtain the characteristic ratios among the selected product ions for isomer speciation. Therefore, knowing the characteristic abundance ratios of pure isomer, a deconvolution of these spectra is possible based on a series of linear regression equations as follows:

$$\left({}^{P_i}/_{R_i}\right)_m = \sum_{x=1}^{n} \left({}^{P_i}/_{R_i}\right)_x * [\%]_x \qquad (1)$$

Where:

$(Pi/Ri)_m$: is the abundance ratio between the product (Pi) vs reference ions (Ri) measured (m) in the sample;

$(Pi/Ri)_x$: are the characteristic abundance ratios between the product ion vs reference ion of pure isomers;
$[\%]_x$: is the concentration (%) of isomers in the sample.
Precision and accuracy of LEDA algorithm were evaluated by the LC-MS/MS analysis of standard mixtures prepared with different composition of the isomer pair. Then, the LC-MS/MS method with LEDA post-processing tool was apply to the determination of 2-IPMA and 3-IPMA in real samples of wine extracts prepared as reported by Ricciutelli et al [4].

Results
The quantitative results obtained applying the LEDA approach on the abundance ratios of data collected from the MS/MS analysis of the standard mixtures are: accuracy 97.3 % and 96.3 % with a precision, expressed as RSD %, of 1,9 and 1,8 for 2-IPMA and 3-IPMA respectively. Also the application of LEDA on the wine samples shows a good correlation between the quantitative data obtained with the classical chromatography separation and linear equations deconvolution analysis of MS/MS spectra.

Conclusions
The proposed LC-MS/MS method coupled with mathematical algorithm (LEDA), applied to deconvolute the MS/MS spectra from unresolved chromatographic peak, was effective, allowing the determination of the isomers presents in wine samples. The obtained results confirm the ability of the LEDA approach to deconvolute a mixture of isomers from chromatographically unresolved peaks, allowing short analytic runs and high throughput in processed samples. It is to emphasize that, in the last decade, many investigation were carried out to develop ancillary MS technique able to give a different analytical dimension to the researcher in the field (e.g. ion mobility methods). In this paper we have shown that the energetic dimension of MS/MS experiments can be fruitfully employed to solve isomers characterization and quantification problems.

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P5

Alkaloid profiling of food tannins using high resolution mass spectrometry

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Summary: *Tannins are natural extracts of plant products. UHPLC coupled with HRMS (Orbitrap) was used to characterise, for the first time, the alkaloid profile of this products highlighting the presence of more than 5000 alkaloids belonging to different chemical classes.*

Keywords: *Tannins, Alkaloids, LC-HRMS*

Introduction

Commercial tannins are polyphenolic compounds with a molecular weight ranging from 500 to 20,000 Da. They belong to several botanical sources, each one having peculiar and different chemical and technological characteristics. Widely used in food and winemaking industries as processing aids or flavouring substances [1], they can be classified in 2 main groups: condensed tannins (proanthocyanidins), mainly extracted from grapes, quebracho and tea, and hydrolysable tannins (gallo- and ellagitannins), whose main botanical origins are oak, chestnut, tara and different galls.

Although tannins are identified as natural extracts of plant products and many of them are documented as containing natural alkaloids (alks), no studies in literature investigated their presence in these extracts. Alks, a very heterogeneous class of compounds produced by plants as secondary metabolites, have long been studied due to their specific toxicological characteristics, as some of them are suspected of having very dangerous properties. Many of them exert a marked physiological action, some are considered to be responsible for the beneficial effects of traditional medicines [2], and others may have the harmful effects of poisons [3]. They are chemically classified into three principal classes, depending on precursors and final molecular structures: atypical and typical (amino acid-derived), and pseudo alks (terpenoid and purine-derived). In this study, a high resolution mass spectrometry (HRMS) method was used to establish the alkaloid profile of a large selection of commercial tannins.

Experimental

Thirty-two single-variety powder tannins were collected on the Italian market, grouped by 4 declared botanical origins (oak, N=14; grape, 8; chestnut, 6; tea, 5) and analysed by UHPLC method that combines a previous online solid-phase purification with a high resolution mass spectrometry (HRMS).

The mass spectrometer operated in positive ion mode and mass spectra were acquired, with full MS-data dependent MS/MS analysis (full MS–dd MS/MS) at a mass resolving power of 140,000.

Results
41 alks were quantified in reference to analytical standards, while the presence of more than 100 non-targeted alks was confirmed on the basis of accurate mass, retention time and fragmentation profile.

Conclusions
The HRMS, enabled the screening of a broad selection of tannin samples. As expected, the study confirmed the presence of a large number of alkaloid compounds in this matrix.

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P6

Characterization of Iris pallida Lam. rhizomes cultivated in Chianti area by HPLC-DAD-MS, HS-SPME-GC-VUV and HS-SPME-GC×GC-TOF analysis

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Summary: *The root of Iris is a precious material for the production of foods and cosmetics. In this work Iris pallida rhizomes samples cultivated in Chianti area were characterized for phenolic and volatile fractions with innovative techniques.*

Keywords: *volatile compounds, isoflavones, liqueur drink, food and cosmetic application*

Introduction

The root of *Iris* is a precious material for the production of perfumes, beauty creams, cosmetics, spirits, herbal remedies, wines and more. Rhizomes find their main application in the alcohol industry, as a base for some of the most important liquors on the market. The characteristic violet-like smelling compounds of the essential oil of rhizomes of *Iris florentina* L. and *Iris pallida* Lam. have been known to be three isomeric irones. It is well established that these ketones do not occur in freshly harvested plants but develop over years by a slow process. The specific hydrodistillation of dried and crushed iris rhizomes leads to an essential oil called iris butter. As this essential oil almost exclusively contains irones and fatty acids with no smell, its commercial value is directly determined by its irone concentration. Similarly, the value of iris rhizomes is closely related to their irone content [1]. Quantification of irones in rhizome is obtained by time and money consuming methods as the official method in iris butter (ISO 18054:2004) and by a solid–liquid extraction followed by CG. An alternative method using HS-SPME-GC on crushed rhizome has been also proposed [1]. In this work innovative methods as HS-SPME-GC-VUV and HS-SPME-GC×GC-TOF are developed. GC-VUV is a highly specific analytical technique for compound identification and previous studies have utilized GC–VUV for a variety of applications, including analysis of pesticides, permanent gases, hydrocarbons in fuels, fatty acids and terpenes [2].

Comprehensive GC-MS (GCxGC-MS) is a powerful technique that provides two-dimensional chromatography data acquisition capability and it's suited for a variety of applications, including profiling of complex matrices such as a natural products, and grouping analysis based on 2D- chromatograph patterns. Rhizomes were even analysed for their polyphenolic content by HPLC-DAD-MS. Characteristic constituents in the rhizome are isoflavones, in particular tectorigenin, which reportedly show anti-inflammatory and anti-oxidative properties [3,4], phenolic acids and xanthones like mangiferin and neomangiferin. Mangiferin has documented antioxidant and anti-inflammatory

effects. Recent studies indicate that it modulates multiple biological processes involved in metabolism of carbohydrates and lipids [5].

Experimental

Iris pallida rhizomes samples were purchased by Frantoio Pruneti srl (San Polo in Chianti, Florence). Samples were deep-freezed with liquid nitrogen and then chopped with mortar and pestle, until a homogenous powder was obtained. Volatile organic compounds (VOCs) were analyzed by both HS-SPME-GC-VUV and HS-SPME-GCxGC-MS-TOF analyses. For both 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-GC-VUV: a GC SRA-Agilent 7890B connected to a VUV 101detector (VUV Analytics) equipped with a 60 Innowax column was used. Standard solution of Methyl-α-ionone (α-IRONE) ≥90% from Sigma Aldrich in Ethanol was prepared. 100 mg of rhizoma powder was dissolved in 5 ml of H2O and ethanol 2%. Sample aliquots were spiked with various amounts of standard solutions ranging from 0.2 to 0.8 μg mL−1 in order to determine concentrations of the target compound using the standard addition method. By this method, the initial concentrations were calculated from the interception of the linear regression with the y-axis (0.99 R^2).

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). A standard solution of iso-Methyl-α-ionone as internal standard was used for the quantification.

HPLC-DAD-TOF: a HP1100L 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 (N2); 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.

Results and conclusions

HPLC-DAD-TOF analysis, allowed identifying and quantifying isoflavones, the most represented compounds (16.51-21.75mg/g), in particular tectorigenin (9.62-10.56 mg/g), phenolic acids (1.02-1.78 mg/g) and xanthonoids (0.61-1.17 mg/g).

Figure 1 shows the contour plot obtained by GCxGC-MS/TOF analysis of peeled orris root (white). Comprehensive two-dimensional GC fingerprint analysis pointed out a different profile between withe and dark (not peeled) root. In particular peeled root showed cis-α-Irone as the main compound whereas the unpeeled root the cis-γ-Irone. Irone content was estimated at 16-132 mg/kg in rhizomes by internal calibration.

A new GC detector based on vacuum ultraviolet spectroscopy (VUV), which

measures full scan absorption in the range of 125–240 nm, was developed and applied to the analysis of irones. Irones isomer pairs, such as α-/γ- irone or cis-/trans-α-irone, are essentially indistinguishable in MS due to the presence of only a very few differences in their electron ionization mass spectra, according to reference mass spectra from the NIST database. The VUV software detect automatically the peak in the sample and it compared them to an internal spectral UV library. The qualitative difference is readily observed in their VUV spectra, in particular for α-/γ- irone, but the spectra for cis-/trans-α-irone are also distinct at lower wavelength using the first and second derivative of the transmission spectra with respect to wavelength. VUV spectroscopy follows the simple linear relationship between absorbance and concentration described by the Beer-Lambert law and confirmed values obtained with GCxGC. The VUV detector showed the capability to discriminate among different isomers and to quantified irones in this high value matrix. GC-VUV complements MS by overcoming its limitations and providing a secondary method of confirmation. It also offers a single instrument alternative to the use of multiple detectors for qualitative and quantitative analysis.
In conclusion, high value product, aimed to food and cosmetic uses, was characterized for its phenolic and volatile fractions with innovative techniques.

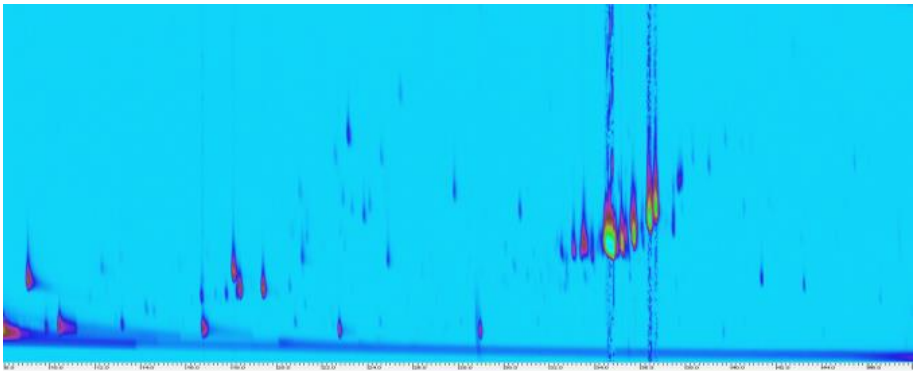

Fig. 1. *Contour plot from GCxGC/TOF analysis of Iris pallida LAM. rhizome.*

Acknowledgement
Authors acknowledge SRA Instruments S.p.A. This work was supported by PIF 2017- sottomisura 16.2 - PSR Toscana 2014-2020 - L'IRIS DI FIRENZE and GAL-START 2018 - sottomisura 16.2- Regione Toscana 2014-2020- IRIS DEL CHIANTI

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P7

Anchovy waste-derived fish oil loaded on periodic mesoporous silica for nutraceutical applications

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Summary: *We have synthesized mesoporous MCM-41 silica as carrier for the fish oil extracted from anchovy waste with the aim to stabilize the omega-3 lipids in a solid formulation suitable for the development of new food supplements. GC-MS analysis was used for fish oil characterization and to assess the effect of MCM-41 on the main oil components stability.*

Keywords: *omega-3, MCM-41, GC-MS*

Introduction

Long-chain polyunsaturated fatty acids (PUFAs) belonging to the omega-3 lipids are the nutraceutical ingredients of dietary supplements (52.9% of global value share in 2017), infant formula (20.2% of the value share) and pharmaceuticals (15.4%) [1]. Generally derived from fish oil, these lipids exert numerous concomitant health benefits since the ability of tissue's self-defense against oxidative stress depends upon its ω-6:ω-3 lipid composition [2].

Shifting the production of fish oil from fish to fish processing waste is an urgent global need to end the contribute of omega-3 ingredient production to overfishing [3]. One promising alternative is the oil is extracted from discards of anchovy fillets using *d*-limonene as green biosolvent in a solid-liquid extraction [4]. In general, long-chain PUFAs are chemically unstable and susceptible to quick oxidative degradation which results in the formation of toxic peroxides and their by-products. For this reason, refined fish oil is encapsulated in gelatin capsules in the presence of antioxidants [5], with the best manufacturers undergoing third-party certification ensuring minimal presence of peroxides and other toxic compounds.

Aiming to protect omega-3 lipids from chemical and photochemical degradation numerous studies have been devoted to the microencapsulation of fish oil mostly using spray drying, coacervation, ultrasonication and membrane emulsification techniques [6]. In 2016, the need for new microencapsulation methods to produce fish oil microcapsules with >60% fish oil content (on dry basis) able to withstand food processing conditions was clearly identified [6].

We have therefore used periodic mesoporous silica nanoparticles MCM-41 as a carrier for the extracted fish oil obtained from anchovy fillet lefotvers [4].

MCM-41 exhibits a high specific surface area (>900 m2/g), a high pore volume (>0,9 cm3/g), a high loading capacity, biocompatibility and stability against biological and thermal attack which allow its utilization as support for the adsorption and delivery of food ingredients and nutraceuticals [7]. However, MCM-41 silica is poorly stable under stimulated *in vitro* digestion conditions [8],

requiring functionalization of the silica cages with amino groups to increases the chemical stability of the material [9].

Experimental

Extraction fish oil

The fish oil was extracted from discards of anchovy fillets using *d*-limonene as green biosolvent in a solid-liquid extraction. Limonene was recovered and reused in subsequent extractions [4].

Preparation and loading of MCM-41

MCM-41 was synthesized by sol-gel process using TEOS (tetraethyl orthosilicate) in the presence of CTAB (hexadecyltrimethylammoniun bromide). The surfactant was removed via calcination at 550 ºC. Loading of the material with anchovy fish oil was carried out by direct adsorption of the oil by stirring the material (100mg) in the presence of the oil (120 µl) in a glass flask for 24 h.

Functionalization of MCM-41and loading with fish oil

Functionalization of MCM-41(300 mg) was achieved by post-grafting under reflux temperature using (3-aminopropyl)trimethoxysilane (AP-TMS, 0,42 mmol) as modifying agent [9]. Loading of the material functionalized with amino groups with anchovy fish oil was carried out by direct adsorption of the oil by stirring the material (100 mg) in the presence of the oil in a glass flask for 24 h.

Characterization

The fatty acid composition of anchovy fish oil was assessed through the standard method involving transesterification of the oil triglycerides and CG-MS analysis of the respective fatty acid methyl esters using a ThermoScientific Trace 1310/ISQ LT single quadrupole GC/MS spectrometer [4]. The MCM-41 blank and functionalized materials were characterized by the use of FT-IR (Bruker, ALPHA model), and thermogravimetric analysis (Mettler Toledo TGA/DSC1). The specific surface area of the material was calculated via the BET method using a Micromeritics ASAP 2020 Plus 1.03 porosimeter.

Results

FT-IR spectra of MCM-41 shows a signals at 3448 cm-1 and at 1637 cm-1 due to the O-H bond of the silanol group and peaks at 1383,1088, 806 and 460 cm-1 corresponding to the Si-O-Si stretching and bending vibrations. FT-IR spectra of MCM-41 functionalized shows peacks between 2990 and 2890 cm-1 corresponding to the CH2 stretching of the chain of AP-TMS and the C-NH2 stretching. While the spectrum of MCM-41 loaded with fish oil shows peaks between 3082 and 2854 cm-1 which correspond to the stretching =C-H of the double bonds. Fig. 1 shows the TGA curves of MCM-41 with a 50% load of anchovy fish oil. Finally, the surface area, the pore volume and the pore size of the material MCM-41 were determined by the BET method and are 825,2490 m2/g, 0,599511 cm3/g and 2,9058 nm respectively.

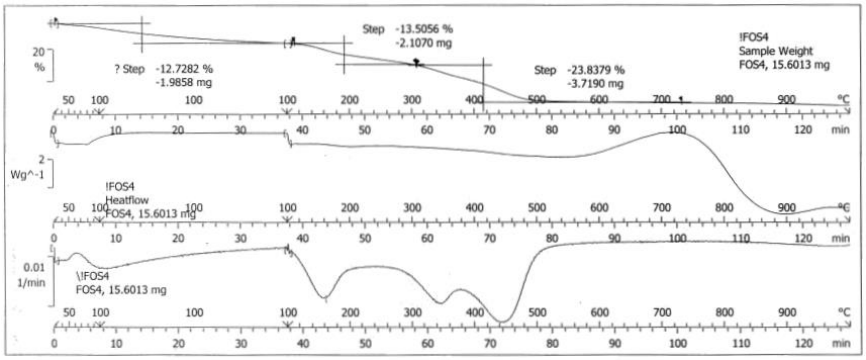

Fig. 1. *TGA curves of MCM-41 with a 50% load of anchovy fish oil.*

Conclusions

A remarkable oil-loading capacity of 50% for the MCM-41 material was demonstrated. No change in colour, consistency and flow properties was qualitatively observed for the biocompatible carrier material loaded with fish oil, opening the route to forthcoming practical applications. Fish oil stability tests in the new functional are currently undergoing GC-MS analysis.

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P8

Characterisation of the methanolic extracts from the ancient apple variety ‘‘Mela Rosa dei Monti Sibillini’’

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Summary: *Our study was carried out to characterize 20 polyphenols and triterpenes in the dried and lyophilized methanolic extracts from the italian apple ‘‘Mela Rosa dei Monti Sibillini’’ using HPLC-DAD-MS in other to valorise and promote this overlooked old traditional apple in the national territory.*

Keywords: *apple-polyphenols-tripernes*

Introduction

The aim of our study was to characterize the profiles of some polyphenols and triterpenes in the ‘‘Mela Rosa dei Monti Sibillini’’, which is an ancient apple variety cultivated in the area of the Sibillini mountains, Marche region, Central Italy since the Roman age.

Fig.1. *Mela Rosa dei Monti Sibillini*

Experimental

Nine apple peel and pulp samples of the ‘‘Mela Rosa dei Monti Sibillini’’ were lyophilized after homogenization with liquid nitrogen or dried at 45°C for at least 18 h. After pulverisation, the dehydrated material (dried and lyophilized) was submitted to extraction using methanol. Extracts were analysed qualitative and quantitative using HPLC-DAD-MS for 20 secondary metabolites (flavan-3-ols, flavonols, dihydrochalcones, hydroxycinnamic acids and triterpene acids) [1] [2]. For comparative purposes, another traditional apple cultivar from the

southern Italy (Annurca) and 2 commercial apple varieties (Golden Delicious and Granny Smith) were analysed as well.

Results

The results revealed that the peel samples were richer in polyphenols and triterpenes than the pulp. The lyophilised samples contained higher amounts of the phenolic compounds epicatechin (2988.5 mg/kg), procyanidin B2 (2186.4 mg/kg) and phloridzin (1399.7 mg/kg) than dried samples (1604.6 mg/kg, 1150.7 mg/kg, 639.7 mg/kg respectively). Whereas the quantity of triterpenes (ursolic and oleanolic acids) was more or less the same in both dehydrated materials. Compared with Annurca and commercial samples, the Mela Rosa dei Monti Sibillini contained higher levels of the afore-mentioned polyphenols constituents.

Conclusions

These results show that the lyophilisation of the raw apple material conserves better the polyphenolic profile of the fruit than the drying method. They support the possible use of this ancient apple variety as a source of nutraceuticals and functional foods due to its high amount in antioxidant compounds. At the same time, they represent an important stage for the valorisation and promotion of this old traditional apple in Italy.

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P9

Influence of boiling cooking on phytochemical profile of orange cauliflower (*Brassica Oleracea* L. var. *botrytis*)

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Summary: *In the present study, the evolution of orange cauliflower phytochemical profile (phenolic acids, flavonoids and glucosinolates) during boiling treatments was monitored. For this purpose was performed an ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS).*

Keywords: *polyphenols, glucosinolates, UHPLC-HRMS*

Introduction

In recent years, diets rich in cruciferous foods have gained popularity due to their content of different bioactive compounds [1]. In this line, a particular attention has been focused in a less investigated Brassicaceae species such as orange cauliflower *(Brassica oleracea* L. var. *botrytis*) which biodiversity is not only visible in its outer appearance but also in term of phytochemical profiles [2]. However, cauliflower phytochemical composition has been proved to be strongly affected by common domestic cooking practices due to its hydrophilic and heat-sensitive nature [3].

Experimental

Fresh cauliflower was boiled at 10 and 25 minutes. After that, solvent extraction (acetone:water:formic acid 70:29.9:0.1 v/v/v) was performed starting from freeze-dried sample. Thus, raw and cooked sample were analyzed by a UHPLC-ESI-HRMS (Q-Exactive Orbitrap) method. The obtained HRMS data was then processed by means of customized target accurate mass databases of phenolic acids, flavonoids, glucosinolates and derivatives using TraceFinderTM 3.3 EFS software. Successively, phytochemical profiles were exploited by principal component analysis (PCA) and partial least squares regression discriminant analysis (PLS-DA) by using PLS_Toolbox 7.8.2 (Eigenvector Research).

Results

PCA (Fig. 1) and PLS-DA (Fig. 2) analysis using 70 phytochemical compounds as descriptors, were performed. As results, the samples were strictly clustered according to the time treatment (0, 10, 25 min).

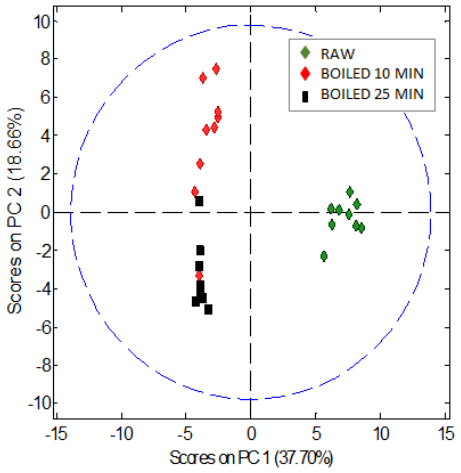

Fig. 1. *PCA score plot of PC1 vs. PC2*

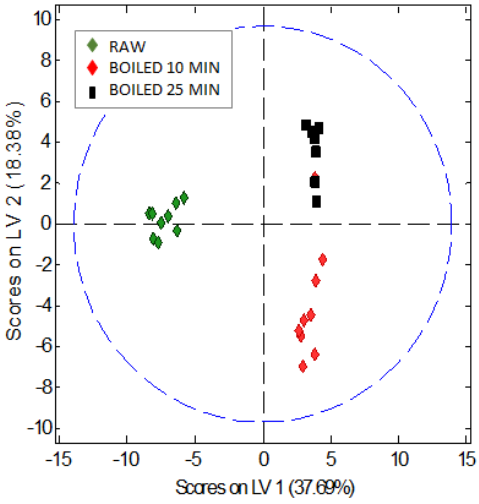

Fig. 2. *PLS-DA score plot of LV1 vs. LV2*

Conclusions

The employed UHPLC-HRMS method clearly distinguished among raw and boiled samples, and also a trend according to boiling time was observed.

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P10

Honey discrimination by Volatile Organic Compounds analysis: comparison between GC-IMS and GC-E-Nose

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Summary: *The intrinsic characteristics of 54 honey samples were assessed by qualitative analysis of their volatile fraction through GC-E-NOSE and GC-IMS. Both systems detected differences among samples, due to botanical origin, breeding techniques and production processes. The application of IMS allowed to identify the discriminating molecules among honey samples.*

Keywords: *Honey, Volatile Compounds, Gas Chromatography*

Introduction

Food safety and quality are becoming more and more considered by food companies and consumers that are daily exposed to food fraud risk.
To face food fraud, the development of new analytical techniques that can identify and recognize the authenticity of food products has become essential. One of the most innovative analytical approaches is the assessment of the volatile fraction combined with chemometric techniques.
The composition of volatile organic compounds (VOCs) from Acacia and Millefiori honey samples were analyzed, to identify molecules relevant for their classification according to the botanical origin. Honey is one of the most exposed food to fraud, due to its economic value, its worldwide production and the current problems associated with the control and maintenance of bees' farms.
The honeys' volatile fraction was analyzed by using two diverse gas chromatography (GC) instruments (GC-E-Nose and GC-IMS), and the data obtained were compared after chemometric processing.

Experimental

Fifty four honey samples (26 Acacia and 28 Millefiori) were selected, prepared and treated under standardized operating conditions. About 1 g of sample was placed in a 20-mL vial, without any further treatment before chromatographic analysis. The two GC instruments used for the study were:

- GC-E-Nose: Ultrafast Heracles GC instrument with 2 GC capillary columns of different polarity and 2 flame ionization detectors (FID), used in untargeted mode.
- GC-IMS: GC instrument with a GC column coupled to an ion mobility spectrometer (IMS), used in both untargeted and target mode.

Finally, the raw data of the samples' volatile composition were subjected to Principal Component Analysis (PCA) statistical analysis.

Results

The VOCs raw data obtained with the two different GC instruments, were separately processed by using PCA statistical analysis.

PCA analysis was able to similarly discriminate samples into the two main honey classes (Millefiori and Acacia) regardless of the type of GC instrument used, as shown in Fig. 1 and Fig. 2. Furthermore, both PCA evidence that a sample of Acacia honey (grouped with a light blue circle) was not correctly positioned with respect to the honey classification. The latter sample is an organic honey, which might contain characteristic volatile molecules that are related to unconventional breeding techniques or a different production process. When the PCA was applied to the data obtained by the untargeted approach, the acacia honey samples were divided into conventional and organic ones (Fig. 3). The non-compliant sample was studied more in depth by GC-IMS, with a target approach, where acetic acid was identified as a "marker" molecule of this type of sample. In fact, according to the EU Reg. 2018/8481, acetic acid can be used against Varroasis in the production of organic honey.

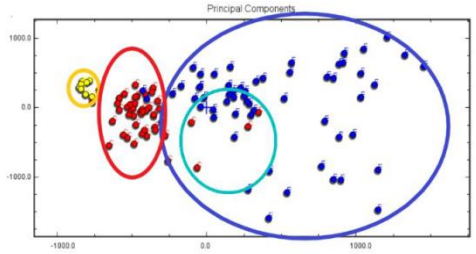

Fig. 1. *PCA Statistical Analysis of data obtained from GC-IMS: Millefiori (blue), Acacia (red), Blank (yellow)*

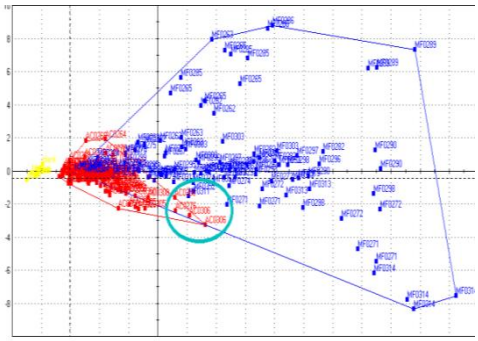

Fig. 2. *PCA Statistical Analysis of data obtained from GC-E-Nose: Millefiori (blue), Acacia (red), Blank (yellow)*

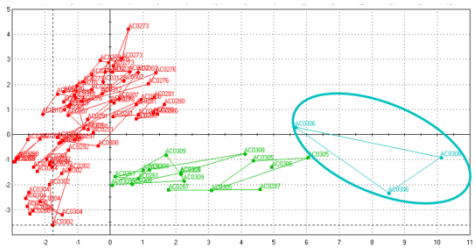

Fig. 3. *PCA Statistical Analysis of Acacia Samples: Conventional acacia (red), Organic acacia (green), Non-compliant Acacia with this classification (blue)*

Conclusions

The results obtained show that the two GC instruments are able to generate very similar outputs, when used under the same operating, analytical, and statistical conditions. The untargeted analytical approach associated with chemometric data processing allows the formation of different sample classes

according to qualitative differences detected in the honey volatile fraction. Clustering of untargeted data proved to be a useful tool for preliminary identification of differences among samples, but it does not allow the identification of discriminating molecules. To recognize the chemical nature of discriminating molecules, it should be used a target approach, such as the one carried out with GC-IMS in the present work. The identification of discriminating molecules allowed to justify the obtained sample classification, being thus useful to distinguish with greater certainty a conventional honey from an organic one.

References

1. Reg EU of 30 May 2018, No 848 relating to organic production and labelling of organic products.

P11

Analytical method for determination of glyphosate and other polar pesticides in vegetables and honey using Ion Chromatography coupled with High Resolution Mass Spectrometry (IC-HRMS)

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Summary: *The aim of this work was the development and validation of an analytical method suitable to quantify glyphosate and other polar pesticides using an extraction and purification procedure able to isolate the molecules of interest and a selective, repeatable and robustness instrumental method such as ion chromatography coupled with high resolution mass spectrometry.*

Keywords: *Glyphosate, Ion Chromatography, High Resolution Mass Spectrometry*

Introduction

Glyphosate and other similar pesticides are used in agriculture to combat weeds that compete with crops. They are generally applied before sowing and as a pre-harvest drying treatment to optimize the ripening process[1]. The use of glyphosate in agriculture is much discussed by the competent bodies in the field of food safety. Some European press have questioned the use of this substance because it could be dangerous for human health, but recently European Food Safety Authority (EFSA) gave a positive opinion on the use of glyphosate in agricultural practices until December 15, 2022. A new evaluation on the toxicity of this molecule and the potential risks on human health will be conducted by a commission of experts before the fixed deadline[1].

Ion Chromatography, recently used for the analysis of these polar compounds in water samples, was chosen as alternative technique to those indicates by European Reference Laboratories for Residue of Pesticides in QuPPe method[2], thanks to the recent and interesting developments in food analysis[3], and the possibility to couple with high resolution mass spectrometry (Q-Exactive).

The aim of this work was the development and validation of an analytical method suitable to quantify different polar pesticides and relative metabolites such as Glyphosate, N-Acetyl Glyphosate, AminoMethylPhosphonic Acid (AMPA), N-acetyl AMPA, Glufosinate, 3-MethylPhosphonicPropionic Acid (MPPA), N-Acetyl Glufosinate (NAG), Ethephon, Ethephon hydroxy, Fosetyl Alluminium and Phosphonic acid using Ion Chromatography coupled with High Resolution Mass Spectrometry (IC-HRMS) in vegetables and honey according to the criteria established by SANTE/11813/2017[4] taking into account maximum residue levels (MRL) indicated by Regulation (EC) No 396/2005[5].

A second purpose of this work was to have a fast, simple and repeatable extraction method that allowed reducing analytical errors and processing times, suitable to routine analysis in an Official Laboratory.

Experimental

Four different commodity groups, from SANTE/11813/2017 Annex A[4], were taken into account during method development and validation. For each group were selected reference matrices on which to perform extraction tests: grapes for 1-high water content and 2-high acid content and high water content; honey for 3-high sugar and low water content; wheat for 5- high starch and/or protein content and low water and fat content.

Before the analysis, all matrices were accurately homogenized; 2.5 g food samples were weighed into 50 ml polypropylene tubes; 10 mL of 1% acidified water with formic acid were added, and then were shaken manually for 1 minute. Accurate amounts of labelled internal standards were added to each sample to take under control analytical procedure and extraction efficiency. Internal standards were also used for quantitative analysis in order to evaluate matrix effect.

10 mL of water:acetonitrile 9:1 v/v were added to the samples; then were shaken mechanically for 20 min and centrifuged at 4000 rpm for 25 min at 4 °C. After centrifugation about 2.5 ml of each sample were filtered on 335 mg HLB Prime Cartridge (Waters); a portion of filtered extract was diluted and transferred in vial. Instrumental analysis was carried out with IC 5000+ (Dionex) ion chromatograph coupled with Q-Exactive Focus (Thermo Fisher) mass spectrometer. Chromatographic separation was performed using Dionex IonPac™ AS19 column (250 mm, 2 mm, 4 µm) equipped with a pre-column Dionex IonPac™ AG19 (50 mm, 2 mm, 4 µm).

Results

All parameters required by SANTE/11813/2017[4] were taken into account in validation study. Specificity was studied through the response obtained from reagents blank and blank control samples, verifying the absence of significant interferences around the characteristic retention times for each pesticide. For each commodity, spiked samples, at different levels, were 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[4]. Matrix effect and linearity were evaluated comparing analytical response differences between solvent standard solutions and matrix-matched standard.

Quantitative analysis, of each analytical validation batch, was carried out using two response factor (RF) calculation approaches: the first consisted in calculating of average RF (RF_M) starting from six matrix-matched standards, the second by repeated injections (min. 6, during instrumental sequence) of one solvent standard; this study demonstrated statistically equivalent results of the two calculation methods.

For this reason, in routine analysis, average response factor (RF_M) was calculated from replicate injection of standard solution during instrumental analysis, avoiding matrix-matched curve for each commodity, reducing analysis time.

Only in specific cases, in which concentration values of some pesticides are close to the MRL, the RF_M will be calculated using matrix-matched standard.

During validation study, for each commodity, precision and trueness were checked at LOQ, 5x LOQ and safety levels; mean recoveries (trueness) ranged between 70 and 105% 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 matrices analysed in the same analytical batch.
Obtained results appeared very satisfying and coherent with criteria indicated in SANTE/11813/2017 Document[4].

Conclusions

Analytical method developed is suitable to quantify glyphosate and other polar pesticides to guarantee the maximum residue limits in current regulation[5]. Matrix effect, which characterizes both ion chromatography and mass spectrometry, is under control thanks to use labelled internal standards; this allows analysing different food matrices in the same analytical batch, using isotope dilution and response factor approach. This procedural method combined with Ion Chromatography coupled with High Resolution Mass Spectrometry is fit for purpose to routine analysis in Official Laboratory.

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P12

UHPLC-HRMS polyphenolic profile for the characterization and classification of nuts

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Summary: *Targeted UHPLC-HRMS (Q-Orbitrap) polyphenolic profiling by means of TraceFinderTM software was applied to the characterization and classification of nuts. Polyphenolic profiles were proposed as chemical descriptors to address nut authentication by multivariate chemometric techniques. The method provided a satisfactory classification of nut samples according to their type and thermal treatment.*

Keywords: *UHPLC-HRMS, polyphenolic profiling, nuts classification*

Introduction

The quality of food products, in terms of the nutritional point of view but also in relation to food safety issues, has become in the last years a topic of great interest within manufacturers, researchers and consumers. However, mainly due to the complexity of the food chain and the difficulty to detect them, illegal practices such as food adulteration are rising. For instance, the substitution of authentic substances with cheaper ones is a common food fraud.

In 2016, a 4% of the food fraud cases reported by European Union-Member States corresponded to nuts, nut products and seeds [1]. Nuts are worldwide consumed and well-known for their health beneficial effects because of their high content of bioactive compounds. In fact, they are the main dietary sources of total polyphenols [2], which are the largest group of secondary metabolites found in plants [3]. Moreover, their concentration levels as well as distribution depend on a wide range of factors as origin, climate or thermal treatment applied. For that reason, analysing them could be useful for authentication purposes.

In this work, a targeted UHPLC-HRMS polyphenolic profiling method was proposed in order to achieve nut characterization and classification. Therefore, polyphenolic data was considered as a potential discriminant chemical descriptor and was studied by exploratory principal component analysis (PCA) and supervised partial least squares regressiondiscriminant analysis (PLS-DA).

Experimental

A targeted UHPLC-HRMS (Q-Orbitrap) method was employed in order to obtain polyphenolic profiles of nut samples. A 35 min chromatographic separation was achieved by using a Kinetex C18 (10 cm × 4.6 mm × 2.6 µm particle size) column and a gradient elution mode using 0.1% (*v/v*) formic acid aqueous solution (solvent A) and methanol (solvent B) as mobile phase components. Regarding the mass spectrometry acquisition, negative ionization,

with electrospray as ionization source, and Full Scan (*m/z* 100-1500) modes were applied.
Data treatment consisted of a targeted approach by means of a customized target accurate mass database of more than 100 polyphenolic compounds using TraceFinderTM 3.3 EFS software. Polyphenolic data was considered as a source of potential chemical descriptors for the characterization and classification of nuts by PCA and PLS-DA.
A total of 149 nut samples belonging to different classes (almonds, cashew nuts, hazelnuts, macadamia nuts, peanuts, pinions, pistachios, pumpkin seeds, sunflower seeds and walnuts), some of them with several thermal treatments (raw, fried or toasted), were analysed. Sample treatment was based on a first extraction with acetone:water 70:30 (*v/v*) followed by a defatting step with hexane.

Results

After the analysis of 149 nut samples by the proposed UHPLC-HRMS, remarkable differences in the chromatographic fingerprints depending on the nut sample matrix, such as the number and distribution of extracted compounds or their signal intensity, were obtained. After being processed by means of a customized target accurate mass database of 109 exact masses, corresponding to 154 polyphenolic compounds, 89 were detected in at least a type of nut. Thereby, polyphenolic data was then studied by multivariate chemometric techniques. Non-supervised PCA provided a slight trend among samples according to their nut typology and, as expected, PLS-DA improved those results. In a first PLS-DA model with all typologies, macadamia nuts, pistachios, pinions, and pumpkin and sunflower seeds, were clearly classified. Therefore, another PLS-DA model was built by removing the discriminated types. A satisfactory classification of the remaining nut classes was reached.
As the adulteration fraud is often produced by replacing a high quality or expensive nut product with a lower quality or cheaper nut, PLS-DA models were built to study some nuts in pairs, i.e. almonds vs hazelnuts, which is shown in Figure 1, almonds vs peanuts or pumpkin seeds vs sunflower seeds.

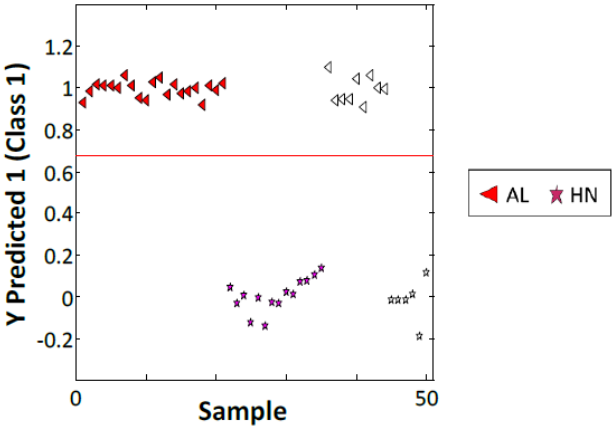

Fig. 1. *PLS-DA classification plot of Sample vs Class for almond vs hazelnut samples. Filled and empty symbols correspond to calibration and validation sets, respectively. Solid red line corresponds to the threshold of separation among almond (top) and hazelnut (bottom) classes.*

These models were also validated by using a 70% of each group of samples as the calibration set, whereas the remaining 30% constituted the validation set. In all cases, a classification rate of 100% was reached.
Other nut features, such as nut format presentation according to the thermal processing treatment (raw, fried or toasted), were also evaluated in order to prove the applicability of the UHPLCHRMS method. A satisfactory classification was also obtained in this case.
It should be taken into consideration that the study of PLS-DA loading parameters, such as variable importance projection (VIP), selectivity ratio or regression vector, could allow the identification of the most discriminant polyphenolic compounds.

Conclusions

The employed UHPLC-HRMS polyphenolic profiling method allowed the perfect classification of nut samples, not only according to their typology but also to their thermal processing treatment. Moreover, the study of PLS-DA loading parameters could allow in the future the identification of specific biomarkers in order to detect determinate nut frauds.

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P13

Preliminary characterization of a candidate reference material for poly- and perfluoroalkyl substances

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Summary: *The concern about the global contamination from poly- and perfluoroalkyl substances (PFASs) is more and more raising. The availability of reliable measurements of their levels is fundamental to support political decisions. In order to promote interlaboratory calibration, the preliminary characterization of a candidate reference material (wild boar liver) was reported.*

Keywords: *poly- and perfluoroalkyl substances (PFASs), wild boar liver, reference material*

Introduction

Numerous poly- and perfluoroalkyl substances (PFAS) have been manufactured and distributed on the world market. Research on PFASs has highlighted their global distribution and impact on ecosystems and human health since these compounds are present in remote environments contaminating humans and wild games. In this context, the production of reference materials (RMs) is fundamental for the quality control and improvement of the worldwide analytical activities. The aim of this study was the preliminary characterization of a candidate RM (wild boar liver) to be used for analysis of PFASs in biological samples.

Experimental

Liver samples of 28 wild boars were collected at slaughterhouse by Veterinary Services of the Italian National Health System. Liver samples were grinded and pooled together with ultra-turrax, poured in 40 mL glass jars and sterilized. Homogeneity study was carried out analyzing 12 jars in duplicate for a total of 24 determinations. The data evaluation was performed following the harmonized IUPAC Protocol for the proficiency testing of analytical chemistry laboratories [1]. The sample extraction and purification were performed according to Kärrman et al. [2] with slight modifications. Thirty-three PFASs were analysed by an LC-Q-Orbitrap system (LC-Q-Exactive, Thermoscientific, San Jose, CA, USA). The quantification was carried out by means of isotopic dilution methodology, introducing 21 labelled internal standards.

Results

In Figure 1, the LC-Q-Orbitrap chromatograms of some of the detected PFASs (jar 8) are shown together with a standard solution (10 ng/mL). As reported by other research groups, wild boar is a good indicator for environmental pollution of PFAS contamination [3-4]. Our results evidenced the presence of 18 PFASs

(>LOQ) out of the 33 tested. The concentrations were in the range 0.18-107 µg/kg. PFOS (Perfluoro-1-octanesulfonate) was the most abundant compound (107 µg/kg), followed by PFOA (Perfluoro-n-octanoic acid) at 76 µg/kg (mean value). The other sixteen analytes were between 0.18 µg/kg and 13 µg/kg. Evaluating the 24 results from the homogeneity study, there was not evidence for outliers (Cochran's test) and, therefore, the complete data set was evaluated. The between-sample standard deviation (ssam) of each PFAS was compared with the critical value (*c*) for the test. Being (ssam)2 always lower than *c*, the produced material was considered sufficiently homogeneous.

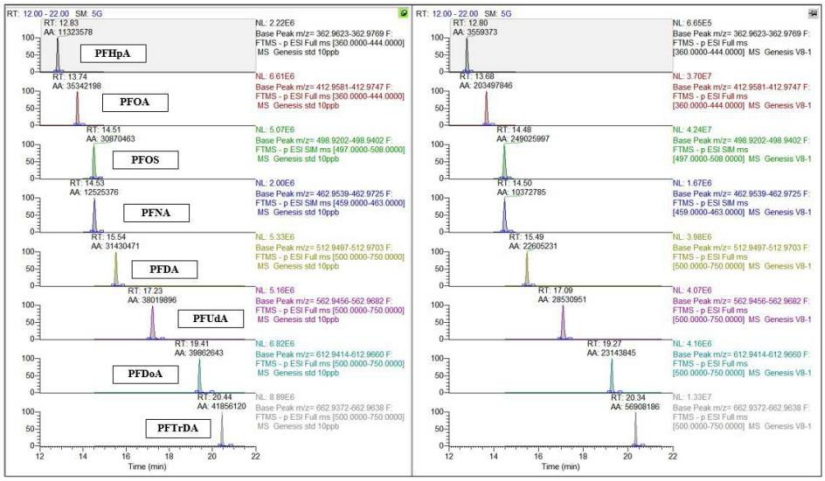

Fig. 1. *LC-Q-Orbitrap chromatograms of: PFASs standard solution at the concentration 10 ng/mL (left side); wild boar liver sample from jar 8 (right side)*

Conclusions

The analysis of pooled wild boar liver samples further support the hypothesis that this species is a suitable bioindicator for environmental pollution of PFASs. Statistical evaluation of the concentrations of the 18 analytes found in the candidate reference material did not demonstrated their inhomogeneous distribution.

Stability studies are in progress. Since, currently, PFAS contamination is representing a global issue, it is more and more important to assure reliable measurements around the world. Our study could give a small contribution to produce suitable materials for the organization of intercalibration exercises promoting standardization among laboratories.

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P14

Perfluoroalkylated pollutants in liver of farm animals by LC-Q-Orbitrap: method development and validation

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Summary: *Perfluoroalkylated substances (PFASs) are widely diffused environmental contaminants. In this work, a method for the determination of thirty-three PFASs at ng/kg levels has been developed and validated in animal liver applying LC-Q-Orbitrap technique. Good performances were obtained in terms of selectivity, accuracy (precision and trueness), detection and quantification limits. The procedure was then applied to analyze liver samples of farm animals finding 50-150 ng/kg of PFOS and lower concentrations of some of other PFASs.*

Keywords: *Perfluoroalkyl substances (PFASs), animal liver, LC-Q-Orbitrap*

Introduction

Perfluoroalkylated substances (PFASs) have been produced since the 1950s and they represent a wide group of highly stable synthetic compounds used in various industrial applications. They are found, for example, in food packaging, non-stick coatings, fireproof foams, paper coatings and fabrics and personal care products[1]. Over the past decade, PFASs have proven to be ubiquitous in water, air, food, wildlife and humans thanks to their high resistance to typical environmental degradation processes. Although studies on their toxicity are not definitive, their action as endocrine disruptors is now clear. The aim of this work was the development and validation of an analytical method for the determination of a considerable number of these substances (33) in animal liver samples.

Experimental

Thirty-three analytes were included in the method scope together with twenty-one labelled compounds used as internal standards. An amount of 2 g each homogenized liver was purified following the protocol suggested by Kärrman et al. [2] with slight modifications. The quantification was performed by liquidchromatography coupled to Q-Orbitrap analyser (LC-Q Exactive, Thermoscientific) using ESI negative ionization mode and full scan/SIM acquisition. The resolving power was set at 17500, 35000 or 70000 (FWHM at *m/z* 200) depending on the reachable number of acquisition points (minimum 10). The mobile phases were water and MeOH both containing ammonium acetate. The analyte separation was achieved on a LC column Kinetex XB-C18 100 Å (100 mm x 3 mm, 2.6 µm, Phenomenex). The validation study was performed spiking liver samples of different animal species (bovine, pig and poultry) at eight concentrations: 2, 5, 10, 25, 50, 100, 500 and 1000 ng/kg. For each level, four

replicates in three different days were carried out, except for 500 and 1000 ng/kg (one validation day).

Results

One of the major drawback of PFAS analysis is their possible laboratory contamination due to the extensive use of these substances in industrial products and, therefore, also in labware and equipments. During the preliminary experiments, it was noticed that the LC system introduced a contamination of some PFASs. Two cartridges (2.1 x 20 mm) packed with weak anion exchange (WAX) and hydrophilic-lipophilic balance (HLB) stationary phase, respectively, were therefore installed between the pumping system and the injector device to minimize PFAS emission. Furthermore, even the SPE WAX cartridges used to purify the sample extracts, were preliminary washed with MeOH. With regard to the optimization of chromatographic conditions, the high number of analytes (33) with very different polarities prevented the achievement of acceptable peak shapes for all the compounds. In order to dilute the high percentage of "strong" phase (80% MeOH) contained in the dissolution mixture of the final extracts, a peek tube was installed between the injector and the analytical column, improving peak symmetry and reducing broadening. This arrangement also allowed to increase the injection volume (from 5 to 20 µL). The method was validated according to Regulation 2017/644 requirements [3] starting from 2 ng/kg to 1000 ng/kg. The results were satisfactory with intra-laboratory reproducibility coefficients of variation lower than 20% and trueness from 80 to 110%. Detection and quantification limits were from 2 to 50 ng/kg. Finally, real liver samples belonging to pig, bovine and poultry species were collected at local slaughterhouses and analysed. In Figure 1, the LC-QOrbitrap chromatograms of a pig liver are shown. The sample was analysed with spiking (10 ng/kg) and without (blank).

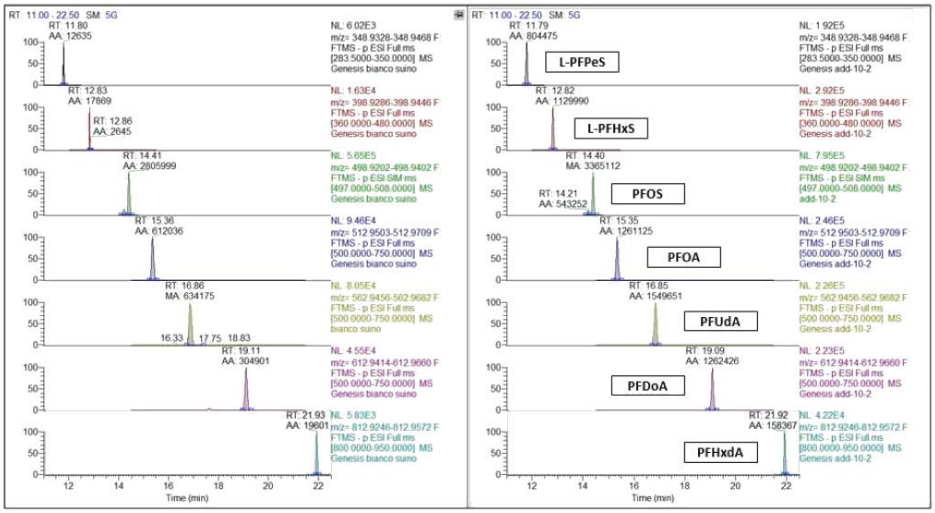

Fig. 1. *LC-Q-Orbitrap chromatograms of a blank pig liver (left) and pig liver fortified at 10 ng/kg (right). The endogenous levels (left side) of PFOS, PFOA, PFUdA, PFDoA were 90, 12, 5 and 4 ng/kg, respectively.*

Some of the determined PFASs such as PFOA, PFOS, PFUdA, PFDoA were "naturally" present confirming the ubiquity of perfluoroalkylated substances also in food producing animals [4].

Conclusions

The measures put in place during the method development allowed to minimize and keep the PFAS laboratory contamination under control. One of the consequences was the possibility of reaching limits of detection and quantification lower than 100 ng/kg. This work demonstrated that the achievement of satisfactory analytical performances not only depends on the available instrumentation, but also on the implementation of rigorous quality assurance practices including the availability of a wide set of labelled compounds to quantify PFASs (isotope dilution).

Acknowledgments

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P15

Fish oil from anchovy by-products via direct and green extraction: characterisation via mass spectrometry of nutraceutical ingredients

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***Summary**: Blue fish oil, is the main source of both omega-3 lipids and vitamin D3 nutrients. One such direct method based on the utilization of biobased d-limonene for the extraction of fish oil from anchovy by-products was lately developed at our Labs. The extract's characterization was performed with the use of GC-MS and HPLC-MS.*

Keywords: *d-limonene, vitamin D3, HPLC-MS.*

Introduction

More than 50% of the total fish capture is not used as food and results in the unwanted production of several million tonnes per year of biological waste across the world [1]. The cost for waste disposal at fish processing companies based in EU countries is significant, currently amounting to around $250-300 per tonne in Italy [2]. The use of fish by-products and discards to source valued marine ingredients such as omega-3 lipids, vitamin D3 and squalene, for the nutraceutical and cosmetic industries has long been proposed as a key sustainability solution to the problem of overfishing [3].

Experimental

Extraction analysis

By using an electric blender, the anchovy waste was ground together with the bio-solvent d-limonene. A semi-solid grey purées was obtained and a part which was extracted with limonene. After evaporating limonene, we obtained 3.0 g of oily extract. A 100 mg sample of the latter oil was evaporated under a flux of nitrogen for removing the residual d-Limonene. Finally, the sample was transesterified to obtain methyl esters of fatty acids from the starting triglycerides [4].

GC-MS method

The GC-MS analysis was carried out using a ThermoScientific Trace 1310/ISQ LT single quadrupole GC/MS spectrometer. The column was a carbovax with an autosampler Triplus RSH Thermo Scientific, using He (5.0) as gas carrier (flow-rate 0.95 mL/min). The temperature ramp used was as follows: the column was held for 2 min at T = 60 °C, after which temperature was raised at 20 °C/min rate up to 250 °C, with a final 5 min isotherm.

Table 1. *Relative abundance of fatty acids in anchovy extract*

Acid (in lipid numbers)	**Retention time**	**Abundance (%)**
Myristic acid (14:0)	9,95	6,98
Pentadecanoic (15:0)	10,38	1,2
Palmitic (16:0)	10,61	33,55
(6,Z)-7 methyl-6-Hexadecenoic	11,04	1,19
Margaric (17:0)	11,1	0,94
Stearic (18:0)	11,34	0,53
Oleic (18:1, n-9)	11,39	23,97
Linoleic (18:2, n-6)	11,6	1,97
alpha-Linolenic (18:3, n-3)	11,78	0,96
Stearidonic (18:4, n-3)	11,86	1,04
Gadoleic (20:1, n-11)	12,18	3,09
Eicosapentenoic (20:5, n-3)	12,07	5,4
11-Docosenoic (22:1, n-11)	13,02	4,66
Docosahexaenoic (22:6, n-3)	13,9	12,39

HPLC-MS

For the vitamin extraction we used a published methodology with a slight variation. The process has been described in detail elsewhere [5].

0,5 g of fish oil was put into a flask and were added respectively 2 ml of 1 % methanolic L(+)ascorbic acid and 5 ml of 0,5 M methanolic potassium hydroxide. Subsequently the sample was vortexed for 30 seconds and then the flask was immersed in a silicone bath at 80 °C (with reflux) for 30 minutes. The sample was cooled in an iced water and added with 5 ml of n-hexane. Finally, the hexane layer was dried under nitrogen flux. Prior to injection, samples of unsaponifiable fraction was brought to dryness and the residue dissolved in 600 µl of methanol. The UHPLC (Dionex UltiMate®3000 Rapid Separation LC system by Thermo Fischer Scientific) system was coupled to a Orbitrap mass spectrometer instrument (Q Exactive) (Thermo Scientific, Germany), equipped with heated electrospray (HESI) ion source.

A novel method for identification of vitamin compounds were applied. Electrospray conditions for analysis in positive ion mode of A, D2, D3, Vitamin E and Vitamin K1: sheath gas flow rate 35 (arbitrary units); auxiliary gas unit flow rate 15 (arbitrary units); spray voltage 3.5 kV; S lens RF level 50; capillary temperature 280 °C; auxiliary gas heater temperature 300 °C.

The UHPL column was a Sinergy Hydro RP 100 Å, 150 x 2.1 mm, 2.5 µm. The column temperature was set at 25 °C and the injection volume at 1.0 µL.

Mobilephase composition: formic acid/water 0.1% v/v (eluent A), formic acid/ methanol 0.1% v/v (eluent B).

Results

Since 2008, has been known in the literature the use of limonene as an extraction solvent for lipids in the food (Chemat et all) [6].

Table 1 shows the relative percentage abundance of fatty acids in the oil. The most abundant component is palmitic acid (33.55%), followed by oleic acid (23.97%). PUFA represent about 20 % of the relative abundance of fatty acid

composition [4]. Limonene successfully extracted also vitamin D3. A content of 82 µg of vitamin D3 per kg oil was assessed via advanced mass spectrometry coupled to HPLC. Mass spectrometry studies will be carried out in high resolution MS/MS to investigate the structure of said isomers in the fish oil extracted from anchovy by-products with biobased limonene.

Conclusion

In conclusion, it is likely that, following optimization of the extraction method, new dietary supplements might soon use blue fish oil extracted from blue fish by-products via the newly developed straightforward process based on edible and health beneficial limonene derived from waste orange peel [7]. In general, fish and fish products are the most important food sources of both vitamin D3 and omega-3 nutrients [8], whose concomitant global deficiency of vitamin D as well as of omega-3 in the population of most world's countries has led many countries to deploy recommended daily intakes and specific nutritional guidelines.

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P16

Evaluation of volatile profile of ten Italian tomato cultivars and relative processed products

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Summary: *Volatile profile of ten Italian tomato cultivars and relative processed products (tomato pulps and purees) was evaluated. The differences in volatile composition among the varieties and between the fresh and processed tomatoes were analyzed by solid-phase microextraction coupled with gas chromatography mass spectrometry (SPME-GC-MS).*

Keywords: *volatile profile, tomato, solid-phase microextraction*

Introduction

Tomato flavor is one of the most important attribute for consumer acceptance. More than 400 volatile compounds have been detected in tomato [1], but only 15-20 volatiles have an impact on human perception [2, 3]. However, tomato flavor is usually affected by thermal treatment. Processing techniques can indirectly alter the content of some volatile compounds by enzymatic and chemical reactions. The endogenous enzymes of tomato catalyze the formation of specific volatile compounds important for overall tomato flavor.

The aim of this study was to evaluate the volatile composition of ten Italian tomato cultivars in order to investigate the effect of two different processing techniques (pulp and puree) to determine and identify the key aroma compounds

Experimental

Ten commercial tomato cultivars were selected, harvested at ripening stage and industrially transformed into final tomato product. Each variety was analyzed at three steps: fresh, pulp and puree. An aliquot (8 g) of each sample was weighed into a 20mL vial and spiked with 50 µL of internal standard. The determination of the volatile profile was carried out by solid-phase micro extraction (SPME) and analyzed with a gas chromatography-mass spectrometry (GC-MS).

The experiments were performed using a 50/30 µm divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) fiber. Volatile compounds were identified by comparing two spectral libraries NIST and WILEY. Standards compounds were injected and analyzed under the same conditions. The data obtained were collected by using the software Bruker Chemical Analysis MS Workstation version 7.0. Statistical analysis were performed using SPSS software ver. 21.0.

Results

The volatile profile is not significantly different among the 10 tomato cultivars. The main class of volatile compounds that were found are the follows: aldehydes, ketones, alcohols, furans and terpenes. It is observed that the

aroma profile changed during processing. The fresh product shown about 50 volatile compounds, while in the pulp and puree products, the volatile compounds are reduced to 39 and 35, respectively. In Table 1 is reported the main aroma compounds found in the samples. The major contributors of fresh products are 1-penten-3-one, hexanal, 2-hexenal and 2-isobuthylthyazole that contribute to the green note. In addition, the compound 5-hepten-2-one-6-methyl conferred the characteristic fruity and sweet note in fresh products. One of the most marked difference between the fresh and processed tomato is the complete loss of 2-hexenal, 2-heptenal, 2-isobuthylthyazole, 1-penten-3-one, 2-pentenal, 1-octen-3-one and beta ionone in the processed products. The decreased levels of many tomato volatiles may due to their degradation and oxidation. In addition, the processed tomato shown volatile compounds related to the Maillard reaction such as furfural and dimethyl sulfide. These compounds are important for the overall aroma of the pulps and especially of the purees because they influenced positively the odor of cooked tomato.
Principal Component Analysis (PCA) was carried out to describe relations between different processing conditions and key volatile compounds. In tomato products, typical changes are total loss of the green note and the formation of furfural and dimethyl sulfide (Figure 1 and Table 1).

Table 1. *Normalized peak areas (compound area/IS area) of the key volatile aroma compounds of fresh tomato and relative pulp and puree (mean of the ten tomato cultivars). nd: not detected*

Peak	Volatile compound	Fresh	Pulp	Puree
A	Butanal, 3-methyl	0.01	0.01	0.01
B	1-Penten-3-one	0.06	nd	nd
C	2-Pentenal	0.02	nd	nd
D	1-Pentanol	0.07	0.01	nd
E	2-Hexenal	0.77	nd	nd
F	Heptanal	0.06	0.001	0.002
G	2-Heptenal	0.19	nd	nd
H	Beta-Pinene	0.08	0.02	0.02
I	1-octen-3-one	0.03	nd	nd
L	5-Hepten-2-one, 6-methyl	4.22	0.55	0.17
M	Octanal	0.07	0.003	nd
N	2-Isobutylthiazole	0.23	nd	nd
O	Nonanal	0.14	0.01	0.01
P	Methyl salicylate	0.18	0.001	nd
Q	alpha-Citral	0.28	0.01	nd
R	Geranil acetone	0.69	0.02	0.005
S	Hexanal	28.91	0.03	0.02
T	Dimethyl sulfide	nd	0.56	0.79
U	Furfural	nd	0.003	0.003
V	Beta-ionone	0.01	nd	nd

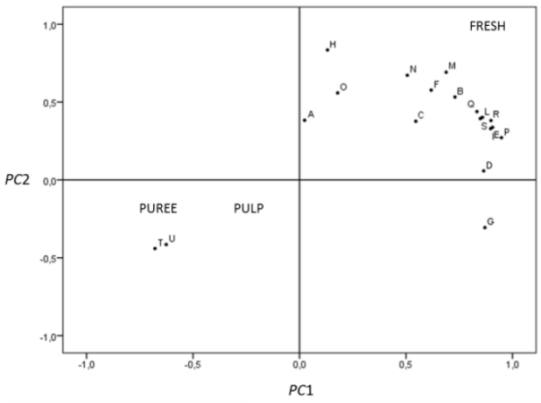

Fig. 1. *PCA plot of the key volatile compounds of the ten tomato cultivars at different processing conditions (fresh, pulp and puree). The total variance explained among the samples is 74.18%. The letters of the plot refers to compounds listed in Table1.*

Conclusions

The main differences in the volatile profile are related to the processing and do not depend on the variety. The high temperature clearly modify the aroma compounds. Important volatiles of fresh tomatoes (hexanal, 2-hexenal, 1-penten-3-one) significantly changes. The level of most aldehydes decreased as the temperature increased. The overall aroma of the pulp is more similar to the puree than fresh tomato.

Further studies are in progress for correlating the tomato volatile compounds with sensory profiles through the organization of a Panel test.

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P17

Transfer of a multiclass method for over 60 antibiotics in food from a high resolution tandem mass spectrometry platform to a low resolution one

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Summary: *Two multiclass methods to determine antimicrobial residues in animal muscle and milk have been developed and validated using liquid-chromatography coupled to tandem mass spectrometry (LC-QqQ). The main performance characteristics were estimated and compared to those previously obtained applying similar procedures implemented on an LC-Q-Orbitrap platform. The validity of the method transfer was ascertained through intra-laboratory and inter-laboratory studies.*

Keywords*: antibiotics, liquid chromatography mass spectrometry, method validation*

Introduction

Antibiotics are widely used in livestock breeding for treating several diseases in food producing animal species. To guarantee public health protection, the European Union requires the member state to implement yearly monitoring plans to control the presence of antibiotic residues in food. Therefore, the surveillance is mainly aimed at controlling the compliance with Maximum Residue Limits (MRLs) fixed in Regulation (EC) No 37/2010 [1]. In the last ten years, the improvement of LC-MS equipments allowed the realization of procedures able to determine simultaneously more than one antimicrobial class in food. Dozens of papers have then published about this approach, reporting the use of different MS analysers and configurations such as triple-quadrupole (QqQ), time-of-flight (TOF), Q-TOF, Orbitrap and Q-Orbitrap. In 2016, also our group developed and validqated multiclass methods for over 60 antibiotics in meat and milk by LC-Q-Orbitrap [2,3]. All that said, multiclass methods are no longer innovative procedures and, at present, there is interest in their widely diffusion which involves the possibility of an effective and efficient transfer from a LC-MS platform to another one. The aim of this work was to demonstrate the validity of the transfer of previously developed LC-Q-Orbitrap procedures to an LC-QqQ platform.

Experimental

More than sixty antibiotics belonging to ten drug families (amphenicols, cephalosporins, lincosamides, macrolides, penicillins, pleuromutilins, quinolones, rifamycins, sulfonamides and tetracyclines) have been included in the method scope. The sample preparation was described elsewhere [2,3]. The measurements were performed by a Surveyor LC pump coupled to a Quantum Ultra Thermo Fisher (San Jose, CA, USA) triple quadrupole mass spectrometer (LC-QqQ). The validation study for the main performance characteristics

(selectivity, linearity, precision, trueness, limits of decision, detection capabilities, LOD and LOQ) was carried out according Commission Decision 2002/657/EC [4].

Results

The LC-QqQ procedures were easily implemented both in meat and milk starting from the previous developed protocols [2,3]. The development has been rapid thanks to the already studied conditions (sample treatment, LC separation and fragment ions). Satisfactory performances were generally obtained with recovery factors in the range 70-100 % for the majority of analytes. Coefficients of variation evaluated in intra-laboratory reproducibility conditions were lower than 20%. Only for valnemulin in muscle and cefacetrile and tulathromycin marker in milk the observed precision was unsatisfactory, restricting the method suitability for screening purposes. The limits of detection were between 2 and 33 µg kg^{-1}. The validation study was concluded re-analysing a series of stored test materials from Proficiency Test Schemes (Table 1). The data are shown in Table 1 involving quinolone, sulfonamide and tetracycline determinations. The concentration found applying the new method were comparable with those obtained with LC-Q-Orbitrap procedure in both matrices. It is worth to note that all the values were within the acceptability range furnished by the Proficiency Test Organizer (Test Veritas, Padova, Italy).

Table 1. *Analysis of test materials from Proficiency Test Schemes: comparison between LC-Q-Orbitrap and LC-QqQ methods*

Method			LC-Q-Orbitrap	LC-QqQ	
Year	Matrix	Analyte	Found concen-tration, (µg kg^{-1})	Found concen-tration (µg kg^{-1})	Acceptability range (µg kg^{-1})
2014	Milk	Sulfamethazine	144	96	57-150
2014	Muscle	Sulfamethazine	88	75	36-102
	Muscle	Sulfadimethoxine	32	23	12-42
2014	Muscle	Ciprofloxacin	5.5	5.0	3.2-8.1
	Muscle	Enrofloxacin	173	152	92-227
2015	Milk	Sulfamethazine	165	131	75-191
2016	Milk	Flumequine	91	111	47-129
2016	Milk	Oxytetracycline	93	55	49-132
2017	Milk	Danofloxacin	91	80	39-109

Conclusions

The validity of the transfer of two multiclass methods for the determination of

antibiotic residues in food from an LC-Q-Orbitrap to an LC-QqQ system has been demonstrated. Since this transfer has been performed on an obsolete LC-MS apparatus (fifteen year-old), the feasibility of multiclass strategy was demonstrated also without very recent equipments, encouraging the complete replacement of single class approach in routine laboratories devoted to official control.

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P18

In depth characterization of dried rings and flakes from Emilia Romagna authocthon onion (Allium cepa L.) by GC-MS and GC×GC/TOF

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Summary: *Volatile fraction of dried rings and flakes from a territorial certified and organic onion for nutraceutical uses, were characterized by HS-SPME-GC-MS and 2DGC-MS/TOF. Forty-nine volatiles were identified; dipropyl disulfide was the main component in both the samples. The amounts of volatiles were significantly higher for onion flakes than onion rings*

Keywords: *volatile compounds, food and nutraceuticals application, HS-SPME-GC-MS*

Introduction

Onion (*Allium cepa* L.) is a vegetable widely used as food ingredient all over the world. It is characterized by a typical fragrance capable to enrich many dishes with a flavor very appreciated by consumers. Flavanols and volatile organic compounds as sulfur compounds are usually the main bioactive constituents [1]. Over the years, dried forms of onion have gained increasingly attention as an alternative to the fresh onion for industrial and domestic uses; indeed, it is a very easy to use functional food ingredient [2], mainly in the powdered form.

We aimed at characterizing phenolic (by HPLC-DAD) and volatile (by HS-SPME-GC-MS) fractions of dried samples of onion rings and onion flakes. Herein, we present results from volatiles characterization, for which we applied HS-SPME-GC-MS as tool of choice for analysis of VOCs and 2DGC-MS/TOF for deep in this characterization [3].

Experimental

Dried onion rings and onion flakes samples were purchased by Officinali Agribioenergia factory, (Medicina, Bologna, Italy). Before GC-MS analysis, samples were deep-freezed with liquid nitrogen and then chopped with mortar and pestle, until a homogenous powder was obtained.

Volatile organic compounds (VOCs) were analyzed by both HS-SPME-GC-MS and 2DGC-MS/TOF analyses. 15 mg of sample were placed into a 20-ml screw cap vial fitted with PTFE/silicone septa, together with 2 g of NaCl, 5 mL of deionized water and (only for HS-SPME-GC-MS analysis) 5 µL of internal standard (4-methylpentan-2-ol, 20 mg/l in water). Samples were analyzed in triplicate. For both HS-SPME-GC-MS and 2DGC-MS/TOF analyses, VOCs were absorbed from the headspace of the 20-ml screw cap vial by the coating material of a 2-cm trivalent fiber (DVB/CAR/PDMS), for 10 min under orbital shaking. The working temperature was 60°C, but some experiments of HS-

SPME-GC-MS were also carried out at 30°C in order to evaluate if different temperatures could affect the volatile profiles. VOCs were then immediately desorbed at 280°C in a GC injection port and separated in a 7890a GC system (Agilent Technologies, Santa Clara, CA, USA) using a DB InnoWAX column (50 m, id 200 µm, df 0.40 µm) and detected by a quadrupole mass spectrometer (Agilent 5975C MSD), operating in EI mode at 70 eV. Relative concentration of each identified compound was calculated according to previous literature [4], using the following formula:

$$[VOC\ (\mu g/g)] = (A_{VOC}/A_{ISTD})*(m_{ISTD}\ (\mu g)/m_{sample}\ (g))$$

GCxGC was performed by a flow modulation apparatus consisting of an 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). Chromatographic separation was performed using a first dimension (1D) HP-5 column (0.18x0.18mm, 20 m) and a WAX second dimension (2D) column (0.23x0.32 mm, 5 m). Compounds were tentatively identified comparing mass spectra of each peak with those reported in mass spectral databases; identification was confirmed by their retention index.

Results and conclusions

HPLC-DAD analysis, carried out before GC-MS analysis allowed identifying and quantifying 11 flavonoids: total amount 4.06 mg/g in onion flakes and 2.91 mg/g in onion rings.

Figure 1 shows the contour plot obtained by 2DGC-MS/TOF analysis of onion rings. Comprehensive two-dimensional GC fingerprint analysis pointed out dipropyl disulfide and dipropyl trisulfide as the most abundant VOCs (figure 1).

HS-SPME-GC-MS analysis showed no differences given by different temperatures during VOCs absorption on the coating material of the fiber. A total of 49 VOCs were identified and quantified by HS-SPME-GC-MS analysis: 3 monosulfides, 16 disulfides, 7 trisulfides, 4 other *S*-compounds, 14 aldehydes, 1 ketone and 4 compounds belonging to other classes. For all these classes of compounds, the amounts were significantly higher for onion flakes than onion rings, with dipropyl disulfide as the main component in both the samples, (49.3 mg/kg in onion flakes and 28.5 mg/kg in onion rings).

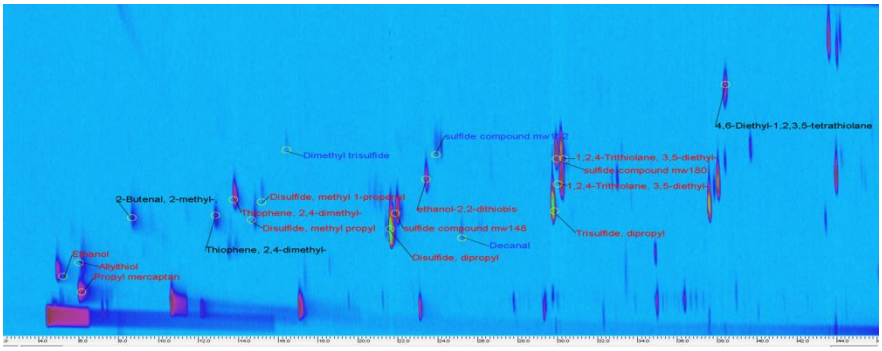

Fig. 1. *Contour plot from GCxGC/TOF analysis of dried onion rings.*

Trisulfides was the category of VOCs that discriminated the most between the

two samples: onion flakes showed a total amount of 42.9 mg/kg, while onion rings only 6.27 mg/kg. Dipropyl trisulfide was the main representative trisulfide (23.3 mg/kg in onion flakes and 3.3 mg/kg in onion rings).
In conclusion, an innovative product from territorial certified and organic onion (*Allium cepa* L.) in two dried different forms, aimed to food and nutraceutical uses, was characterized for their phenolic and volatile fractions.

Acknowledgement
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P19

Differentiation of Italian land snails (Helix aspersa) from foreign samples, using C and N stable isotopes analysis: preliminary results

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Summary: *Land snails' farms are recently increasing in Italy, and producers are interested in protecting their products. Stable isotopes are useful to trace food and differentiate the origin areas. We applied this methodology to samples coming from Italian and foreign farms, founding that nitrogen isotopes are the most discriminating.*

Keywords: *land snail, food traceability, stable isotopes*

Introduction

In recent years, the number of land snails' farms is increasing in Italy and in the whole world, with an increase in the commercial demand, which regards not only food, but cosmetic industry too. To protect their own products, the Italian breeders have created the Italian Heliculture Protection Consortium (Consorzio di Tutela dell'Elicicoltura Italiana – COTELI). In this context, differentiate local snails from foreign one, is one of the fundamental goal of the consortium. Stable isotopes technique are useful to trace food [1], and to characterize the animals' tissues: $\delta^{13}C$ and $\delta^{15}N$ of snail muscle reflect the isotopic signal of the vegetable eaten [2], and so of the local cultivations. In particular, the carbon isotopic analysis are able to distinguish C3 photosynthetic pathway plants from C4 one [3]. Instead, vegetable nitrogen isotopic signal depends on different factors, as climate or agriculture practice [4].
In this study, we isotopically analyzed different samples of land snail (*Helix aspersa*), collected from Italian and foreign counties, with the aim to distinguish their origin.

Experimental

In this first phase of the project, samples of land snail (*Helix aspersa*) were collected from the breeding of four Italian sites and two foreign one (Tunisia and Poland). Snails' muscles were extracted from the shell, repeatedly washed with distilled water, frozen at -80°C and lyophilized. After homogenization, the samples were weight in tin caps (0,8-0,9 mg) for $\delta^{13}C$ and $\delta^{15}N$ analysis, performed with an EA-IRMS system (Thermo Scientific), at the iCONa lab (isotopic carbon, oxygen and nitrogen analysis) of the University of Campania "Luigi Vanvitelli".

Results and discussion

From preliminary results, we found a differentiation of all the sampled sites (Italian and foreign), based on the $\delta^{15}N$ values (range from 2,4‰ to 7,5‰),

confirming the fact that nitrogen strongly depends on the local signal. In fact, the δ^{15}N average of Italian samples was statistically different from the foreign one ($p < 0.001$).
Instead, the δ^{13}C (range from -26,7‰ to -24,4‰) is the less discriminating of the origin area, probably due to the similarity of the vegetables used for the animal feedings.

Conclusions
We analysed different land snails samples, coming from Italian and foreign counties, to distinguish the provenience on the bases of the isotopic signals. In this preliminary analysis, we found that the areas of production can be differentiate due to the δ^{15}N signals, while the δ^{13}C is the less discriminant.
More measurements and statistics are planned in the future studies.

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P20

A comprehensive analysis of volatile profile of Cannabis sativa L. variety Futura essential oil by TotalFlowUnderVacuum GCxGC-TOF and GC-VUV

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Summary: *Hemp aroma is a result of a specific and harmonic combination of multiple class of compound, in low and high concentration. Characterization of this type of complex sample require an high chromatographic peak capacity plus sensitive and specific detection. UnderVacuumTotalFlow GC×GC allow direct coupling of second dimension flow to MS_TOF giving robust, sensitive routin operation. VUV detection give additional information to identify, in presence of a large number of isomer, the correct peak.*

Keywords: *volatile compounds, UVTF_GC×GC food and cosmetic application*

Introduction

Hemp (*Cannabis sativa L.)* is an annual species, native of central Asia and spread in Europe and Africa, source of hundreds of biological active compounds such as cannabinoids, terpenoids, flavonoids and polyunsaturated fatty acids. Hemp essential oil, with its unique smell is, at present, used in cosmetic and perfume products, aromatherapy and as beer flavouring agent. The sesquiterpenes fraction is very complex, since the mass spectra of the different components are often very similar and do not allow an unbiased discrimination. In the present work we used the fingerprinting capability of Vacuum Ultra Violet Detector (VUV) to help to discriminate between the isomers.

Experimental

Essential oil of cannabis Futura obtained by steam distillation was analyzed by GC-MS using two different and innovative techniques: A comprehensive GC×GC-TOF with flow modulator and A GC- chromatography with Vacum Ultra Violet (VUV) detector. The GC×GC system consisted of an Agilent GC-MS 7890B, with capillary flow modulator device for comprehensive separation, coupled to an TOF-DS Markes detector. The system was set up as follows: a first dimension HP-5 column 20 m 0.18 mm i.d. × 0.18 µm d.f. Operating at Helium flow of 0.4 ml/min and a second dimension innovax 5 meters 0,32mm i.d. × 0.32 µm d.f. operating at 10 ml/min. Under vacuum chromatography was obtained in the second column thanks to a 15 cm 0.1 mm restriction placed

immediately before the second column.
In all samples all Compounds were tentatively identified comparing mass spectra with those reported in mass spectral databases; identification was confirmed by their retention index.
The samples were also injected in a GC Agilent 7890B connected to a VUV 101 detector (VUV Analytics) equipped with a 60 m HP-Innowax 0.25 mm i.d. 0.5 µm d.f. column operating in standard mono dimensional chromatography. In order to verify the VUV potential in compound recognition we injected a mixture of 6 analytical standard sesquiterpenes.

Results and conclusions

Comprehensive GC×GC-MS is currently adopted as separation technique not only because of its high separation power and sensitivity but also for its ability to produce more widely distributed and rationalized peak patterns [1] for chemically correlated group of analytes. Hemp essential oil showed 3 separated regions corresponding to the classes of monoterpenes, sesquiterpenes and oxygenated sesquiterpenes. The blob detection of the GC×GC Image software after subtracting base line blobs and background interferences detects a large number of blobs (110), but the region corresponding to the sesquiterpenes group, showed many blobs with similar mass spectra giving uncertain identification.
The VUV detector tested on a standard mix of sesquiterpenes, was able to identify of each peak in the standard solution by software comparison with an internal spectral UV library. When applied to the chromatogram of the hemp essential oil, the VUV detector showed the capability to discriminate among different sesquiterpene isomers.
The GC×GC-TOF analysis showed a very high sensitivity, but for some compounds the mass spectrum is not specific enough to ensure an unbiased identification. The Retention Index reference is useful to predict the elution order, but the possibility to use a different detector can give better results. VUV detector give additional information to identify, in presence of a large number of isomer, the correct peak.

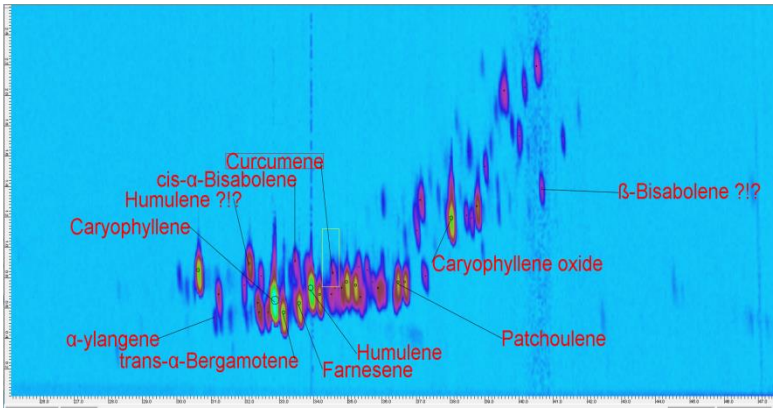

Fig. 1. *Zoom of sesquiterpene region from GC×GC-TOF contour plot analysis*

Δ-9-tetrahydrocannabinol (THC) could not be detected in the essential oils and the amount of other cannabinoids was very poor [2]. Nevertheless, a GC×GC-

TOF method was optimized to determine the possible presence of cannabinoids, by varying temperature ramp and analysis time. The data confirmed the poor values of the non psychoactive cannabinoid, i.e. cannabidiol (CBD), and THC.
In conclusion, high value product, aimed to food and cosmetic uses, was characterized for its volatile fractions with innovative techniques.

Acknowledgement
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P21

Chemotype diversity of commercial essential oils

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Summary: *Fourteen commercial essential oils were screened for their different chemical profiles by GC-MS analysis. The successive multivariate analysis, PCA, generated six groups showing distinct chemotype.*

Keywords: *Essential oils, PCA analysis, chemotyping.*

Introduction

Aromatic plants (APs) have been used since antiquity as a potential source of drug in folk medicine, and as preservatives in foods. APs contain many biologically active compounds, mainly essential oils (EOs) and phenolics. In particular, EOs are highly complex mixtures involving several tens to hundreds of different types of volatile compounds such as terpenoid, oxygenated terpenes, sesquiterpenes, and hydrocarbons, which are responsible for characteristic aroma of APs. EOs have been widely used for their virucidal, bactericidal, fungicidal, anticancer, antioxidant, antidiabetic activities that are strictly linked to their chemical composition [1-3]. The aim of the present work was to study the chemotaxonomy of plants by analyzing the chemical composition of the EOs, comparing fourteen commercial EOs by means of Principal Component Analysis (PCA).

Experimental

EOs are usually prepared by the following extraction techniques: distillation (hydro or steam distillation), cold pressing, extraction (maceration), or by means of supercritical carbon dioxide extraction. The unique aroma of all EOs was derived from many volatile compounds that were identified and quantified by gas chromatography-mass spectrometry (GC-MS).

Results

Based on this multivariate analysis, the EOs clustered in six groups (Fig. 1): the first consisted by eucalyptus and yin yang Chinese, both with high content of eucalyptol, whereas the second one was made up of bitter orange and pepper, which had as main constituents D-limonene and o-cymene. The cluster III, consisting of star anise and fennel, was characterized by large quantities of anethole and estragole, while the group IV was clustered by the presence of sesquiterpenes and was composed by cloves, palmarosa, and ylang ylang. Furthermore, it is observed the EOs turmeric, lavender, and oregano (Group V)

were closer due to the presence of eucalyptol and o-cymene. In the same way, the similarity between tee tree and yin yang japanese can be observed (Group VI) due to the content of the components eucalyptol and D-limonene.

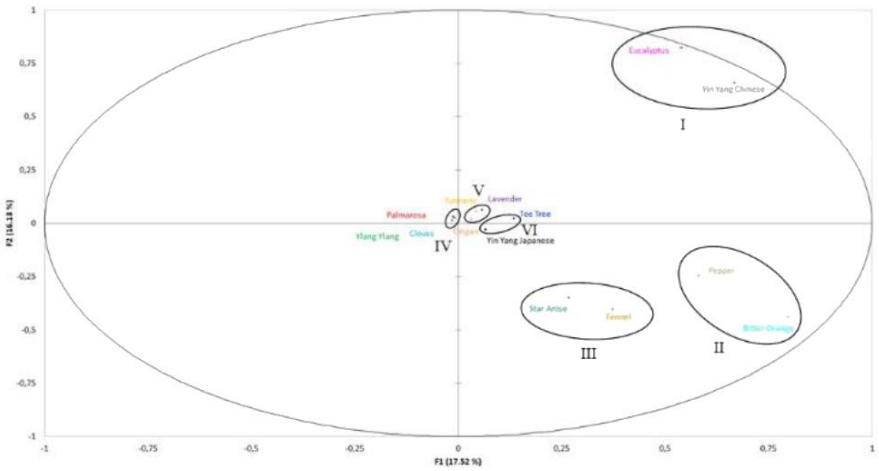

Fig. 1. *PCA analysis*

Conclusions

Overall, according to the chemical compositions and their relative contents analysed by means of PCA, the 14 EOs were classified into six chemotypes. This PCA analysis of all compounds present in the EOs is an important preliminary step with the future goal to correlate the chemotypes with biological activity, thus allowing their potential applicability as natural substances from plant origin to use as antifungal and antioxidant substances.

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P22

Fully automated determination of 3-MCPD and glycidol in edible oils and fats by GC-MS based on the commonly used methods ISO 18363-1, AOCS Cd 29(a&c)-13, and DGF C-VI 18

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Summary: *3-MCPD, Glycidol and related fatty acid esters are process contaminants that can arise from edible oils refining and they are suspected harmful to human health. This work describes a solution for their fully automated determination in edible oils and fats based on the reliable indirect method AOCS Cd 29(a&c)-13, as well as a partial automation of method AOCS Cd 29(b)-13.*

Keywords: *MCPD, Glycidol, AOCS Cd 29c-13*

Introduction

3-MCPD, Glycidol and related fatty acid esters are process contaminants that can arise from edible oils refining. Because of their proven or suspected potential human carcinogenetic and genotoxicity, they're nowadays subjected to EU regulations. This work describes a solution for their fully automated determination in edible oils and fats based on the reliable indirect method AOCS Cd 29(a&c)-13, as well as a partial automation of method AOCS Cd 29(b)-13.

Methods

The complete automated sample prep (including IS addition, derivatization/ quenching, evaporation, reconstitution and injection in GC-MS), was performed on a GERSTEL MPS DualHead. Key modules are QuickMix (which performs a reliable LLE) and mVAP (wich provides the significant benefit of removing excess derivatization reagent, allowing at the same time to reach required LOQs using a single Quad MS).

Results

The linearity was checked by a 5 level calibration for both assay A and B, spiking an olive oil with different amounts of Glycidol and 3-MCDP ranging from 0.12 to 1.9 mg/kg. The repeatability was tested as well, with a RSD% < 7% for both analytes. Finally, the matrix effect was taken in account: three different (reference) edible oils were analyzed with excellent results in terms of accuracy. It is also important to underline that the described sample prep allows to quantify, in the same run, even the amount of 2-MCDP

It's shown that AOCS method can be automated using the GERSTEL MPS and that the results obtained correlate well with reference data. The excellent RSD% achieved for the complete process, including GC-MS analysis, speaks in favor of the presented sample prep station. Moreover, the evaporation step ensures that, for most matrices, the required limits of detection can be reached

using a single quad.

Remark

Although GC-MS technique has been proven to be suitable for "general purpose" analysis, the need to reach lower LOQs (i.e. few µg/kg for baby-food, as required by EU regulation 290/2018), suggests to move towards a GC-MS-MS approach, which is able to reach such limits of quantitation. Furthermore, the same analytical platform is suitable for PAH quantitation in edible oils and fats according to EU regulation 1881/06 (and subsequent amendments).

P23

Quality assessment of Tuscan extra virgin olive oil by HS-SPME-GC-MS analysis of volatile compounds, HPLC-DAD analysis of phenolic compounds and chemometrics

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Summary: *The quality of Tuscan Extra Virgin Olive Oils was assessed through their volatile and phenolic profiles. The most of Tuscan oils were suitable for the EFSA health claim, while quantitative data collected by HS-SPME-GC-MS allowed correctly (up to 98%) classifying the samples according to their origin and commercial category.*

Keywords: *extra virgin olive oil quality, volatile compounds, phenolic compounds*

Introduction

Virgin olive oil is extracted from the olive fruit only through physical/mechanic means [1]. It is mainly produced in the Mediterranean basis and represent only a minor part of the vegetable oil production all over the world. In spite of this, when it is classified into the highest quality category [Extra Virgin Olive Oil (EVOO)], it is extremely popular mainly thanks to its delightful taste, functional health promoting and nutritional attributes [2]. The EFSA also approved a health claim for olive oil polyphenols [3].

These properties, which make EVOO strongly different from other edible oils, derive from the presence of polar phenolic compounds and non-polar volatile organic compounds (VOCs) and, usually, EVOO quality is strongly linked to its phenolic and volatile profile [4]. From the qualitative point of view, Italy is considered as a golden area for cultivating olive tree and producing high quality extra virgin olive oil.

Aim of this study is the quality assessment of Tuscan EVOOs, through: i) evaluation of the total content of tyrosol and hydroxytyrosol in free and bound forms, for confirming the samples fit the requirements for the EFSA health claim [5]; ii) evaluation of sensory attributes by the panel test and the volatile profiles by a previously validated HS-SPME-GC-MS method [6]; iii) application of chemometric approaches previously developed for confirming the geographical origin and the commercial classification of samples according with the panel test.

Experimental

Fifty-eight Italian oil samples (51 from the Tuscany region, one from Campania

region, 6 from Sicily region) were collected during the 2018-2019 olive oil campaign. All samples belong to the EVOO category. The positive attributes of samples were evaluated by a professional panel of A.N.A.P.O.O. consisting of a panel leader and 8-12 trained tasters. The content of phenolic compounds was evaluated both before (following the IOC official method) and after acid hydrolysis [5].
The volatile organic compounds were analyzed according to the multiple internal standard method, previously validated [5], using a 6890N GC system equipped with a MS detector, model 5975 by Agilent, and a HP-Innowax capillary column 50 m × 0.2 mm ID, 0.4 µm DF. 4.3 g of sample and 0.1 g of internal standard solution were added into a 20 ml screw cap vial fitted with a PTFE/silicone septa. A SPME fiber 50/30 *µm* DVB/CAR/PDMS was exposed for 20 min in the vial headspace under orbital shaking at 400 rpm after equilibration for 5 minutes at 45°C, then the adsorbed VOCs were desorbed in the injection port of the GC system. Mass detector worked in scan mode within the range of 30-350 Th, 1500 Th/s at ionization energy of 70 eV. Each VOC was quantified using a calibration curve in which the area ratio (ratio between areas of that VOC and the selected ISTD) was plotted versus the amount ratio.
Suitable chemometric approaches (PCA-LDA and ANOVA-LDA) previously developed were applied to the obtained set of data.

Results and Conclusions
Results from analysis of phenolic compounds after acid hydrolysis showed that 39 out of the 58 EVOO samples (67%) had a phenolic content higher than the 5 mg/20 g_{oil} recommended by the EFSA for the health claim. Interestingly, none of the 6 Sicilian EVOOs reached such value, despite before hydrolysis the content was higher than 5 mg/20 g_{oil} for 5 out of the 6 samples. Further researches seem to be necessary to understand if Tuscan EVOO fit with EFSA health claim better than EVOO from other Italian region.
Analysis of volatile compounds pointed out amount of VOCs up to 98 mg/kg. In Tuscan samples, *E*-2-hexenal, typically related to green and fruity notes, was present in the 52-87% range of percentages on the total content, while in the sample from the Campania region it was 25% of the total amount and in the samples from the Sicily region it was present in the 43-57%. Figure 1 shows a comparison of the chromatographic profiles (TIC) of a Tuscan and a Sicilian sample.
The PCA-LDA approach previously developed for supporting the panel test in virgin olive oil classification [7] correctly classified 57 out of the 58 samples in the EVOO category, confirming that this HS-SPME-GC-MS method gives reliable chemical data suitable for oil classification.
At the same time, our previous ANOVA-LDA model, developed for classifying virgin olive oil according to its geographical origin (Italy, Spain, Tunisia, Portugal, Greece, Other) was applied to the 58 samples. The method used quantitative data of only 25 out of the 73 quantified VOCs, and 57 out of them were correctly classified in the Italian category, only one sample was misclassified as Tunisian.
These findings confirm that the chemometric approaches developed using chemical data from HS-SPME-GC-MS quantification of 73 VOCs in virgin olive oils are suitable for the quality assessment of Italian EVOOs, in that 98.3% of samples were correctly classified according with both their geographical origin and classification in the EVOO category.

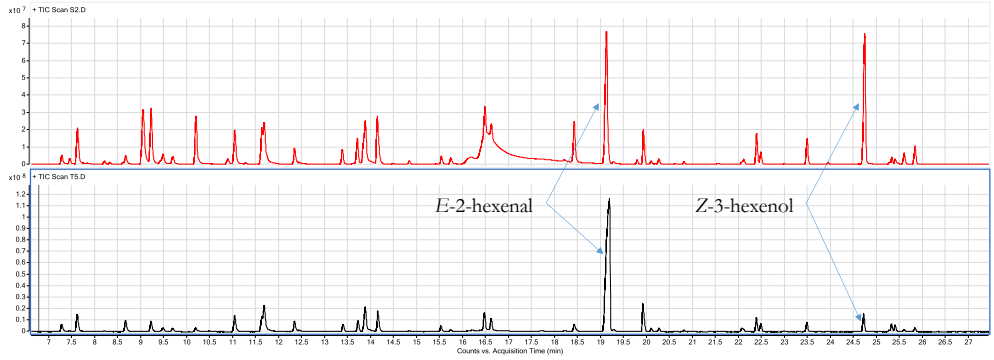

Fig. 1. *Comparison of chromatographic profiles (TIC) of a Tuscan (black) and a Sicilian (red) extra virgin olive oils*

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P24

Determination of ten mycotoxins on Moroccan medicinal and aromatic plants by liquid chromatography coupled to tandem mass spectrometry

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Summary: *Medicinal plants are subjected to contamination by various fungi producers of mycotoxins. The present investigation gives the first data on ten mycotoxins distribution in medicinal plants samples from Morocco. AFs, OTA, ENs and BEA were analyzed and carried out by liquid chromatography tandem mass spectrometry (LC-MS/MS). Results showed that AFG1 (9%), AFG2 (3%), ENB (3%) and ENA1 (5%) were present in analyzed samples (n=40).*

Keywords: *medicinal plants, Morocco, mycotoxins*

Introduction

Medicinal and aromatic plants (MAP) are widely used as home remedies and raw materials for the pharmaceutical industries. In Morocco, herbal remedies are used in the prevention, treatment and cure of disorders and diseases since ancient times. However, during harvesting, handling, storage and distribution, medicinal plants are subjected to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins. Aflatoxins (AFs) and ochratoxin A (OTA) have been widely studied, nevertheless, emerging *Fusarium* mycotoxins mainly enniatins (ENs) and beauvericin (BEA) have been studied in the last decade. In fact, EFSA has elaborated a scientific opinion about the risk to humans related to the presence of ENs and BEA in European food [1]. Very limited information has been provided regarding the occurrence of mycotoxins in Moroccan medicinal plants.

Due to the fact, that aromatic plants are a staple in Moroccan diet and it are often consumed as digestive and as home remedies, it is interesting to study the presence of mycotoxin in MAP from Morocco. The presence of AFB1, AFB2, AFG1, AFG2, OTA, ENA, ENA1, ENB, ENB1 and BEA was studied in samples of medicinal plants largely used in Morocco.

Experimental

A set of 40 samples which included *Rosmarinus officinalis* (n=6), *Matricaria chamomilla* (n=5), *Origanum vulgare* (n=12), *Verveine officinale* (n=5), *Mentha* (n=2), *Myrtus communis* (n=6) and *Lavandula Intermedia* (n=5) were collected from different local markets in Rabat (Morocco) analyzed in order to investigate the presence of mycotoxins.

For such evaluation, a method developed by Pallarés et al. (2019) [2] based on a dispersive liquid–liquid microextraction procedure (DLLME) was followed;

analysis was carried out by liquid chromatography tandem mass spectrometry (LC-MS/MS) using a LC Agilent 1200 couplet to a 3200 QTRAP®ABSCIEX equipment. Sensitivity was evaluated by limit of detection (LD) and limit of quantification (LQ), which were estimated for a signal-to-noise ratio (S/N) ≥ 3 and ≥ 10, respectively, from chromatograms of samples spiked at the lowest level validated. Recoveries and matrix effect were studied. Matrix effect (ME) was assessed for each analyte by comparing the slope of the standard calibration curve (a standard) with that of the matrix-matched calibration curve (a matrix), for the same concentration levels. For linearity evaluation, matrix-matched calibration curves with all the analyzed mycotoxins were constructed at concentration levels between 0.25 and 250 µg/kg.

Results

LD values were between 0.01 (ENB) and 0.955 (AFB2) µg/kg and LQ values were between 0.04 (ENB) and 3.2 (AFB2) µg/kg, the average linearity was 0.993. Good recoveries and ME were observed, which were above (97±15)% and (15±3)%, respectively.

The results of the detected mycotoxins in analysed samples are shown on Fig. 1. AFG1 was the most detected mycotoxin with an incidence of 9%. However, the highest levels detected were for AFG2, in a *Rosmarinus officinalis* plant sample with 2.5 µg/kg (Fig. 2).

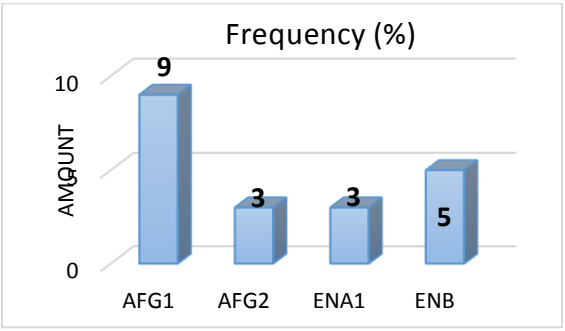

Fig. 1. *Frequency of detected mycotoxins in analyzed samples*

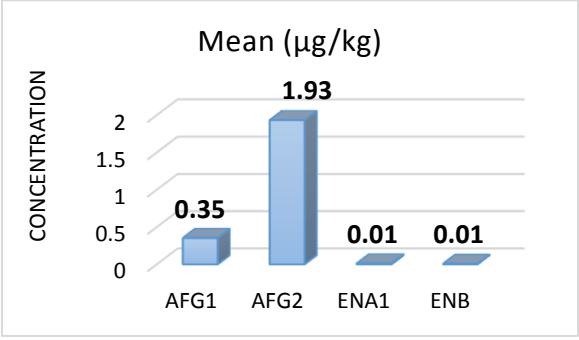

Fig. 2. *Mean values from detected mycotoxins in contaminated samples.*

Conclusions

The analytical procedures used herein were suitable to quantify AFs, OTA, ENs and BEA mycotoxins in medicinal plants.

The present investigation gives the first data on ten mycotoxins distribution in medicinal plants samples from Morocco. Our findings showed that only two AFs (AFG1 & AFG2) and two ENs (ENB & ENA1), at lower levels than the European MRLs [3], were present in the samples.

In our opinion, further studies are required for strict food security policies worldwide. Still, a focus on plants is also relevant to reach larger spectra of mycotoxins contamination knowledge and their contribution to any possible toxicological issues.

Acknowledgement: This research has been supported by the Spanish Ministry of Economy and Competitiveness [AGL2016-77610-R].

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P25

16-O-methylated diterpenes in green *Coffea arabica*: UPLC-MS/MS method optimization and validation

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Summary: *A new UHPLC-MS/MS method has been developed for a rapid identification and quantification of 16-O-methylated diterpenes, such as 16-O-methylcafestol and 16-O-methylkahweol, present in the unsaponifiable fraction of green C. arabica coffee beans.*

Keywords: *coffee diterpenes, mass spectroscopy, green coffee arabica beans*

Introduction

Coffee diterpenes are the main constituents of the coffee oil unsaponifiable fraction. The three most important diterpenes are cafestol, kahweol and 16-O-methylcafestol (16OMC) and they are produced only by plants of *Coffea* genus. The 16OMC for a long time has been considered present exclusively in Robusta and so, it has been reputed an excellent authenticity marker for the presence of Robusta in coffee products [1]. Recently, in addition to these three major diterpenes, 16-O-methylkahweol (16OMK) has been identified and quantified, for the first time, in Robusta coffee [2]. For the quantification of coffee diterpenes, NMR has proved very useful: the diagnostic NMR peak at 3.16-3.18 ppm, whose area has been extensively used to detect the presence of Robusta in a coffee sample, derives from the H21 methyl groups of both esterified 16OMC and 16OMK [3,4]. Moreover, to improve the detection limit of Robusta in roasted coffee samples, *Gunning et al.* (2018) [5] recently made a change to the sample preparation procedure used up to that point to quantify the 16OMC by NMR. This improvement in limit of detection has led to the surprising discovery of very low levels of 16OMC and 16OMK in Arabica coffees, in contrast to previous studies.

The aim of this work is to develop a new reliable LC-MS/MS method for identification and quantification of 16-O-methylated diterpenes in green *Coffea arabica* beans, able to distinguish both 16OMC and 16OMK.

Materials and Methods

Arabica green coffee beans from different geographical origin were used. The 16-O-methylated diterpenes were extracted following the *de Souza & Benassi* method [6] with some modifications and then LC-MS/MS analysis was performed.

UPLC-MS/MS analyses were performed with a Agilent 1290 UPLC interfaced to a SCIEX Triple Quad™ 4500. The chromatography column was a Waters ACQUITY HSS T3 C18 (2.1 x 100 mm, particle size 1.8 µm) maintained at 30 °C with a flow rate of 500 µl/min. A two solvent system was used. Solvent A: 0.1% formic acid in water, solvent B: acetonitrile. The linear gradient was as follows: 0 min, 40% A; 6.5 min. 40% A; 9.5 min, 20% A; 10 min, 40 % A. The injection volume was 10 µl.

The mass spectrometer was run in positive ion electrospray, acquiring in Multiple Reaction Monitoring (MRM) that guarantees the specificity of the method. The source temperature was 350 °C.
The method was validated in terms of specificity, linearity, concentration range, limit of detection (LOD), limit of quantification (LOQ) and repeatability [7].

Results and discussion

To better distinguish between 16OMC and 16OMK in Arabica green coffee extracts, we developed a quantitative analysis by means on UPLC-MS/MS acquiring in Multiple Reaction Monitoring (MRM).
Specific compounds transitions (331 → 299 m/z; 331 → 281 m/z) and (329 → 297 m/z; 329 → 279 m/z), which indicate the presence of 16OMC and 16OMK respectively, were confirmed based on literature data and comparison with reference solutions of 16OMC and kahweol, respectively for the 16OMC and the 16OMK. Extracted ion chromatograms confirmed retention time of 1.65 min for kahweol, 3.28 min for 16OMK and 3.54 min for 16OMC. The quantification of 16OMC in all samples was performed using a 16OMC standard solution calibration curve (concentration range of 2 - 100 µg/ml; regression coefficients > 0.998) and the data were treated with MultiQuant software. The LC-MS analysis also revealed the presence of 16OMK, which, together with 16OMC, contributes to the 3.16 ppm ^{1}H NMR marker peak. The quantification of 16OMK in Ethiopia green coffee sample was performed by using a kahweol standard solution calibration curve (concentration range of 1 – 10 µg/ml; regression coefficients > 0.995) since 16OMK standard is not commercially available.
The specificity of the method was guaranteed by the use of the MRM mode. The relative standard deviation of the MRM1/MRM2 signal ($RSD_{MRM1/MRM2}$) and the operating conditions were optimized using a 16OMC standard solutions. To evaluate the linearity of the method, eight standard solutions with concentrations ranging from 2 to 100 µg/ml 16OMC and 1-10 µg/ml kahweol were analyzed on three different days. The repeatability of the method was determined by extracting ten times 16-O-methylated diterpenes from Ethiopia green coffee beans.

Conclusions

The developed UPLC-MS/MS method allows to rapidly identify and quantify both 16OMC and 16OMK, in Arabica green coffee beans, and then to overcome the limitation of using NMR methods in determining the 16OMC/16OMK ratio. Method validation in terms of specificity, linearity, concentration range, limit of detection (LOD), limit of quantification (LOQ) and repeatability was accomplished.

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P26

UHPLC-MS/MS method for rapid quantification of chlorogenic acids in roasted coffee

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Summary: *The aim of this work is the development and validation of an analytical method for the quantification of major chlorogenic acids in coffee. The method was validated in terms of specificity, linearity, concentration range, limit of detection (LOD) and limit of quantification (LOQ), precision and accuracy, checking the obtained results against an intercalibration circuit for roasted coffee.*

Keywords: *UHPLC-MS/MS, caffeoylquinic acids, roasted coffee*

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), feruloylquinic acids (FQA) and p-coumaroylquinic acids (p-CoQA) [1].
There's a growing interest for complete and unambiguous identification of this class of compounds that take part in the generation of color, flavor and aroma of coffee during roasting and that in the last decades were also object of numerous studies to evaluate their impact on human health. The aim of this work is to develop a new reliable and fast LC-MS/MS method for quantification of major chlorogenic acids in roasted coffee, optimizing the procedure described by the validated method [2] and following the AOAC guidelines [3].

Experimental

Acetonitrile, methanol and formic acid were purchased by Sigma Aldrich (HPLC analytical grade), chlorogenic acid (5-CQA) was purchased by PhytoLab (Germany). Water obtained from milliQ system (Millipore, France).
Roasted ground coffee was provided as reference material for proficiency test LVU Lippold (LVU, Germany) and extracted with a mixture of methanol and water and ultrasonic bath at 60°C for 15 min.
Before injection the coffee extracts were centrifuged and filtered, where different membrane filters were evaluated: Phenex nylon membrane 0,20 µm (Phenomenex), Reliaprep RC regenerated cellulose membrane 0,20 µm (Ahlstrom), mini-UniPrep PTFE filter media 0,20 µm and mini-UniPrep PVDF filter media 0,20 µm (Whatman).
LC-MS analysis were performed on a Agilent 1290 system coupled to a Sciex triple quad 4500. Chromatographic separation was achieved with a 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 µl/min with the following elution programme: 0 min 95% A, 5% B; 8 min 85% A, 15% B; 11 min 60% A,

40% B; 11,5 min 0%A, 100%B; 13 min 0% A, 100% B; 13,5 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-caffeoyl quinic acid solution and 3,5-dicaffeoylquinic acid solution. For quantification two MRM transitions were evaluated for every compound (1 quantifier and 1 qualifier) based on previous analyses and literature data. Collision energies (CE) were optimized to maximize the transition signals (precursor ion to product ion). Multiquant 3.0.2. software was used for data processing.
Standard stock solution (200 mg/L) of caffeoylquinic acid was prepared with H2O/MeOH 75/25 v/v. Working solutions of 5-cqa over the concentration range 0,5 – 20 mg/L were obtained from stock solution with water. Stock solution were stored at 4°C for 48h, calibration standards were stored at 4°C for 24h.

Results

After extraction and centrifugation the samples were filtered: four types of membrane filters were used, chlorogenic acids recoveries after filtering were tested with a sample solution of roasted coffee: nylon filters resulted to have the worst performances, PVDF filters were the best options in terms of retention efficiency and RSD %. 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 [4].

Table 1. *method validation parameters*

Parameters		5**-caffeoylquinicacid**
Retention time (min)	Mean	3.98
	RSD %	0.4
MRM1/MRM2	Mean	1.6
	RSD %	3.0
Linearity range (µg/mL)		0,05 – 13,5
	Calibration curve	Y = 568843x+140999
	Correlation coeff (R^2)	0.993
LOD	3 $S_{y/x}$/b	0.013
LOQ	10 $S_{y/x}$/b	0.04
Accuracy		100.0 ± 4.1
Repeatibility	0.54 – 2.7 – 5.4 µg/mL	9,5% - 8,7% - 6,4%

Conclusions

In conclusion we developed and validated an analytical method for the quantification of major chlorogenic acids in coffee. This is the first step of a wider project that aim to complete the identification and quantification of minor compounds of this class and that could be applied not only for roasted coffee but also green coffee and other plant parts in order to characterize the antioxidants profile of leaf, pulp and silverskin.

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P27

Omega-3 enriched *Hermetia illucens* as novel ingredient for insect-based food for the future: influence of growth substrate based on coffee-roasting by-product and microalgae

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Summary: *The aim of this work was to find alternative eco-sustainable rearing substrates for the growth of omega-3-enriched Hermetia Illucens (HI) as future ingredient for insect-based food. High percentages of unsaturated fatty acids, particularly of omega-3, were detected in HI prepupae reared on coffee-roasting by-product (coffee silverskin) enriched with 10%, 20% or 25% of microalgae Schizochytrium sp.*

Keywords: *Hermetia illucens; coffee silverskin; microalgae; fatty acids*

Introduction

Due to the rapid increase in world population, the production of enough food for humans represents a serious challenge for the future. Insects may represent a valuable alternative ingredient for food production in a new interesting approach of sustainable circular economy, since they show high reproductive rate and nutritional value and can grow on organic by-products [1]. EFSA Scientific Committee in 2015 proposed a list of insect species with the greatest potential as food and feed ingredients in the EU, including *Hermetia illucens* (HI, Diptera, Stratiomydae). The aim of this work was to find environmentally friendly rearing substrates for the growth of PUFA-enriched HI, to be used as a perspective food ingredient. At this purpose, insect feeding substrates based on the re-use of coffee silverskin, the main by-product of the coffee-roasting industry, enriched with various percentages of microalgae as PUFA-source (i.e., *Schizochytrium* sp. or *Isochrysis* sp.), were tested.
The fatty acid (FA) profile of HI prepupae were determined by Gas Chromatography-Mass Spectrometry.

Experimental

Rearing of Hermetia illucens larvae. Nine feeding substrates were tested: 1 control (coffe silverskin 100%), 4 added with 5, 10, 20 or 25% *Schyzochytrium* sp., and 4 added with 5, 10, 20 or 25% *Isochrysis sp*. Larvae were reared at a density of 0.3 ind./cm^2 in a climatic chamber at a 27±1°C temperature, 65±5% relative humidity, in continuous darkness. Prepupae were manually collected and stored at -80°C for further analyses.
Lipid extraction and fatty acids analysis. To determine the total lipid content and the overall FA profile of prepupae, samples were thawed, and homogenized. Aliquots of 200 mg were added with 100 µl of Internal Standard (methyl ester of nonadecanoic acid,), and extracted overnight [2] Lipid extracts

were evaporated under laminar flow inert gas (N_2) until constant weight and determined gravimetrically. GC-MS analysis was carried out on three aliquots *per* sample. The extracted lipids were resuspended in n-epthane to transesterify fatty acids with sodium methylate. FAMEs were determined on an Agilent-6890 GC equipped with a split-splitless injector and coupled to an Agilent-5973N quadrupole Mass Selective Detector. Instrumental conditions were as reported in Truzzi et al. [3]. The method performances were as those obtained in Vargas et al. [1]. Moreover, the method showed a good accuracy and precision.

Results

In general, the inclusion of microalgae determined in both feeding substrates and in HI prepupae an increase of total lipid content. The inclusion of *Schizochytrium* sp. in substrates (Fig. 1a) determined, in HI prepupae, a statistically significant decrease in saturated fatty acids (SFA) and a statistically significant increase in polyunsaturated fatty acids (PUFA), in particular of omega-3 (n3) such as 22:6n3 (DHA) and 20:5n3 (EPA), with respect to prepupae HI E, reared on control substrate. Moreover, prepupae HI Bs, HI Cs, and HI Ds showed a similar FA composition, especially in relation to unsaturated FAs. The same trends can be noted for prepupae reared on coffee silverskin added with *Isochrysis* sp. (Fig. 1b), but they showed lower percentages of PUFA and higher percentages of SFA than prepupae reared on substrates enriched with *Schizochytrium* sp.

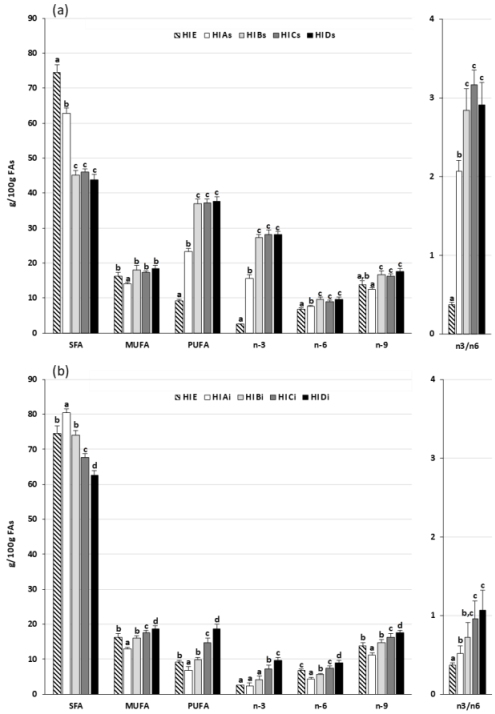

Fig. 1. *FA classes (g/100 g FAs) of HI prepupae reared on control substrate (HI E) and on substrates enriched with 5% (HI As), 10% (HI Bs), 20% (HI Cs) and 25% (HI Ds) of Schizochytrium sp. (a) or with 5% (HI Ai), 10% (HI Bi), 20% (HI Ci) and 25% (HI Di) of Isochrysis sp. (b).*

Conclusions

Schyzochytrium sp. looks to be a very good PUFA source, given that not only the percentage of n-3 in the larvae was increased, but also the total lipid content. The substrate enriched with a 10% inclusion level of *Schizochytrium* sp. should be considered the most convenient one since a greater inclusion of microalgae does not bring benefits in terms of nutritional value of HI prepupae. Thanks to fat quality (specifically in terms of PUFAs), these PUFA-enriched HI prepupae deserve a special attention as food ingredient in the future.

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P28

Tips and tricks for handling high purity water in the LC-MS laboratory

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Summary: *As ultrapure water is the most-frequently used solvent in LC-MS laboratories, its purity plays a critical role in analyses. The data presented in this study will show the potential risks involved in poor ultrapure water handling practices in an LC-MS laboratory, and give useful tips to avoid them.*

Keywords: *LC-MS; Ultrapure water; Contamination*

Introduction

Ultrapure water is highly prone to contamination, e.g. it easily leaches contaminants out of container surfaces and absorbs contamination from the laboratory environment. As ultrapure water is the most frequently used solvent in any LC-MS laboratory, its purity plays a critical role in analyses. There are a number of high purity water handling pitfalls that result in degradation of its quality. To help analysts critically evaluate the potential risks involved in poor ultrapure water handling, we discuss here

- the effect of laboratory environment and long high purity water storage
- the effect of the container material used to collect ultrapure water
- the effect of laboratory ware and equipment cleaning
- the effect of poor practices of water purification system usage.

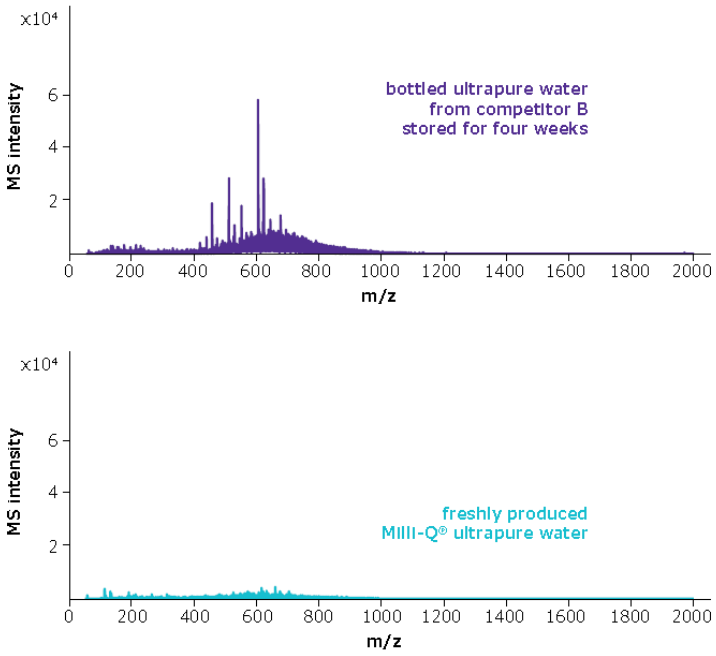
Laboratory Environment and Water Storage

Different types of contamination are present in laboratory environment (organics, alkali metals, bacteria, particles), thus water exposure to the lab atmosphere and usage of various decanting steps results in water quality degradation. The practice of long-time storage of ultrapure water increases the risk of water contact with the lab environment. This results in contamination leading to high MS background noise, adduct formation and signal suppression.

Container Material

Use of plastic devices such as bottles or funnels during handling and storing of water leads to leaching of ubiquitous additives (anti-static agents, stabilizers and plasticizers) and causes ghost peaks and increased background noise. It is recommended to collect ultrapure water in either surface treated brown glass bottles or in borosilicate glass. In standard glass bottles silica and alkali dissolve and form adducts.

Glassware Cleaning
Dishwashers are operated utilizing strong bases and surfactants. The former lead to dissolution of alkali and silica from glassware, while traces of the latter remain on the glass surface.



Water Purification System Usage
Water stagnating in the water purification system may degrade with time. Also contaminants present in the lab environment can become absorbed by the final polisher membrane and contaminate water during its collection. Thus, it is recommended to properly flush a system prior to water collection, e.g. with several liters after the weekend or ~ 250-500 mL when system is used every day.

Conclusions
Properly installed and well-maintained Milli-Q® water purification systems meet stringent LC-MS requirements for solvent purity. The best results are obtained when using freshly collected ultrapure water without long storage or exposure to the lab environment. Alternatively, ultrapure water should be collected in clean borosilicate glass not processed in a dishwasher.

P29

Innovative sampling approaches for drinking water in the analysis of emerging pollutants by liquid chromatography - tandem mass spectrometry

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Summary: *Two innovative sampling and preconcentration methods were optimized, employed and compared for the determination of trace levels of emerging pollutants in waters by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS).*

Keywords: *drinking water, sampling and preconcentration, emerging pollutants*

Introduction

In recent years, growing attention has been paid to safety of food and drinking water, making necessary the adoption of policies for water sources protection and the development of sensitive and rapid analytical methods to identify micropollutants. In this framework emerging contaminants represent one of the most challenging issue because of their occurrence, fate and concentration levels in the different environmental compartments [1]. The Drinking Water Directive (*Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption*) is intended to protect human health by indicating healthiness and purity requirements which must be met by drinking water within the Community. Among the wide range of emerging pollutants, perfluorinated compounds and various pharmaceuticals, such as nonsteroidal anti-inflammatory drugs, are probably the most studied. Usually, expected levels of these analytes in water are particularly low; therefore, sensitive and selective analytical techniques are required for their determination.

Results and discussion

Sampling and preconcentration are fundamental steps to reach the low detection limits required [2]. In this work, two innovative sampling approaches, namely polar organic chemical integrative sampler (POCIS) and thin film microextraction (TFME) were employed and compared for the determination of various emerging pollutants in water by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Some of the considered analytes were: diclofenac, ketoprofen, mefenamic acid, naproxen, ibuprofen, perfluorooctanoic acid, perfluorooctanesulfonate and caffeine. The two sampling and preconcentration methods were developed optimizing the most relevant variable involved, spiking tap water with known amounts of the analytes. Application to real water samples will be also presented and discussed.

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P30

Analysis of Bisphenol A in foods using solid phase micro-extraction with an overcoated fiber

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Summary: *The use of SPME was revisited in order to develop a quick, easy and sensitive method for analysis of BPA in a variety of food products*

Keywords: *SPME, Food, BisPhenol A*

Bisphenol A (BPA) is commonly used for food packaging applications such as polycarbonate bottles, and the linings of metal cans used for soups, juices, etc. It is a suspected endocrine disruptor, and thus low level, long term exposure as a result of migration into food from packaging materials is a concern. Extraction methods for determination of BPA in food include both solvent and solid phase extraction, with the later more commonly used with liquid samples and the former for solid samples. Analysis can be done by either LC or GC, and both have been used throughout the literature. Solid phase microextraction (SPME) has been used for the determination of BPA in water, but has not been widely used for this application in food matrices due to sensitivity and fiber ruggedness issues associated with exposure to matrix components such as fats and proteins. In this work, the use of SPME was revisited in order to develop a quick, easy and sensitive method for analysis of BPA in a variety of food products. Matrix and fiber durability issues with immersion SPME were addressed through the use of an overcoated (OC) divinylbenzene (DVB) fiber. The overcoating, which consists of polydimethylsiloxane (PDMS), protected the DVB layer from contamination, and increased the physical robustness of the fiber. SPME extraction using the OC-DVB fiber was followed by GC/MS/MS analysis for optimum sensitivity. The steps taken in method development and optimization will be described, as well accuracy in a variety of matrices. Data will also be presented on method ruggedness compared to a standard, non-overcoated DVB fiber.

P31

Analysis of pesticides in paprika - Development of an SPE cleanup method

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Summary: *An SPE cleanup using a new dual layer, multi-sorbent cartridge was developed for cleanup of paprika extracts in the analysis of pesticide residues by LC/MS/MS and GC/MS/MS*

Keywords: *Spices, Pesticides, clean-up*

Paprika is a spice made from the drying of sweet peppers, and used for flavor and color in many types of cuisine. Pesticides applied to the peppers during cultivation can carry through the drying process, ending up in the dried paprika spice. Paprika is a commonly used spice, thus, in the interest of food safety, testing for the presence of pesticide residues is of great interest. For pesticide analysis, the "Quick, Easy, Cheap, Effective, Rugged and Safe" (QuEChERS) approach has become a popular method for extraction of various commodities, including spices. However, the background resulting from dried spices can be problematic. Conventional QuEChERS cleanup may not be thorough enough for these types of samples. In this work, an SPE cleanup using a new dual layer, multi-sorbent cartridge was developed for cleanup of paprika extracts in the analysis of pesticide residues by LC/MS/MS and GC/MS/MS. The cartridge differs from conventional dual layer products containing carbon and PSA or aminopropyl silica in that it is much smaller, requiring less solvent for processing. It also contains blends of sorbents optimized to reduce oil and pigment background, while producing better pesticide recoveries than larger cartridges containing graphitized carbon black. The steps undertaken to develop the cleanup method for paprika extracts are described, and method accuracy and reproducibility are reported using replicates spiked at 50 ng/g with a variety of pesticides.

P32

Flavonoids annotation using a product ion-dependent MS^n data acquisition method on a Tribrid Orbitrap mass spectrometer

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Summary: *The new LC-MS^n workflow enables improved throughput, identification coverage and confidence for flavonoids profiling experiments*

Keywords: *Metabolomics, Flavonoids, Orbitrap*

Introduction

Flavonoids are secondary metabolites that play important biological roles in plants. Flavonoids are found in almost all fruits and vegetables, and are powerful antioxidants with anti-inflammatory and immune system benefits. The untargeted profiling of flavonoids provides insights into their biological functions and potential health benefits for humans. However, comprehensive identification of flavonoids remains challenging because of the limited availability of authentic standards and the structural diversity of this class of compounds. Previous studies relied upon extensive expert knowledge about fragmentation rules and structures of flavonoids. Plus, simple MS-MS based analyses are often not sufficient for complete structural characterization. Here we present a new flavonoid profiling workflow that uses comprehensive fragment ion information from HCD/CID MS-MS and higher order CID $FTMS^n$ for rapid flavonoid annotation on an Orbitrap ID-X™ Tribrid™ mass spectrometer.

Method

All the MS and MS^n data were collected with Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer. We collected MS/MS spectra on precursor ions detected in the survey MS scan within a 1.2 second cycle time. High order $MS^{n\ (3\text{-}4)}$ spectra were only collected when the MS detected the sugar neutral loss fragment ions in MS/MS and/or $MS^{3\text{-}4}$ data. The MS^n spectral tree data were processed using Thermo Scientific™ Mass Frontier™ 8.0 and Thermo Scientific™ Compound Discoverer™ 3.0 software. Multiple databases were employed in the processing workflow including mzCloud™, ChemSpider, Arita lab flavonoid structure database[1] and a custom flavonoid mass list for unknown flavonoid structure annotation.

Results and Conclusion

Collecting more structurally relevant fragment ion information from the high order of $MS^{n\ (3\text{-}5)}$ enables the identification of more unknown flavonoid compounds from natural products. Two times more flavonoids were annotated using this new LC-MS^n workflow from three fruit and vegetable juice samples.

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P33

Multidisciplinary approach to determine botanical and geographical origin of durum wheat, semolina and pasta

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Summary: *The multidisciplinary approach described (DNA microsatellite analysis, stable isotope ratio analysis and untargeted metabolomic fingerprinting) is useful to support brand protection and to satisfy consumers' expectation regarding food quality as it allows to discriminate durum wheat semolina samples of 100% Italian origin and samples from other countries.*

Keywords: *DNA, Isotope Ratio, Metabolomics.*

Introduction

In the food industry, the value of raw materials and finished products can be linked to their botanical and/or geographical origin.
This study is an example of how a proper sampling procedure together with a multidisciplinary analytical approach are able to assist food industry in brand protection and to meet consumers' expectations on food quality.

Experimental

The determination of the geographical origin of durum wheat (Triticum durum) was analyzed from different points of view in order to obtain a global view. The multidisciplinary approach involves three types of analysis: variety identification, stable isotope ratio analysis and untargeted metabolomic study. The sampling design took into account both the geographical origin and the harvest period.
First step involves PCR technology (fragment analysis) from durum wheat samples by determining the distribution of microsatellites (DNA fragments with repetitive sequences) specific for single variety.
The second step involves the analysis of the isotopic ratio of the main light elements (C, H, O, N, S) constituting the entire sample that can be correlated with specific physical and geological parameters of the territory where it was cultivated.
Metabolomics with Mass Spectrometry (the study of targeted and non-targeted metabolites), when based on robust and significant experimental design of construction and systematic maintenance of the reference database - completes the picture by providing a useful and final framework of the sample in relation to its botanical and geographical origin.

Results

Microsatellite analysis, stable isotope ratio analysis and untargeted metabolomic fingerprinting together with a suitable method for multivariate classification permit to verify food integrity from multiple perspectives such as

variety identification, growing conditions of the plants (climate conditions and soil composition) and analysis of plant metabolite

Conclusions

The results presented show that the multidisciplinary approach described is useful to support brand protection and to satisfy consumers' expectation regarding food quality as it allows to discriminate durum wheat semolina samples of 100% Italian origin (from "Armando" production chain) and samples from other countries (the samples analyzed came from the 2017-2018 production year).

P34

LC simulation software employment for complex chromatographic method development in food and pharmaceutical analysis

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Summary: *LC simulation software employment for complex chromatographic method development and for reduction of development time in food and pharmaceutical analysis.*

Keywords: *Chromatography, Impurities, Degradation Products*

Introduction

In food and pharmaceutical analysis, the chemical characterization of potential toxic substances is a key requirement, for example in development of forced degradation and shelf-life studies on raw materials and finished products. The identification of unknown molecules is one of the cornerstone applications of mass spectrometry, but chromatography remains a critical prerequisite technique to separate components during stability indicating method development.

However, chromatographic method development is not a trivial process, especially when attempting to achieve efficient selection, setting, and optimization of all parameters. Currently, there are powerful software solutions that simulate LC separations in order to assist researchers by significantly reducing the time needed to achieve sufficient resolution of key analytical signals.

Experimental

In the present work(already presented in HPLC 2019 Symposium), the software ACD/Method Selection Suite has been applied to optimize chromatographic separation and resolution of 11 key analytical signals [active pharmaceutical ingredient (API), API-derived impurities and matrix interference peaks] that were detected by Reverse Phase UHPLC-UV/DAD. The need for a method for the unambiguous determination of assay and impurities in finished drug products is critical when dealing with correlated substances on APIs and thus with the need of stability indicating method. The presented approach can also be applied in food analysis to evaluate if purity criteria of ingredients, substances added for nutritional purposes and food additives used in the manufacture of food supplements, novel foods and for food fortification and enrichment comply expected acceptance criteria.

Results

A complete separation of all peaks was obtained performing few experiments.

Conclusions

The selected software and methodology proved to be the keystone for the development of an efficient chromatographic method and for the reduction of development time.

P35

Characterization of the main phenolic compounds in agri-food by-products by HPLC-DAD-MS

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Summary: *The work is targeted to investigate on the composition of by-products obtained from the production of fresh vegetables for the market. The nutraceutical properties of green-red pepper and basil productions were studied defining the phenolic profile by HPLC-DAD-MS of their aqueous extracts obtained by a TIMATIC extractor.*

Keywords: *flavonoids, rosmarinic acid, extraction optimization*

Introduction

The processing operations of fruits and vegetables produce significant amounts of wastes (approx. 25%-60%), nowadays known as by-products [1]. Indeed, rather than as wastes, they are recognized as a source of valuable bioactive molecules, as carotenoids, phenolic compounds, dietary fiber and vitamins with several beneficial health attributes. Food by-product promotion became one of the most interesting trend for eco-sustainability of several crops [2], with the final goal to have productions with almost zero wastes. Their valorisation involves at least some key steps, as extraction optimization and chemical characterization of the extracts. The aim of this study was the characterization by HPLC-DAD-MS of extracts obtained by the application of green and efficient extractions to by-products from green-red pepper and basil productions. The final goal was to provide the best phenolic extraction yields by using the TIMATIC extractor.

Experimental

The extraction conditions have been previously applied at laboratory scale to predict the extraction capability of the TIMATIC system (Technolab Perugia). The tested parameters were time (60, 120 and 180 min), temperature (30°C, 50°C, and 90°C) and solvent used (from water 100% to ethanol 10%). The extractions by TIMATIC were performed through several extractive-cycles under pressure (max 7 bars). All extractions were carried out applying a drug/solvent ratio of 1:20 (w/v). The extracts obtained in all the tests were analyzed by an HPLC system coupled with a DAD and a LTQ (Thermo Scientific, Germany) ion trap mass spectrometer. A Restek column 150 x 3 mm, 5 µm (Raptor, USA) was employed. Both negative and positive ionization were applied for optimizing the fragmentation conditions of the different compounds present in the extracts.

Quantification of phenolic compounds has been performed by DAD using calibration curves of three external standards: gallic acid (280 nm), quercetin (350 nm) and rosmarinic acid (330 nm).

Results

The main phenolic component of the basil extracts was rosmarinic acid, with value up to 120 mg/g in the 100% aqueous extract obtained at 90°C with Timatic. The chromatographic profiles also showed the presence of other cinnamic acid derivatives, the most abundant of which was chicoric acid. Figure 1 reports the mass spectra of rosmarinic and chicoric acids from basil extracts. Regarding pepper extracts, the total phenolic content was lower with respect to basil, with the maximum value of 11.2 mg/g dry extract obtained with water extraction at 50°C; this extract was rich in highly methylated polysaccharides (approx. 7%). Several flavonoids were detected and the content was estimated in the range of 15-20% of the total phenolic amount. The identification of these flavonoids and their glycosides by the EI profiles and mass spectra allowed pointing out the presence of several C glycosides of luteolin, apigenin together with chrysoeriol-6-*C*-hexoside-8-*C*-pentoside and quercetin-3-*O*-rhamnoside-7-*O*-glucoside [3] as shown in Figure 2.

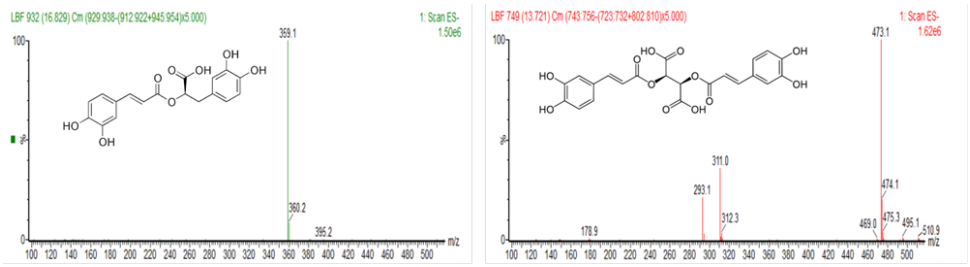

Fig. 1. *Mass spectra in negative ionization mode for rosmarinic (left) and chicoric (right) acids from basil extracts*

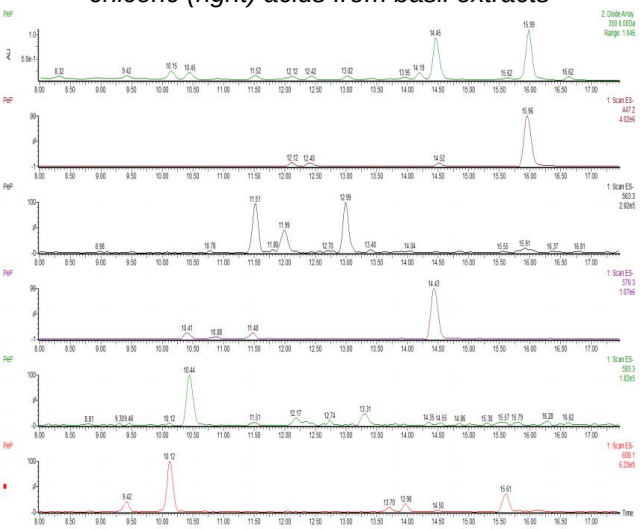

Fig. 2. *Chromatographic profiles of pepper extracts; all EI chromatograms are in negative ionization mode. From top to bottom: 1) UV-Vis profile at 350 nm; 2) EI at 447 Th (luteolin C-glucoside and isobars); 3) EI at 563 Th (apigenin C-exoside, C pentoside and isobars); 4) EI at 579 Th (luteolin 7-O-gluco-pentoside); 5) EI at 593 Th (chrysoeriol-6-C-hexoside-8-C-pentoside), EI at 609 Th (quercetin-3-O-rhamnoside-7-O-glucoside and its isobars).*

Conclusion

The results described in this work highlight the possibility to reuse some agricultural by-products by sustainable extraction processes, to obtain extracts potentially suitable as new functional food ingredients.

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P36

Characterization and valorization of ancient cereals by HPLC-UV and ICP-MS

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Summary: *Climate change, often associated with the increases in biotic and abiotic stress that occur with crop failure in some regions, becomes a limiting step in global grain supply. A possible solution to counter this effect improving the productivity of the agricultural sector could be the reintroduction of old crops varieties.*

Keywords: *Ancient grains, ICP-MS, HPLC*

Introduction

The ancient varieties proved more versatile than the main cereals due to their high tolerance to various biotic and abiotic stresses. In fact, they would be more resistant to temperature variations and since they require less water and fertilizer, they would reduce the impact of agriculture on the environment. Ancient cereals could be the answer to the current poor state of food security, satisfying the nutritional needs of a growing population and the necessity to use species without gluten, for subjects who are not celiac but sensitive to gluten [1, 2].

However, given the low or irregular performance of ancient cereals, careful research in this field becomes a priority to improve their cultivation.

The aim of this study is to select the varieties of ancient cereals suitable for local environmental conditions, in terms of resistance, productivity and nutritional value, in order to reduce the use of pesticides and human interventions and to identify a source of superior quality food from the nutritional point of view.

Experimental

Different varieties of cereals, ancient grains and not, were characterized from the chemical-nutritional point of view, determining the content of amino acids and minerals. These parameters are of particular interest to investigate plants used both as food and forage.

Trace and major elements were determined by inductively coupled plasma mass spectrometry (ICP-MS thermos Scientific iCAP RQ), according to Ranaldo et al. (2015) [3]. Samples were treated at minimum in fourfold and then measured 3 times. Seventeen elements such as iron (Fe), sodium (Na), potassium (K), calcium (Ca), manganese (Mn), Cu copper (Cu), boron (B), aluminium (Al), lithium (Li), selenium (Se), rubidium (Rb), phosphorus (P) and sulfur (S) were determined in all cereals samples.

The contents of amino acids (AA) in all the samples was determined by HPLC (High Performance Liquid Chromatography) equipped with an UV detector.
The method involved the acid hydrolysis of the samples and the derivatisation of the amino acids with 9-Fluorenyl-methyl chloroformate (FMOC). The quantification of AA was made using norleucine as internal standard.

Results

A significant difference in the nutritional profile of cereals were highlighted, both in the element and AA determinations. Particularly, the elements which showed the highest variations, were Mn, Zn, Fe, K, Ca. Spelt is rich in Fe, Zn, K, and Sr, wheat has a content of minerals similar or lower than ancient cereals, while oats is richer in Ca. The analysis in terms of major and trace elements composition evidence a similarity of P and S content in all the cereals.
It is interesting to analyse also the AA profile (Fig.1).

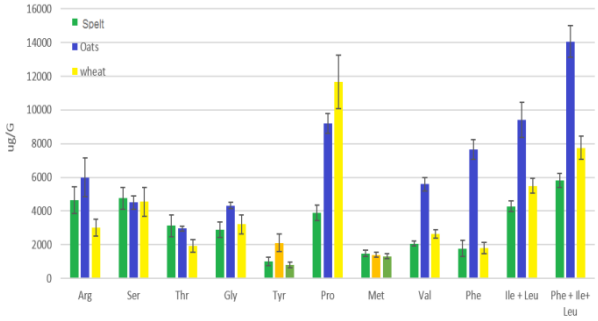

Fig. 1. *Amino acids concentrations in spelt (green line), oats (blu line) and wheat (yellow line)*

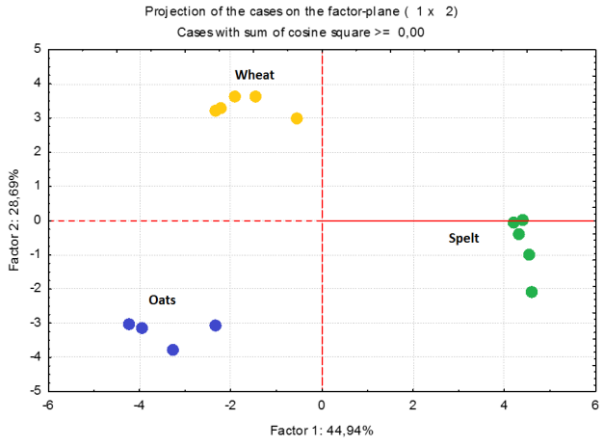

Fig. 2. *Scatter plot of samples on the first two PCs where yellow circle symbols designate wheat, blu circle symbols oats and green circle symbols spelt.*

Oats is reach in Glycine, Valine, Phenylalanine, Leucine and Isoleucine, while wheat presents a high content of Proline.

The evaluation of differences among plant groups based on all the chemical parameters was carried out by means of a principal component multivariate statistical method.

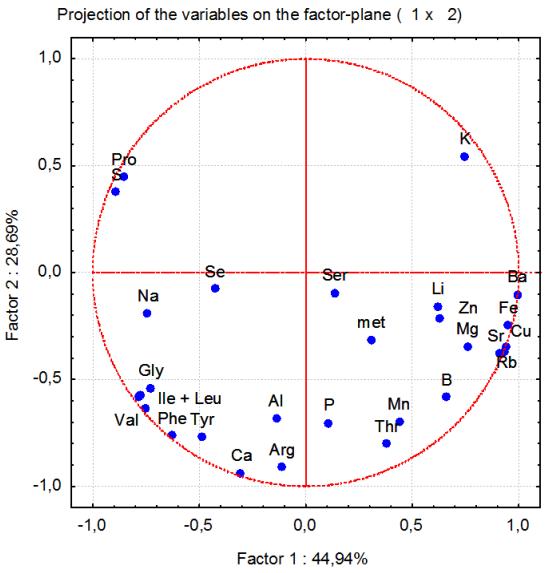

Fig. 3. *Plot of the component weights on the first two PCs*

The statistics used to summarise the most important results was the proportion of the total variance explained by the first two principal components (Fig. 2 and Fig. 3).
The first component explained the 45% of variance and divided spelt from the other two cereals. The second component defined the separation between wheat and oats. The variables that characterise spelt are Fe, Zn, Sr and K, while some amino acids as Isoleucine, Leucine, Phenylanine and Tyrosine are specific of oats.

Conclusions

This study allow to state that spelt an ancient grain, is richer in nutrient content with respect to oats and wheat. In particular, Fe and Zn result of great interest because of their role in the immune system, while Sr is studied in the process of stimulation of bone-producing cells.
On the other hand, oats presents a major concentration in amino acids.
Particularly, elevated is the content of Phenylanine, Tyrosine, Isoleucine and Leucine which are involved in proteins synthesis, regulate the sugar levels in blood and produces energy during intense physical activity. Wheat has a profile similar to those of oats but with a lower content of Arginine and Threonine. On the contrary, the concentration of Proline, which is fundamental in the production of collagen, is more elevated than in other cereals.
It is evident that our approach in which more parameters are detected with different techniques, associated to a statistical analysis can be very useful to study and identify cereals that could represent a source of superior quality food

from the nutritional point of view. At the same time, this method can be useful to detect those varieties that are more resistant in particular climatic conditions.

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P37

Identification of emerging contaminants in wastewater and uptake in *Beta vulgaris var. cicla* and *Allium cepa* by HPLC-HRMS

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Summary: *Emerging contaminants are often not completely removed in wastewater treatment plants and could be present in irrigation water. In this study the untargeted analysis conducted in effluents and in plants hydroponically grown in the same water demonstrate the presence of potentially harmful bioactive compounds and the importance of monitoring activity.*

Keywords: *HPLC-HRMS, emerging contaminants, bioaccumulation*

Introduction

Emerging contaminants have recently been discovered to be present ubiquitously in the environment; these compounds, which are often not completely broken down by wastewater treatment plants, include drugs, pesticides, surfactants, plastic derivatives [1]. Many emerging contaminants and their metabolites are commonly presents in shallow waters even if their emission levels are not regulated. The presence of these compounds in irrigation waters, as already highlighted by some studies, could produce accumulation phenomena on the foodweb, with negative effects on food quality and potentially compromising food security [2, 3]. Therefore the better comprehension of the presence and distribution of these compounds in irrigation waters, their uptake in plants and their potential accumulation through the foodweb it is a crucial point in order to assess the risk for human health connected to food consumption. In this study the untargeted analysis of emerging contaminants was conducted in the effluents of a wastewater treatment plant, generally used as irrigation water. Morover analysis were conducted on plants hydroponically grown in the same effluent. The results demonstrate the presence of potentially harmful bioactive compounds and the importance of improving the monitoring of effluents.

Experimental

Wastewater effluents has been roughly filtrated with filter paper; 250 mL has been extracted by solid phase extraction (SPE) with Isolute Multimode (500 mg/ 3 mL) previously activated with 5 mL of MeOH and 5 mL of H_2O; samples has been washed with 6 mL of water and eluted with 6 mL of MeOH. Finally these solution were concentrated to a final volume of 0.5 mL and analyzed. Plants of *Beta vulgaris var. cicla* and *Allium cepa* were hydroponically grown on wasterwater effluents for two months. After collection, plant samples were fast-cleaned with distilled water and freeze-dried in an Edward freeze-drying

machine. Samples were grinded and homogenized by using a ball mill (MM 400, Retsch, Verder Scientific, Haan, Germany) equipped with two PTFE vessel and grinder balls; samples were ground for 5 min with a vibration frequency of 20 Hz to achieve a final fineness of ≈ 5 µm.

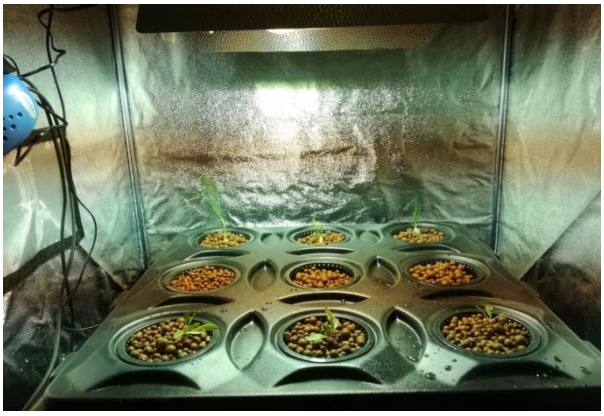

Fig. 1. *Shoots of Beta vulgaris var. cicla*

The sample treatment procedure was based on the protocol of De Vos et al. 2007 [4] as already described by Scalabrin et al. 2017 [5]. Briefly 50 mg of plant material were extracted for 30 min in an ultrasonic bath with 1.5 mL of MeOH/H_2O 75:25 (v/v) acidified with formic acid 0.1 % and then centrifuged for 20 min at 14,000 rpm; the supernatant was collected and filtered with PTFE syringe filters (Ø 25 mm, 0.2 µ m). The solution obtained was finally analyzed, together with extracted wastewater samples, by means of a HPLC-LTQ Orbitrap XL (Ultimate 3000 Dionex-Thermo Scientific). The column was an SB-Aq Narrow Bore RR 2.1 x 150 mm, 3.5 µm (Agilent Technologies, Wilmington, USA) eluted with H_2O 0,01% Formic acid e ACN 0,01% Formic acid. MS analysis was performed in Fullscan modality, at a resolution of 60000, in both positive and negative polarities, in order to identify the higher possible number of compounds. Data dependent analysis were also performed, to obtain a complete fragmentation spectra for compounds and permit their identification, according to Sumner et al., 2007 [6]**.**

Results

The results showed the presence of a number of m/z ions identified as pharmacological compounds or their metabolites in wastewater effluents, confirming the hypothesis that these substances are not completely removed during abatement processes. Moreover, plastic additives and a pesticide were also observed. Levels of these compounds varied during the different seasons; the highest concentrations were detected during spring and late summer. This trend could be particularly dangerous because plants are thought to uptake contaminants especially during the vegetative period and fruit maturation. This tendency, therefore, could lead to accumulation phenomena, especially in the edible part of plants and introduce contaminants in the foodweb, with unknown effects on human health.

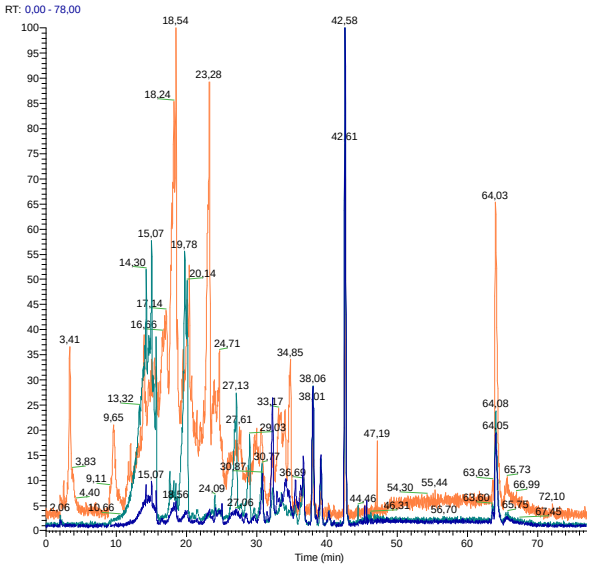

Fig. 2. *Representative chromatograms of wastewater effluents analyzed*

Conclusions

The results indicate the importance of monitoring the presence and concentration of emerging contaminants in wastewater plant effluents and in water used for irrigation. These potentially harmful bioactive compounds could accumulate food and compromise human health and food security. These first results need a more in-depht analysis (which is in course) in order to evaluate the real risk for human food consumption.

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P38

Chemometrics combined with untargeted mass spectrometry for the study of saffron adulterations

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Summary: *Triple quadrupole mass spectrometry was applied by using only Q1 to scan the entire mass spectra of several samples of saffron and other spices. The aim is to combine this untargeted approach to chmemometrics to detect, both qualitatively and quantitatively, possible adulteration of saffron with the other spices.*

Keywords: *Saffron, Chemometrics, Untargeted analysis*

Introduction

Saffron (*Crocus Sativus L.*) is probably the most expensive spices in the world, due to limited areas of production, the laborious process required to obtain the final product and, most of all, to its particular flavor. Therefore, it can be subject to fraud, as for example the undeclared addition of a cheaper spices, with the aim of illegally increase the seller's gain.
Turmeric, safflower, marigold, and garlic are, generally, the spices most used for saffron adulteration. Such spices are cheaper and their flavor can be confused with the saffron one, when mixed with it in low percentages.

Experimental

Single quadrupole mass spectrometry was used as untargeted analysis to check for possible adulteration of saffron with turmeric, safflower, marigold, or garlic. Several samples of each spice were analyzed. However, instead of looking at specific compounds, as it is common by coupling MS with chromatography, samples were injected (properly prepared) directly into the mass spectrometer. Before injection, samples were extracted with a solution of ethanol and water with proportions 80:20.
The whole mass spectra of samples were collected and chemometrics was applied to obtain both qualitative and quantitative models for adulteration analysis.

Results

Principal Component Analysis was applied to full mass spectra, showing a good separation of the five spices (Figure 1)

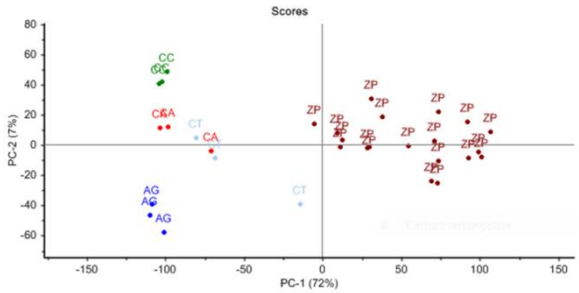

Fig. 1. *PCA scores plot. Good discrimination of saffron (ZP) samples from the other spices is shown*

By PCAs carried out on saffron alone with another spice type (for example saffron and safflower only), the most discriminative mass peaks for each adulterant spice were identified from the loading plot (Figure 2).

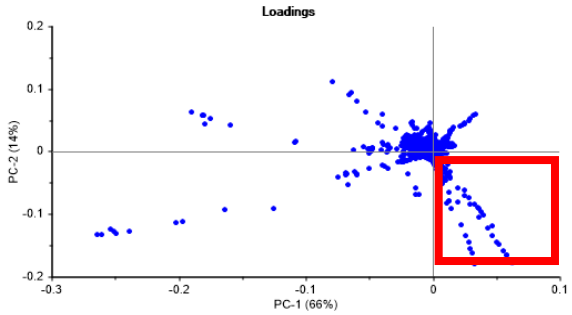

Fig. 2. *Loagins plot of a PCA carried out on saffron and safflower samples. Variables in the red rectangle are the most discriminative for safflower samples.*

Variables in the red rectangle in Figure 2 correspond to some mass peaks, which in turn correspond to the molecules that mostly discriminate safflower from saffron. Thus, a saffron sample with high concentration of such molecules can be suspect of being adulterated with safflower. The same procedure was carried out with all the other adulterants. In this way, the untargeted method can also support the targeted analysis, by confirming the presence of known discriminant molecules and adding new possible analytes to adulteration studies.

Moreover, some saffron samples were manually adulterated with a known amount of each spice, and the mass spectra were used to quantify the added spice. This task was carried out by a Partial Least Square (PLS) Regression and good models were obtained confirming the possibility of using this untargeted method also for quantification purposes.

Conclusions

Good results were obtained, both qualitatively and quantitatively. Considering also the simple extraction method needed and the low analysis time, the results encourage the possibility to apply our untargeted method to routine analyses in food authenticity control, also for other food matrices.

P39

Liquid chromatography coupled with high-resolution ESI-LTQ-Orbitrap MS and high-sensitivity ESI-Qtrap MS for the analyses of different extracts of black currant leaves

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Summary: *Different extracts obtained from black currant leaves were analysed by (HR)LC-ESI-LTQ-Orbitrap-MS(MS), highlighting the presence of phenolics, mainly flavonoids like quercetin and kaempferol glycosides thus suggesting the black currant by-products as a rich source of natural antioxidants that can be employed in nutraceutical and pharmaceutical formulations*

Keywords: *Black currant, metabolomic profiling, Multiple Reaction Monitoring*

Introduction

Liquid preparations such as tinctures and infusion derived from *Ribes nigrum* L. (black currant) leaves are widely used in Europe [1]. Despite the various uses reported for the infusion and hydroalcoholic extracts of the leaves of this species, their chemical profile has not yet been defined. In the present study, the infusion, hydroalcholic and methanolic extracts prepared from *R. nigrum* leaves were analyzed by LC-ESI-LTQ-Orbitrap-MS. Moreover, the identified polar metabolites were quantified by using High-Sensitivity ESI-Qtrap MS in MRM (Multiple Reaction Monitoring) mode.

Experimental

Ribes nigrum dried leaves were extracted with (1) methanol and (2) ethanol/water (1:1) by two procedures: 1) the plant was extracted first using ultrasound for 1 hour and then left in extraction for one night at 20°C; 2) the matrices were extracted for one night at 20°C without using ultrasound. The ratio between drug and solvent was 1g : 20 mL. The process was repeated in triplicates for each matrix, obtaining in total 12 samples of *R. nigrum*. Infusion of *R. nigrum* was also prepared in order to compare the phenolic content of the different preparations. Polar metabolites were identified by an analytical HPLC method coupled with an hybrid mass spectrometer, which combines the linear trap quadrupole (LTQ) and OrbiTrap mass analyzer, and then quantified by using LC system in line with a QTrap MS in Multiple Reaction Monitoring. Multivariate Data Analysis with Principal Component Analysis (PCA) was also performed in order to discriminate the extraction methods.

Results

LC-MS analyses of *R. nigrum* extracts allowed the characterization of 31 phenolic compounds mainly belonging to organic acids, flavonoids, catechins and its oligomers. Quantitative results proved that these preparations are a rich source of polyphenols and flavonoids and among them the infusion presented the highest polyphenol levels. A metabolomics approach was also used for the

comparison and evaluation of the chemical composition of the different extracts obtained from *R. nigrum* leaves. The phytochemical analysis revealed that infusion and hydroalcoholic extracts have high polyphenols and flavonoids contents.
In this work, the multivariate approach was successfully applied to distinguish samples extracted in different way and the metabolites characterizing each extraction procedure applied.

Conclusions

This research allowed to observe as black currant leaves are a relevant source of natural compounds, mainly flavonoids like quercetin and kaempferol glycosides, the most required flavonoids in human diet due to their antioxidant activity. Infusion of black currant seems to contain also acids like quinic acid and coumaroyl-quinic acid, in addition to flavonoids. Quantitative analysis evidenced that ethanolic extract and infusion of black currant leaves contain major quantity of phenolic compounds.
This study suggest the black currant by-products as a rich sources of natural antioxidant that can be employed in nutraceutical and pharmaceutical formulations.

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P40

Metabolite profiling by (HR)LC-ESI-LTQ-Orbitrap-MS(MS) of *Ocimum basilicum* plants grown in microcosm under different light exposure

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Summary: *Ocimum basilicum plants have been grown in a controlled system with different light regimes, respectively white and blue/red light. A metabolomics approach based on (HR)LC-ESI-LTQ-Orbitrap-MS(MS) combined with chemometric tools was applied to study the phenolic composition of leaves coming from plants grown in different controlled environmental conditions.*

Keywords: *Ocimum basilicum, metabolomic profiling, plant growth*

Introduction

Lighting is among the most important environmental factors that affect plant growth and development. The amount of photosynthetic active radiation (PAR) reaching plants directly affects photosynthesis rate but light spectral features also affect plant morphology as well as transition from vegetative to reproductive growth. Several studies in literature reported analysis of metabolome of basil plants based on volatile compounds [1], however a few data are so far available about phenolic composition of basil. The main phenolic so far reported in basil extracts are phenolic acids and flavonol-glycosides, in addition to rosmarinic acid, chicoric acid and caftaric acid, identified in fresh leaves along with other cinnamic acid monomers, dimers and trimers found in minor quantities in both stems and leaves [2,3]. Since phenolic composition is expected to be affected by different light conditions during plant growth, in this work we investigated the effect of different lighting regimes, respectively white and blue/red light on phenolic composition of basil grown in an innovative field simulator (ENEA-FOS microcosm) reproducing crop conditions similar to the ones basil plants face under cultivation [4].

Experimental

Basil plants var. Genovese were grown in the ENEA-FOS microcosm (European patent 3236741) at 18-22°C / 22-26°C (hypogeal/epigeal), 16/8 hours light/dark and under either white or blue/red wavelengths at, both at 3-4 x 10^{-6} W/n in the blue region and 160 and 490 µmol/m^2/s, respectively. After about 60 days cultivation, top and middle leaves and inflorescences were harvested and extracted with a solution of ethanol/water (1:1). The extracts were sep-packed to remove chlorophyll and analysed by LC-ESI-Orbitrap-MS(MS) with Data Dependent Scan mode. Data were treated with *mz-mine* and the data matrix obtained was subjected to Multivariate Data Analysis with PCA and PLS.

Results

Basil plants grew vigorously under both the different lighting regimes and without any visible stress symptoms, but plants grown under red/blue light 490 µmol/m^2/s showed a greater growth rate and yielded greater biomass. The extracts obtained from the different parts of plant were analysed by liquid chromatography coupled to high resolution mass spectrometry, one of the most sensitive analytical technique generally used in metabolomics [1]. Different metabolites mainly belonging to flavonoids and phenolic acids were putatively identified by using accurate mass, MS/MS fragmentation pattern and comparing the data with literature and database. The multivariate approach was successfully applied to distinguish samples exposure to different light.

Conclusions

Metabolomics approaches were successfully applied to distinguish samples of *O. basilicum* grown in a microcosm under different light exposure. Mass spectrometry allowed the detection and identification of phenolic acid and flavonoids, compounds well known for their health benefits in human health. Moreover, PCA and PLS allowed to group the samples in classes and distinguish samples of plants grown under red/blue light and white light.

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Work supported by Project ISAAC [Programma Operativo Nazionale "Imprese e Competitività" 2014-2020 FESR - F/050119/03/X32].

P41

Evaluation of thermo oxidation on cholesterol oxidation products in salmon and pork loin

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Summary: *The effect of oven baking at 180°C on cholesterol oxidation products (COPs) formation was evaluated on two different food matrices, salmon and pork loin. As results, the cooking treatment did not increase the COPs levels, in pork loin and did not promote their formation in salmon.*

Keywords: *oxysterols, animal food, GC-MS*

Introduction

Animal foods such as fish and pork meat are rich in cholesterol. Their consumption requires a cooking process, that affects the content in cholesterol by enhancing its extraction and/or degradation. Cholesterol can undergo to oxidation when it is submitted to heat treatment during cooking, forming oxysterols. COPs are potentially cytotoxic, mutagenic, carcinogenic and atherogenic [1].

Foods are complex systems where the oxysterols formation depends on the chemical composition: presence of polyunsaturated fatty acids and antioxidants (tocopherols and carotenoids). In view of this, the present study was aimed to evaluate the effect of thermo oxidation in terms of COPs on two different food matrices: salmon, a food with high degree of polyunsaturated fatty acids and rich in antioxidants, and pork loin, a matrix with high levels of saturated fatty acids and poor in antioxidants [2]. Thermo oxidation was inducted by using traditional oven baking reproducing real patterns of consumption.

Experimental

Fresh salmon fillet (*Salmon salar*) and pork loin (Large White) were purchased from local distributor. Salmon slices of 200g±20g were submitted to oven traditional baking at 180°C for 20min (core temperature of 56-60°C). Pork loin pieces (750±75g) were oven baked at 180°C for 55min (core temperature of 74-76°C).

Samples, raw and cooked, were undergone to fat extraction. Fish oil was extracted along Bligh-Dyer method [3], pork fat was extracted along Folch et al. method [4]. A cold saponification was applied to the fat/oil to determine cholesterol, while COPs were purified on SPE along a tailored Larkeson et al. method [5]. All samples were derivatized and injected in the GC/EI-MS system, equipped with a quadrupole analyzer and a column Rtx-65TG. The identification was performed by comparison with standards. Quantification was made by internal standard, 5α-cholestane for cholesterol and 24-Hydroxycholesterol for COPs. The extracted fat of all samples was analyzed using UPLC/FL system for α-tocopherol quantification.

Results

The results, showed in Table 1, demonstrated that cholesterol content was not affected by the heat treatment for both matrices. Raw salmon contained less amount of cholesterol then pork loin, 327.81±54.26 and 637.49±126.70 mg/100g fat, respectively. The identified COPs in all samples were 7α-Hydroxycholesterol (7α-HC), 7β-Hydroxycholesterol (7β-HC), 5,6β-Epoxy-cholesterol (β-CE), 5,6α-Epoxycholesterol (α-CE) and 7-Ketocholestrol (7KC), which were typically found in animal foods [6]. However, in raw and cooked salmon, the total COPs were below the limit of quantification (LOQ of 0.18 mg/100g). Differently, in raw pork loin 7β-HC and 7-KC were quantified. Anyway, values were not significantly different from the oven baked samples. Thermo oxidation, derived from cooking as real pattern of consumption, did not affect the level of COPs in both food matrices.

Furthermore, raw and cooked salmon displayed a content in α–tocopherol (antioxidant) of 26.67±1.2 and 27.67±1.18 mg/100g fat, respectively. In contrast, α–tocopherol in loin was not detected.

Conclusions

The oven baking treatment usually performed by the consumer did not increase the COPs levels, in pork loin and did not promote their formation in salmon. Further studies will be focused on others heat treatments and on profiling carotenoids and polyunsaturated fatty acids.

Table 1. *Cholesterol, COPs and α–tocopherol content in raw and cooked salmon and pork loin.*

	Raw salmon	Oven baked salmon	Raw pork loin	Oven baked pork loin
Cholesterol	327.81±54.26 a	327.70±28.69a	637.49±126.70 a	745.53±37.80a
7α-HC	<LOQ	<LOQ	<LOQ	<LOQ
7β-HC	<LOQ	<LOQ	0.74±0.54a	0.55±0.11a
β-CE	<LOQ	<LOQ	<LOQ	<LOQ
α-CE	<LOQ	<LOQ	<LOQ	<LOQ
7-KC	<LOQ	<LOQ	1.73±0.25a	1.94±0.84a
Total COPs	<LOQ	<LOQ	2.51±0.84a	2.75±1.10a
(Σ COPs/cholesterol)*100	-	-	0.39±0.05a	0.41±0.13a
α-tocopherol	26.67±1.21a	27.67±1.18a	not detected	not detected

Values are expressed as mean ± SD (n=3) as mg/100g fat

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P42

Tracking sugar addition in food and beverage using isotope fingerprints

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Summary: *Isotope fingerprinting is becoming an increasingly useful tool for assessing the authenticity and origins of food and beverage. This paper will provide and overview of the official methods of isotopic analysis employed in the identification of natural versus artificially added sugars in food and drink items.*

Keywords: *Sugar addition, Isotope fingerprinting, IRMS*

Complexities in the food and beverage supply chain from the production site through to the consumer have presented significant, and at times relatively easy, opportunity for economically motivated fraudulent activities to occur and be undetected. Consequently, there is an increase in retailer and consumer demand to proof that food and beverage products are what the label claims them to be, including origin, authenticity and ingredient verification.

One of the most known adulteration processes involves the addition of sugar to food and beverages. Detecting the added sugar can be achieved using stable isotope measurements because stable isotopes can differentiate between the sugar already present in the sample from the sugar which is added artificially. Carbohydrates carry an isotope fingerprint, a unique chemical signature which identifies their origin. To visualize this fingerprint, Isotope Ratio Mass Spectrometry (IRMS) can be used, identifying the isotope fingerprint of the product.

In this presentation the application of stable isotope fingerprints in detecting sugar addition to food and beverage samples is explored. Data show how stable isotopes offer conclusive answers on questions associated with origin, adulteration and correct labeling of food and beverage products. An overview of the interpretation of isotope fingerprints and the official methods using isotope fingerprints for food and beverage analysis are also provided.

P43

Multiresidue determination of pesticides in soil and edible crops by using HPLC-MS/MS triple quadrupole

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Summary: *In organic production, the use of pesticides is significantly restricted, but certain plant protection products are allowed under well-defined conditions. An analytical method for the simultaneous determination of various classes of pesticides was developed and only in 7 soil and 6 edible crop samples pesticides were detected in low amount.*

Keywords: *pesticides, HPLC-MS/MS, GC-MS/MS*

Introduction

The high crop yields obtained in agriculture at present rely on the wide use of pesticides. As a consequence, these chemicals are frequently found in soil and other environmental matrices where the risk they may pose has to be controlled [1]. Pesticides are widely used in agriculture to control pests and diseases with the goal of increasing productivity and improving the quality of products (animal or vegetable) [2]. However, the presence of pesticide residues can harm many organisms in different environmental compartments. Soil, which is a vital agricultural resource, has a high capacity to retain and store chemical substances such as pesticides. Once adsorbed onto soil particles, these compounds may be rapidly degraded or in the case of persistent chemicals, may be slowly released into the atmosphere, subterranean aquatic systems and living organisms [2]. Organic production is a system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method using natural substances and processes. The use of pesticides is significantly restricted, but certain plant protection products are allowed under well-defined conditions. Pesticide residue testing is one aspect of official controls on organic production [3]. The control authorities or control bodies must take and analyse samples for detecting products not authorised for organic production, for checking production techniques not allowed under organic production rules or for detecting possible contamination by products not authorised for organic production. Since January 2014, Article 65 of Regulation (EC) No 889/2008 requires that the number of samples to be taken and analysed by the control authority or designated control body every year shall correspond to at least 5% of the number of operators under its control [4]. The selection of the operators where samples have to be taken shall be based on the risk of noncompliance with the organic production rules. No criteria are established at EU level for the

sampling procedures of organic products, the pesticides to be included in these checks, or the sensitivity of methods. Fileni is the third-placed player in the poultry sector on a national scale and the leading producer of organically-reared white meat in Italy. In order to be organic, the chicken should eat organic feed only, grow on organic farmland and meet all the requirements envisaged by strict applicable regulations. Thus, in this work, an analytical multiresidue method for the simultaneous determination of various classes of pesticides in soil and edible crops was developed by using HPLC-MS/MS triple quadrupole and GC-MS triple quadrupole.

Experimental

Samples were homogenized with blender plus dry ice and weighed in 50 ml centrifuge tubes (5 g for edible crop and 2.5 g for soil). Then, they were hydrated with 10 ml of water at 4 °C for 10 min, then 10 ml of acetonitrile and ceramic homogenizers were added, and samples vortexed for 1 min. Afterwards, quechers salts were added and samples vortexed again for 1 min. Then, they were centrifuged at 5000 rpm for 5 min, supernatants were transferred in the appropriate dispersive SPE pigment samples, homogenized with ceramic homogenizer and vortexed again for 1 min. The residue was filtrated through a 0.45 µm membrane filter and then directly injected into the HPLC-MS/MS or GC-MS systems. LC-MS/MS studies were performed using an Agilent 1290 Infinity II series instrument, made from an autosampler, a binary solvent pump, with a mass spectrometer (MS Agilent 6495 LC/TQ) equipped with an electrospray ionization (ESI) source. The analyte separation was achieved on a Zorbax RRHD Eclipse Plus C18 (2.1 x 150mm, 1.8 µm). The mobile phases were water (A) and methanol (B) both containing 0.1% formic acid and ammonium formate 5 mM (B) 95:5 v/v working in the gradient mode at a flow rate of 0.4 mL min^{-1}. The solvent composition varied as follows: 0 min, 5% B; 3 min, 30% B; 17 min, 100% B; 20 min 100% B, then the column was reconditioned. The column temperature was set at 40 °C and the injection volume was 1 µL. The ESI source was operating in negative and positive ionization mode and the mass spectrometer in Dynamic MRM acquisition mode. A gas chromatograph and mass selective detector were used in combination (GC Agilent 7890B along with MS Agilent 7000C) and the separation was performed on two HP-5MS column connected each other by a backflush system, the dimensions of which were 30 m length x 0.25 mm id, 0.25 µm film thickness. The flow rate of helium was 6 ml·min-1 in solvent vent mode and the injector temperature was 280°C. The column temperature program began at 60°C (1 min) then increased to 170°C at 40°C·min^{-1}, then increased to 310°C at 10°C min. The mass spectrometer used the electron impact (EI) mode with an ionisation voltage of 70 eV, and Dynamic MRM acquisition mode to produce the spectra of the separated compounds.

Results

After method validation, 173 soil samples and 128 different edible crop were analysed. In soil, in only seven samples nine different pesticides were found, i.e. clortoluron, p,p'-DDE, imidacloprid, azoxystrobin, oxadiazon, tetraconazole, cyproconazole, difenoconazole and metalaxyl at levels ranging from 18 to 111 µg L^{-1}. In edible crop, in only six samples six different pesticides were found, i.e. terbutilazine, pyraclostrobin, tebuconazole, azoxystrobin tetraconazole and cyproconazole at levels ranging from 11 to 147 µg L^{-1}.

Conclusions

In this work, an analytical multiresidue method for the simultaneous determination of various classes of pesticides in soil and edible crops was developed by using HPLC-MS/MS triple quadrupole and GC-MS triple quadrupole. A high number of samples were analysed, i.e. 173 soil samples and 128 different edible crops, and only in 7 soil and 6 edible crop samples, pesticides were detected in low amount.

During the previous years, these substances have never been detected during the analytical controls carried out in raw materials at the feed mill. Nevertheless the Fileni company, following a process of continuous improvement, wanted to put in place a very widespread series of controls going up the agricultural supply chain to the cultivation fields, to guarantee an even higher level of safety and quality of the raw materials used to produce the feed for their organic chicken line of products.

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P44

Lipidomics profiling of donkey milk from Asinara by MS/MS^{ALL} technique

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Summary: *Donkey milk has gained even more interest in the scientific research due to its attractive nutrient and functional contents as well as its chemical composition similar to human milk which makes donkey milk a valid alternative in the case of allergy. Then a more in depth investigation on lipids is required. By using infusion MS/MS^{ALL} acquisition, a rapid lipidomics profiling of donkey milk sample can be captured*

Keywords: *lipids, shotgun high resolution mass spectrometry, donkey milk*

Donkey milk has gained interest also in scientific research as a substitute for cow milk, because cow milk is thought to be responsible for food-borne allergies in children below one year. Donkey milk posesses a chemical composition similar to human milk and exhibits hypoallergenic properties and antimicrobial as well as immunomudulatory activity, and is therefor considerd a viable alternative vor babies suffereing from multiple allergies against cow- , goat- and soy milk. While the main lipid class of milk is the triglyceride class, only few publications to date have explored the phospholipid class content of donkey milk. However, this lipid class also possess beneficial nutritional effects for human health as well as important biochemical characteristics2.
Here use a shotgun lipidomics analysis approach termed MS/MS^{ALL} for the lipidomic analysis of donkey milk, using a TripleTOF 6600 mass spectrometer with direct infusion by flow injection analysis (FIA). While Ultra-performance liquid chromatography mass spectrometry (UPLC-MS) is the most widely used technique employed to investigate the lipid composition in milk3,4, FIA-MS/MS^{ALL} has a multitude of advantages over the classical UPLC-MS approach. Because it is quantitative at the MS2-level, it allows for the direct differentiation and quantitation of isomeric lipid molecular species. It fragments all ionizable lipid species in one acquisition, resulting in a digital record of the sample, thus allowing retrospective data processing for information not considered at the time of data acquisition. It is more amenable to quantitative approaches because standards and analytes have identical ionization conditions thoughout the run, and sample carry over is minimal due to the lack of a HPLC column. The aquired MS/MS^{ALL} data is processed with LipidView software. The results show the presence of different classes of phospholipids.

Introduction

Over the last years, donkey milk has gained interest in scientific research due to its attractive nutrient and functional contents. In fact, generally, milk is a common responsible of food allergies among children under one year of age.

And since donkey milk possess a chemical composition similar to human milk, as well as hypoallergenic properties together with antimicrobial and immunomodulation activities, it is considered a valid alternative for babies suffering from multiple-allergies (cow milk, hydrolysed cow milk proteins, goat milk, and soya)[1]. On the point of view of lipids profiling, donkey milk shows a lower fat content compared to human milk, characterized mostly by polyunsaturated fatty acids (PUFA) (ω6 and ω3). Only few papers report the content of minor compounds as phospholipids (PL), which also possess beneficial nutritional effects on for human health as well as important biochemical characteristics[2]. Ultra-performance liquid chromatography mass spectrometry is the most useful technique employed to investigate the PL composition in milk[3,4]. But faster and straightforward methods with no or minimal sample preparation are the main focus of researcher to collect more data in a short time and maximizing analytical throughput. Infusion "shotgun" MS/MS^{ALL} acquisition, for lipidomics, gives the MS/MS of all possible candidates. This technique, in combination with LipidView software, allows to get at same time identifications, quantitative and retrospective analysis of the data and was applied to investigate PL components in milk from Asinara white donkey.

Experimental

Data were acquired using a TripleTOF® 6600 System (SCIEX) coupled to a high flow liquid chromatography system and employing the Infusion MS/MS^{ALL} acquisition to collect one survey scan and then MS/MS of every single precursor within a given mass range. Mass ranges were evaluated from 400 to 1000. Data were processed using LipidView™ Software. Statistical analysis was completed in MarkerView™ Software. The analysis were performed with the instrument of the unitech OMICs, a facility of the University of Milano.

Results

The most abundant PLs in donkey milk, expressed as percentage of total PLs detected in positive ion mode, are phosphatidylcholine (PC) (46.3% of total PLs), phosphatidylethanolamine (PE) (22.4%), sphingomyelin (SM) (13.4%), LPC (10.4%), phosphatidylserine (PS) (4.5%) and phosphatidylinositol (PI) (3.0%). Instead in negative ion mode, PLs are mainly made up of PE (80.4% of total PLs), followed by PS (12.3%) and PC (5.3%).

Conclusions

MS/MS^{ALL} approach for lipidomics profile of milk results a powerful and rapid tool to obtain huge and complementary data of numerous samples even if lipids present only at very low levels in biological samples.

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P45

Application of PTR-MS, HS-SPME GC-MS and HILIC-HRMS to study the chemical changes during storage of ultra-high-temperature (UHT) lactose-free milk produced with different lactase preparations

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Summary: *The study investigated the employment of different commercial lactase preparations for the production of UHT lactose free milk (LFM) through the assessment of the volatiles profiles (by HS-SPME GC-MS and PTR-TOF-MS) and the release of free amino acids (by HILIC-HRMS) during shelf-life at 20°C.*

Keywords: *lactose-free milk, mass spectrometry, volatile organic compounds (VOCs), free amino acids (AAs), shelf-life*

Introduction

Nowadays around 70% of the worldwide population suffers from lactase deficiency (1). Low-lactose (LLM) and lactose-free (LFM) milk products represent a convenient solution for these consumers (2)**.** Nevertheless, manufacturing of UHT LFM is not trivial and poses challenges for optimal production. For example, UHT LFM is more sensitive to chemical changes due the presence of glucose and galactose as well as the occurrence of proteolytic side activity in the commercially available lactases (3). These secondary proteases are particularly problematic because they contribute to the milk protein hydrolysis which can causes gelation, bitterness and off-flavor development (4) . HS-SPME GC-MS has been largely demonstrated as a successful method to isolate and assess the volatile organic compounds (VOCs) in dairy products (5,6). In comparison to other extraction techniques, the SPME requires less sample manipulation and guarantees a lower formation of artefacts along the GC run (7). However, GC technique has some limitations, such as the duration of analysis. PTR-MS was pointed out as a valuable alternative as allows rapid, direct and sensitive monitoring of VOCs (8). The aim of the present research is to investigate the changes in VOCs and free amino acids in UHT LFM produced with different lactase preparations during shelf-life at 20°C for 120 days. Three mass spectrometry (MS)-based techniques were employed: HS-SPME GC-MS and PTR-TOF-MS for VOCs assessment and LC-HRMS for free amino acids (AAs) quantification. All together the results provide a comprehensive overview of the phenomena occurring along the shelf-life of UHT LFM with emphasis on the impact of the proteolytic side activity present in lactase preparations in the definition of final product quality.

Experimental

Three commercial lactases were tested. UHT LFM samples were produced by the “in-batch” procedure according to (2). For each lactases, production was repeated three time to include an estimation of the batch- to-batch milk variability. UHT LFM were stored into a climate chamber set at 20°C for 120 days and samples were collected every 30 days. Free amino acids were analyzed according to (9) interfacing an HILIC separation to an Exactive Orbitrap HRMS (Thermo Fisher Scientific, Bremen, Germany). Analytes were detected through a heated electrospray interface (HESI-II) operating in positive mode. For the analysis of the VOCs a PTR-TOF-MS 8000 (Ionicon Analytik GmbH, Innsbruck, Austria) was used. The instrument was set up according to (10). Besides, the VOCs in the headspace of the UHT LFM were also measured by SPME GC-MS following the methodology described by (11).

Results

Principal Component Analysis (PCA) was performed on the HILIC-HRMS dataset to explore pattern in the distribution of the UHT LFM samples based on their AAs profile. Data revealed a separation of the UHT LFMs dependent on the lactase preparation used and independent from the time of storage. The concentration of Ile/Leu, Trp, Val, Phe, Tyr, Arg, His, Pro, Gln, ProOH, Glu and Asp remained constant throughout the shelf-life indicating the inactivation, at least of most, of the proteolytic side activities of the lactase preparations as a consequence of the UHT treatment. Conversely, storage played a crucial role in the definition of the “volatilome” of the UHT LFM samples, as shown in Figure 1. Mass peaks detected by PTR- TOF-MS and associated to methyl ketones increased significantly during storage for all the UHT LFM.

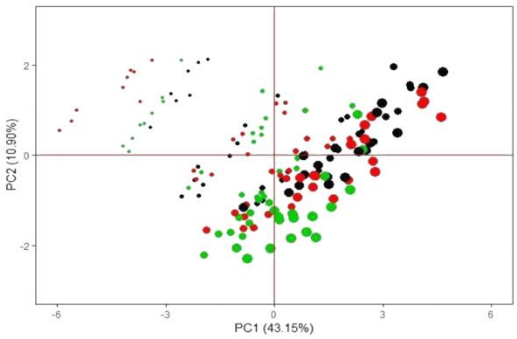

Fig. 1. *Score plot for the 1st (PC1) and 2nd (PC2) component of PCA performed on the PTR-MS data. Different colors represent the three replicates of production (black: 1st; red: 2nd; green: 3rd) while the progressive increase in the dots size defined the proceeding of the shelf-life in the UHT LFM samples.*

Additionally, benzaldehyde (m/z = 107.048) was the only detected VOC whose positive trend changed as function of the tested lactase. This pattern was confirmed by HS-SPME GC-MS as well. Along with benzaldehyde, the latter techniques revealed an effect of the lactase preparations also on the temporal evolution of 2-methylbutanal. Both compounds are particularly important for UHT LFM as their formation occurs mainly at the intermediate stages of

Maillard reaction, especially by Strecker degradation, a reaction well known to be responsible for the flavor develoment in UHT milk (12).

Conclusions

The results of the study suggested that, when lactase was added before the heat treatment, most of its proteolytic activity was minimized. Nevertheless, the positive trend of VOCs associated to Strecker degradation (2-methylbutanal and benzaldehyde) indicated that the survival of some proteolytic side activity could not be excluded. The trends differed as function on the lactase employed suggesting a different degree of purity of the preparations. Overall, the results supported the application of PTR-TOF-MS, HS-SPME GC- MS and LC-HRMS as valuable tools for unrevealing the pathway of chemical changes linked to quality losses in UHT LFM depending on the typology of lactase preparations employed.

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P46

Food and beverage fraud prevention using isotope fingerprints

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Summary: *Isotope fingerprinting is becoming an increasingly powerful tool in establishing the authenticity and origins of food and beverage. This presentation will provide an overview of the methods of isotopic analysis employed in the identification of original versus adulterated food items and beverages.*

Keywords: *Authenticity, Isotope Fingerprinting, Food Fraud*

Stable isotope measurements can differentiate between food and beverage samples which otherwise share identical chemical composition: this is called the isotope fingerprint. Using the isotope fingerprint of food and beverage products is a reliable technique for fraud detection offering conclusive answers on questions associated with origin, adulteration and correct labeling of food and beverage products. An overview of the interpretation of isotope fingerprints and the technology used is provided.

The food and beverage industry suffers from fraudulent activities that include incorrect labeling of products and adulteration, which has a significant impact on food and beverage safety, brand names and reputation and the market economy. Preventing food and beverage fraud is a key challenge that requires a reliable, cost-effective analytical process able to detect whether the labeled product is authentic, if it has been changed after the final manufacturing process, or alternatively if it has been independently produced, using alternative ingredients, but labeled as an original product.

Is your wine watered down? Are your vegetables grown using organic farming? Is the honey you bought naturally sweet? Did you purchase authentic Tequila? Explore how you can use carbon, nitrogen, sulfur, oxygen and hydrogen isotope fingerprints to trace unique answers to these and many more questions regarding origin and authenticity of your foods.

P47

Intelligent MS^n workflow for improved metabolome coverage and increased confidence in unknown identification

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Summary: *Compound identification is a bottleneck in untargeted metabolomics. Here we describe a data-informed workflow that maximizes the number of metabolites interrogated by MS/MS and MS^n, while minimizing the acquisition of uninformative spectra. This workflow was used to analyze human plasma resulting in high confidence identifications and enhanced biological knowledge generation.*

Keywords: *Intelligent MS^n, Metabolomics, Unknown ID*

Introduction

Compound identification is a bottleneck in untargeted metabolomics, hindering biological interpretation of results. Here, we describe a data-informed workflow that maximizes the number of metabolites interrogated by MS/MS and MS^n, while minimizing the acquisition of uninformative spectra. This workflow was used to analyze human plasma resulting in high confidence identifications, deeper metabolome coverage and enhanced biological knowledge generation.

Approach

Human plasma was purchased from NIST. Metabolites were extracted with methanol and injected on a Thermo Scientific™ Hypersil GOLD™ column. Instrumentation included a Thermo Scientific™ Vanquish™ UHPLC system and a Thermo Scientific™ Orbitrap Tribrid™ Mass Spectrometer with modified instrument control and data acquisition software. Data were analyzed using Thermo Scientific™ Mass Frontier software and Thermo Scientific™ Compound Discoverer™ software.

Results

During data-dependent MS/MS, ions are selected based on abundance, without any knowledge of biological relevance or type of ion. In a typical DDA experiment, we determined, that >40% of MS/MS spectra could be attributed to background ions. By enabling the automatic generation and implementation of a background exclusion list based on real-time feature detection in LC-MS data, background ion MS^2 spectra were practically eliminated (<0.1%), allowing for the analysis of more true sample components.

Small molecules form different types of adducts and cluster ions during electrospray ionization. Highly abundant compounds may prevent the fragmentation of metabolites of lower abundance. By populating the inclusion list with the preferred ion for each metabolite, more compounds can be sampled by MS/MS and MS^n in a single run. Additionally, by automatically

updating inter-run inclusion and exclusion lists during analysis, we can ensure that compounds not selected for MS/MS and MS^n will be prioritized during a subsequent injection.

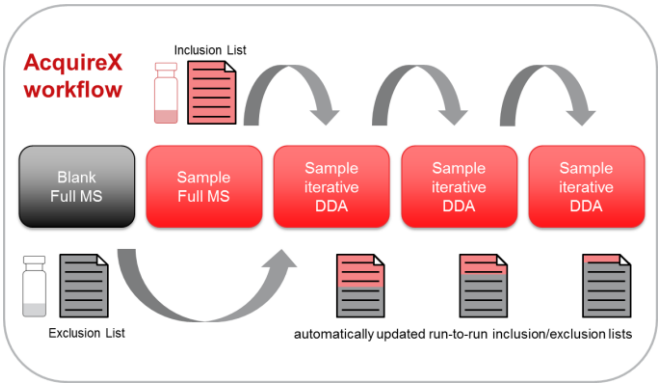

Fig. 1. *AcquireX represents a new acquisition paradigm. First, an exclusion list is generated from a blank run. Then, an injection of the sample followed by feature detection and component assembly populates the inclusion list with compounds detected in the sample. A series of iterative DDA injections follow. Each injection is informed from the previous one, minimizing redundant fragmentation spectra and maximizing relevant spectra and metabolite annotations.*

Conclusions

The combination of MS^n and automatically generated inter-run inclusion and exclusion lists resulted in fragmentation of more unique metabolites and a greater number of metabolites confidently annotated. Application of this innovative workflow addresses the identification bottleneck of untargeted metabolomics studies and enables confident biological interpretation of the results.

Novel Aspect

Automated workflow for information-rich fragmentation data acquisition, designed to minimize irrelevant spectra and maximize metabolome coverage.

P48

In the "spirit" of VOCs: sampling automation and concentration strategy for aroma profiling of alcoholic beverages

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Summary: *This study demonstrates the use of a multi-hyphenated system for fully automated, highly-sensitive aroma profiling of alcoholic beverages. High-capacity sorptive extraction coupled with GC×GC-FID/TOF MS/SCD delivers three complementary datasets per analysis for unparalleled levels of detail and confident identification of odour taints.*

Keywords: *aroma, GCxGC, sorptive extraction*

Introduction

The aroma profiles of alcoholic beverages are composed of a broad range of chemical classes, including terpenes, phenolics, fatty acids, esters, lactones, aldehydes, as well as nitrogen- and sulfur-containing compounds. It is important to be able to confidently identify these volatiles, for quality control and authentication purposes, as well as in the engineering of new aromas.

Historically, a wide variety of sampling methods have been used to extract volatiles from alcoholic spirits, with a key driver being the need to improve upon inefficient solventextraction methods.

One such improved method is high-capacity sorptive extraction (HiSorb™), which is highly efficient sampling approach for a wide range of applications. It involves use of robust, inert metal probes fitted with a relatively large volume of sorptive phase, allowing high sensitivity to be achieved.

Experimental

HiSorb extraction probes with PDMS stationary phase (65 µL) was used in conjunction with secondary refocusing on thermal desorption trap to sample of various alcoholic beverages and their raw ingredients, including beer, wine and hops. This approach offers excellent sensitivity, as well as the ability to re-collect a portion of the sample for repeat analysis in a fully automated workflow.

Nevertheless, the aroma profiles are often highly complex, with important compounds, such as trace-level off-odours, frequently masked by higher-loading components. The enhanced separation capacity of comprehensive two-dimensional gas chromatography (GC×GC) is now frequently used to tackle this challenge.

We apply multi-hyphenated analytical system to obtain comprehensive aroma profiles. The use of parallel detection by three different techniques ensures that three complementary datasets are obtained from a single run:

- Flame ionisation detection (FID) for robust quantitation of high-loading species
- Time-of-flight mass spectrometry (TOF MS) for highly-sensitive, confident identification of aroma-active species
- Sulfur chemiluminescence detection (SCD) for highly specific detection of sulfur odour taints.

Results

In the GCxGC-FID analysis of hops (including Citra, Mosaic and Amarillo varieties) eight major terpenes were found to make up ~70% of the overall composition in each case. β-myrcene was present at levels at least two orders of magnitude greater than the other terpenes, while β-farnesene was found in much lower abundance, and not detected in 'Mosaic' at all. The GCxGC-TOF MS data, on the other hand, provided a more detailed chemical signature of the other aroma-active-species, to uncover more subtle differences in the aroma profile, for example between the "sweet, fruity" compounds methyl nonanoate and undecan-2-one. Finally, the GCxGC-SCD dataset provided an insight into the presence of unpleasant odour taints in hops and the final product beer (Fig 1), caused by sulfur components, such as 3-methylbut-2-ene-1-thiol (3-MBT), which is well-known to cause an undesirable 'lightstruck' or 'skunky' character in beer.

The combination of all three datasets, obtained via parallel detection in a single analysis, gives unparalleled insight into the aroma profile in a realistic timeframe.

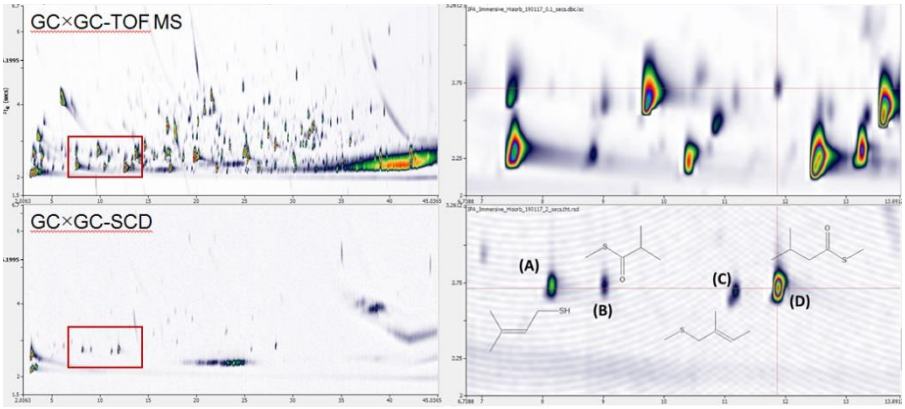

Fig. 1. *GC×GC plots for TOF MS (top) and SCD bottom for immersive sorptive extraction of a pale ale. The boxed region is expanded to show identification of some key sulfur species (A) 3-Methylbut-2-ene-1-thiol, (B) S-Methyl 2-methylpropanethioate, (C) 1-Methylthio-2-methylbut-2-ene and (D) S-Methyl 3-methylbutanethioate.*

Conclusion

In this study, we apply flow-modulated GC×GC within a completely cryogen-free configuration that allows parallel detection by three methods, all in a single run.

We will show how this setup is confident but affordable for the analysis of aroma profiling with fully automated workflows and novel data processing.

P49

Non-targeted food fingerprinting of typical food products of the Basilicata region (Italy)

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Keywords: *Food Fingerprinting, Metabolomics, High Resolution Mass Spectrometry*

Introduction

Food fingerprinting is a non-targeted analysis with the intrinsic aim to detect as many components of the sample as possible with a single direct analysis. Food fingerprinting approaches are typically based on a high throughput screening of samples with differentiation or classification purposes, providing high potential with regard to the characterization and identity verification of food [1]. Therefore, this kind of non-targeted analysis obtained increasingly importance during the recent years. Moreover, the ability of Ultra-High Resolution Mass Spectrometry to investigate multiple objectives with only one direct analysis is a clear advantage for the non-targeted food fingerprinting over the classical targeted approaches [2]. Thus, in this work, a non-targeted food fingerprinting of several typical food products of the Basilicata region (Italy), i.e. Peperoni di Senise PGI (Protected Geographical Indication) peppers, Fagioli Bianchi di Rotonda PDO (Protected Designation of Origin) beans, Melanzane Rosse di Rotonda PDO eggplants and Fagioli di Sarconi PGI beans, was performed to have a complete overview of relative metabolic profiles and to obtain unique food fingerprints.

Experimental

To achieve this goal, sampling of selected food products (belonging to different species) was performed and a proper metabolite extraction procedure was optimized for each of them. To obtain a metabolic profile of samples, direct High Resolution Mass Spectrometry was used. In this work, a Bruker solariX XR Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS) equipped with a 7T superconducting magnet, an ESI and a MALDI sources (Bruker Daltonik GmbH, Bremen, Germany) was used.

Results

Ultra-High Resolution Mass Spectrometry data obtained from the analysis of sample extracts were used to perform a non-targeted food fingerprinting by converting accurate m/z values in putative elemental formulas. Food fingerprints, i.e. Van Krevelen diagrams, were obtained, each of which is unique for every analysed food product and lead to a direct visualization of different classes of metabolites present in food samples under study [3]. In this way, it was possible to desume the presence of important classes of

metabolites, i.e. fatty acids, amino acids, peptides, carbohydrates and polyphenols, and to have some insight on the number of derivatives belonging to each of them.

Conclusions

This study provided a unique and innovative tool to characterize Italian typical food products, able to improve their value in terms of quality and health-promoting properties, useful to shed light on their chemical composition.

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P50

Nutraceutical potential of Corylus avellana daily supplements for obesity and related dysmetabolism

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Summary: *In this study, the nutraceutical potential of two hazelnut varieties (Turkey and Italy) were examined by studying several aspects including the phytochemical properties, antioxidant potential, cell viability and ex vivo neuroprotective potential.*

Keywords: *dysmetabolism, hazelnuts, nutraceuticals*

Introduction

Corylus avellana (common hazel) belongs to the genus Corylus. The common hazel is widely distributed along the coast of southern Europe and the Black Sea region of Turkey, and cultivated in commercial quantities for its nut (hazelnut) which is edible and generally eaten raw/roasted, or ground into a paste. There are reports highlighting the beneficial effects of hazelnut consumption in humans largely due to the high fatty acid concentration, which is composed mainly of mono-saturated fatty acids (MUFA, 82-83%), and fat soluble bioactive compounds like tocopherol and phytosterols.

Experimental

All the extracts were analysed for quantitative and qualitative determination of polyphenols and flavonoids, performed by means of reverse phase HPLC-PDA reported by Di Sotto and co-workers [1]. Fatty acids methyl esters (FAMEs) preparations were obtained by esterification into methyl esters, by saponification with 0.5molL^{-1} methanolic NaOH and transesterification with 14% BF3 (v/v) in methanol. Phenolic and flavonoid compounds of the obtained extracts were analysed by using well established procedures such as Folin-Ciocâlteu and AlCl3 tests, respectively. The antioxidant and enzyme inhibitory effects of the studied extracts were determined through metal chelating, phosphomolybdenum, FRAP, CUPRAC, ABTS and DPPH tests. The enzyme inhibitory activity was detected against acetylcholinesterase, tyrosinase, α-amylase and α-glucosidase [2].

Results

Results showed that the Turkish hazelnut contains the largest concentration of phenolic acids and flavonoids. It shows an higher antioxidant capacity and enzyme inhibition properties and lower saturated fatty acid concentration than the Italian sample. The *in vivo* studies revealed that compared to the Italian hazelnuts, the addition of Turkish hazelnuts to high fat diet was associated with a more significant decrease in body weight, food consumption, atherogenic index, lipid peroxidation levels and biochemical/morphological markers of liver injury. The two hazelnut varieties were protective against β-amyloid-induced neurochemical changes and high-fat diet induced alteration of metabolic indices.

Conclusions

This study has demonstrated the *in vitro*, *ex vivo* and *in vivo* differences between the specific analysed samples of hazelnuts grown in Turkey and Italy [3]. We have also corroborated scientific evidence showing that hazelnut supplementation can mitigate biochemical, morphological and morphometric indices of metabolic syndrome and neurochemical alterations in Alzheimer's type neurodegeneration in rodents.

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P51

Polyphenol composition of 12 apple cultivar grown in South-Tyrol (Italy) determinate by ultra-high-performance liquid chromatography mass spectrometry (UHPLC-MS)

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Summary: *Twelve apple cultivars, grown in South-Tyrol, were collected from the Laimburg experimental fields (Vadena, Italy) and analyzed by ultra-high-performance liquid chromatography coupled to triple quadrupole mass spectrometer in electrospray mode (UPLC-ESI-MS/MS QQQ). This study reports the quantification of individual metabolites with a beneficial role in human health. The goals are to provide comprehensive data on phenolic profile of different apple cultivars.*

Keywords: *apple polyphenols; apple varieties;* UPLC-ESI-MS/MS QQQ

Introduction

Apple (*Malus domestica* Borkh) is the fourth most consumed fruit in the world with an annual consumption of approximately 84.63 million metric tons [1]. South-Tyrol is one of the best productor of apples in Italy and it is surrounded by 18,500 hectares of apple trees [2]. The proverb “an apple a day keeps the doctor away” is supported by current epidemiological and experimental studies [3]. In fact, behind the beautiful colours of this fruit, there are a lot of interesting healthy compounds such as polyphenols. They are the most studied molecules in fruits because of their antioxidant activities [4]; furthermore, they play an important role in the prevention of chronic illnesses (e.g. cardiovascular disorders, cancer, diabetes, vascular inflammation and liver diseases) which are increasing in the world [3]. It has been estimated that the polyphenols concentration in new apple cultivars is lower than the old ones [5].
Our research is focused on the apple pulps being the most part consumed by the customers in order to highlight the health benefits of this fruit. [6, 7].
In this study new and old apple varieties grown in South-Tyrol are deeply investigated including the red-fleshed cultivars due to their promising healthy potential [2].

Experimental

In this study, 12 cultivars representing the current market, including one with scab resistance and two with red flesh fruit, have been investigated. They were collected from Laimburg experimental fields, harvested at the optimal harvest time, grown in the same site under identical climatic and agricultural conditions [8]. The protocol used for the analysis of the phenolic metabolites was adapted from Valls et. al. [9]. In detail, each cultivar was split into 10 biological replicates and 25 mg of freeze-dried apple pulp were extracted with 1.83 mL of a mixture of water and methanol (80:20 v/v) acidified with H_3PO_4 and containing sodium fluoride. The polyphenolic profiles of the pulp were analysed

using UPLC-ESI-MS/MS QQQ (Thermo Scientific UltiMate 3000 HPLC coupled to TSQ Quantiva).
The metabolites quantification was carried out by using an external calibration curves for each compound. To evaluate the performance of the extraction method and analysis, two internal standards (IS) were added to monitor fluctuations in intensity of the peaks. The results were normalized for IS and expressed as mg*100 g-1. Analyses were made in the multiple reaction monitoring (MRM) mode. Data analysis was carried out with Thermo Scientific Xcalibur 3.1 Qual Browser and Thermo Scientific TraceFinder software.

Results

Aware that environmental factors, harvest time, geographic location and storage conditions influence the accumulation of polyphenols [5, 10], the goals of this work are to find difference between new and old cultivars grown under same conditions and estimate their health-related potential as polyphenols content.
The method of Valls *et. al* was performed to detect specific polyphenols in the apple cultivars investigated. In detail, 20 polyphenolic compounds of six sub-classes (phenylpropanoids, dihydrochalcones, flavanones, flavan-3-ols, flavanols and anthocyanin) were quantified.
A future study will be carried out adding two isotopically labelled compounds as internal standards to provide more accurate information in mass spectra avoiding some ionization issues and reducing variations in sample extraction step to make it as comparable as possible [11].

Conclusions

Our findings will provide evidence for consumers about the benefits related to consumption of apples grown in South Tyrol and how to balance the intake of the several cultivars.

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P52

Monitoring of biogenic amines in organic and conventional chicken by HPLC-ESI-QTOF-MS analysis

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Summary: *This study presents a HPLC-QTOF-MS method to monitor 8 Biogenic amines in organic and conventional chicken breast samples. This innovative method guarantees a high sensitivity without any pre-column derivatization. Starting with similar levels at T0, BAs increment resulted lower in organic samples after 10 days of storage (T10).*

Keywords: *HPLC-QTOF-MS, Biogenic amines, Organic chicken*

Introduction

The recent development of organic production in the poultry industry is guided by the increasing consumers interest for healthier food products. However, limited research has been performed to confirm that organic chicken (OC) products are healthier than conventional ones (CC) [1]. This could be done through the comparison of chemical quality markers levels such as Biogenic amines (BAs) between OC and CC. Indeed, BAs are a group of low-molecular-weight nitrogenous compounds present in different food matrixes. They are mainly formed through the bacterial decarboxylation of amino acids and are reported to be harmful to human health with several toxicological effects [2]. BAs determination is an analytical challenge which could be simplified and improved using high-performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (HPLC-QTOF-MS). The present study aims therefore to: a) assess the higher quality of OC by monitoring and comparing the levels of BAs in OC and CC samples during their shelf-life; b) to compare the BAs analysis through HPLC-QTOF-MS and HPLC-DAD.

Experimental

8 BAs were simultaneously determined: spermine (SPE), spermidine (SPD), cadaverine (CAD), putrescine (PUT), histamine (HIS), tyramine (TYR), 2-phenylethylamine (PHE) and tryptamine (TRY). Organic and conventional chicken breast samples were packed and stored at 4 ˚C for 2 weeks. Consecutively, BAs levels were monitored the day of production (T0) and after 10 (T10) days of storage in both chicken types. BAs were extracted with an aqueous solution of Trichloroacetic acid 5%. HPLC-QTOF-MS analyses were performed using an Agilent 1290 series instrument (Santa Clara, CA, USA), equipped with an ESI source operating in positive ionization mode with a vaporizer temperature of 350 ˚C, a nebulizer gas pressure of 55 psi; a drying gas (nitrogen) flow rate of 11 ml min^{-1} and a capillary voltage of 3500 V. The separation of BAs was performed using a Synergi Polar analytical column (150 × 2 mm I.D., particle size 4 µm) and a gradient mobile phase made of

water (A) and acetonitrile (B) at a flow rate of 0.3 ml min^{-1}. The injection volume was 1 µl. The mass analyzer was operating in Full Scan mode by extracting accurate mass $[M+H]^+$ for each molecules, i.e.89.1073 *m/z* for PUT, 103.123 *m/z* for CAD, 122.0964 *m/z* for PHE, 138.0913 *m/z* for TYR, 112.0869 *m/z* for HIS, 161.1073 *m/z* for TRY, 203.225 *m/z* for SPE, 146.1652 *m/z* for SPD.

Results

From the analyses performed on OC and CC samples, the total levels of BAs increased from T0 to T10. However, this increment was lower in OC respect to CC samples. Indeed, PHE, PUT and CAD, which were not detected at T0 in both chicken types were present at T10 but only in CC samples. TYR instead, was observed only at T10 but was higher in CC samples. On the other hand, HIS was present in both sample types at T0. However, after 10 days of storage (T10), HIS level drastically increased in CC samples while it remained constant in OC samples. HIS and TYR are considered the most toxic BAs and are predominantly relevant for food safety. SPM level was similar in both types of chicken sample and remained constant through the shelf-life. Contrarywise, SPM level decreased in CC samples and slightly increased in OC samples. The lower increment of BAs in OC samples was also observed after HPLC-DAD analyses. Nevertheless, HPLC-QTOF-MS showed the advantage that BAs were directly analysed after extraction without the need of an intermediary pre-column derivatization step as before HPLC-DAD analyses [3]. Moreover, the HPLC-QTOF-MS method showed a higher sensitivity.

Conclusions

This study allowed to compare the levels of BAs in organic and conventional chicken during the shelf-life. From the analyses performed in HPLC-ESI-QTOF-MS, OC resulted healthier than the CC having lower levels of toxic BAs than CC after 10 days of storage.

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P53

A new HPLC-MS/MS analytical method for isoflavone and lignan quantification in 25 green coffee samples

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Summary: *an analytical method for quantification of lignans (lariciresinol, matairesinol and secoisolariciresinol) and isoflavones (biochanin A, daidzein, daidzin, genistein, genistin and formononetin) in green coffee has been developed by using HPLC-MS/MS. Results showed that the best extraction process was a base hydrolysis followed by enzymatic digestion and lignans were more abundant than isoflavones.*

Keywords: *green coffee, phytoestrogen, HPLC-MS/MS*

Introduction

Coffee is one of the most important agricultural products in the international trade and last year 68 millions 60 kg bags of green coffee were produced [1]. Green coffee beans, the starting raw material for roasted coffee and coffee beverages, are constituted by carbohydrates (55-65.5%), lipids (10-18%), nitrogen containing compounds (11-15%), purine alkaloids (0.8-4.0%), chlorogenic acids (6.7-9.2%) and minerals (3-5.4%). Other molecules that are found in lower percentages are non-volatile aliphatic acids (citric, malic and quinic acids) and phenols such as phytoestrogens [2]. The most studied and best-known phytoestrogens in foodstuffs are isoflavones and lignans. Both classes have been investigated in coffee powder and beverages but, to the best of our knowledge, none has quantified them in green coffee. Hence, we sought to develop a simply and fast method to quantify three lignans (lariciresinol, matairesinol and secoisolariciresinol) and six isoflavones (biochanin A, daidzein, daidzin, genistein, genistin and formononetin) in green coffee beans by using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). For that purpose, firstly, we evaluated different extraction processes and the best one was chosen for lignan and isoflavone extraction. Secondly, we set up an efficient and fast HPLC-MS/MS method for simultaneous quantification of target molecules in green coffee. Finally, after validation the selected method was applied to 25 green coffee samples.

Experimental

HPLC-MS/MS studies were performed using an Agilent 1290 Infinity series and a Triple Quadrupole 6420 from Agilent Technology (Santa Clara, CA) equipped with an electrospray ionization (ESI) source operating in negative and positive ionization mode. The separation of target compounds was achieved on a Kinetex C18 analytical column (50 mm x 2.10 mm i.d., 2.6 µm) from

Phenomenex (Castel Maggiore, Bologna, Italy). The mobile phase for HPLC-MS/MS analyses was a mixture of 85% water (A) and 15% HPLC-grade acetonitrile (B), both with 0.1% formic acid. The separation was obtained by flowing at 0.4 mL/min with this gradient elution: 0 min (15% B), 5 min (40% B), 8 min (15% B) and then constant until the end of the run (10 min). Detection was performed in the "multiple reaction monitoring" (MRM) mode. Different extraction processes, such as acid and base hydrolysis, enzymatic digestions, organic solvent extraction and a combination of these, were tested and recovery and quantitative data were examined as well. The most efficient was chosen and applied to 24 *Coffea arabica* samples having different geographical origins and 1 *Coffeea canephora*. Chemical differences among the various coffee samples, in terms of lignan and isoflavone contents, were analysed by PCA using the statistical software STATISTICA v.7.1 (Stat Soft Italia S.r.l., Vigonza, Italy).

Results

A new analytical method for simultaneous quantitation of three lignans and six isoflavones was developed by using HPLC-MS/MS triple quadrupole. For isoflavones in glycosidic form, the precursor ions were protonated molecules $[M+H]^+$ in positive polarity, whereas for the aglyconic form and lignans the precursor ions were deprotonated molecules $[M-H]^-$ in negative polarity. The method showed good linearity (R^2 for all target compounds were equal to or higher than 0.9952), sensitivity (LODs for isoflavones and lignans ranged from 0.1 to 15 μg L^{-1}) and the separation of studied molecules was obtained within 6 min. The best performing process was a double extraction composed of base hydrolysis in methanol and enzymatic digestion with clara-diastase, since it showed good recovery levels, ranging from 74 to 94%, and the highest total concentration of all the compounds studied (1193.4 μg kg^{-1}). Therefore, this process was chosen and applied to 25 green coffee samples. Results showed that lignans (286.5-8131.8 μg kg^{-1}) were more abundant than isoflavones (3.4-300.0 μg kg^{-1}) and secoisolariciresinol (172.6-5714.1 μg kg^{-1}) and lariciresinol (113.9-2417.7 μg kg^{-1}) were the most abundant compounds, followed by genistin (12.6-204.8 μg kg^{-1}). After PCA analysis we found that Ethiopian samples differed significantly from the other origins for the higher content of secoisolariciresinol and lariciresinol, indicating that the lignan content can be influenced from the country of cultivation and that these green beans could be used as a good source of lignans.

Conclusions

For the first time, a new analytical method for the simultaneous quantitation in green coffee of three lignans (lariciresinol, matairesinol and secoisolariciresinol) and six isoflavones (biochanin A, daidzein, daidzin, genistein, genistin and formononetin) has been developed, validated, and then applied to 25 green coffee samples. This work provided new knowledge into two important phytonutrients (isoflavones and lignans) in green coffee, one of the most important agricultural products in the international trade.

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P54

UHPLC-ESI-MS/MS for the analysis of primary aromatic amines in plastic food contact materials

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Summary: *In this work, an UHPLC-ESI-MS/MS method (pentafluorophenylpropyl (PFPP) column and acidic mobile phase) was developed for the analysis of 23 PAAs. This sensitive and selective method avoids the inconveniences of ion-pair reagents in MS. The proposed method was applied to determination of PAAs in migration studies of black plastic kitchen utensils.*

Keywords: *primary aromatic amines, food contact materials, ultra-high-performance liquid chromatography-tandem mass spectrometry*

Introduction

In the last few years, the migration of chemical compounds, coming from different packaging and plastic food contact materials, is among the top food safety concern. In addition to plasticizers and mineral oils, special attention is being paid to primary aromatic amines (PAAs) frequently used to manufacture azo dyes and certain polymers and adhesives in the food packaging industry.

PAAs can be produced during the undesired hydrolysis of isocyanates in polyurethane adhesives, used in multilayer films, and could be present in recycled packaging paper, because their use as reagent in its production process [1]. PAAs can also be originated from the decomposition of azo dyes, used as inks, that can migrate for example, from black polyamide kitchen utensils, especially in case of contact with hot acid solutions [2].

It is known that certain PAAs show a toxicological concern as they have been identified as carcinogenic compounds. PAAs stand out among the non-intentionally added substances (NIAS) found in flexible packaging. This means that special attention must be paid to ensure that they do not migrate into food at detectable levels. European Union limit the maximum migration of PAAs from food contact materials to 20 µg aniline equivalents/kg food or food simulant [3]. To detect and monitor the migration of PAAs from different food contact materials reliable analytical methods able to detect and quantify these compounds at low concentration levels are necessary.

Currently, one of the most suitable confirmatory technique for the determination and confirmation of these polar PAAs is liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Until now, many methods have been developed in the literature for the identification of PAAs in food contact materials, but most of them use ion-pair reagents to retain PAAs in a reversed phase column (C18) [4]. Nevertheless, the used of non-volatile substances produce maintenance and ionization problems when using mass spectrometry.

Experimental

A UHPLC-ESI-MS/MS (QqQ) method was developed to quantify 23 PAAs in food contact materials. The UHPLC-ESI-MS/MS method was achieved using a pentafluorophenylpropyl (PFPP) column (100 × 2.1 mm; 2.7 µm) with a ternary mobile phase (acetonitrile:ammonium formiate: acetic acid) and working in positive ion mode and multiple reaction monitoring mode (MRM).

Several food contact materials were analyzed to study the applicability of the developed method. The study was carried out on twenty-four different commercial plastic objects used in kitchen. All products were produced in Asian countries and they were obtained in local markets from Barcelona. The migrations tests of all samples were done following the guidelines described by the EU regulation 10/2011 [5] to carry out the migrations tests in Food Contact Materials (FCM).

Results

The chromatographic separation of 23 primary aromatic amines (PAA) is achieved in less than 10 min under gradient elution with a ternary mobile phase. All PAAs are ionized with ESI in positive ion mode generating the protonated molecule [M+H]+ as base peak of the mass spectra. Moreover, no significant in-source CID fragment ions are observed although most of the target compounds generate the adduct ion of [M+H+ACN]+ (Rel. Ab.%, 30%). To improve the detectability and to ensure the identification and quantitative determination of target compounds, tandem mass spectrometry was evaluated. Tandem mass spectra of PAA ions generated ESI were studied and the corresponding product ions were characterized. Thereby, the two most selective and abundant products were selected for quantitative and confirmatory purposes when working in multiple reaction monitoring (MRM) mode.

Instrumental limit of detection (ILOD) and limit of quantitation (ILOQ) were determined (calculated using standard solutions, LODs based on a signal-to-noise ratio of 3:1 and LOQs based on a signal-to-noise ratio of 10:1) and ranged from 0.002 to 9.2 ng/g aniline. Run-to-run precision was estimated (concentration levels: 20 and 200 ng g–1) obtaining relative standard deviation values (n = 5, RSD%) lower than 10% in all cases. Trueness was also evaluated obtaining satisfactory results, with relative errors lower than 10%. Moreover, day-to-day precision (RSD%) was <10%. These results indicate that the proposed UHPLC–ESI–MS/MS method shows good performance and can determine the selected 23 PAAs in migration studies.

In this work, 24 commercial plastic objects used in kitchen were studied following the marked guidelines for migrations tests for FCMs. As these guidelines indicate, the test should be carried out three consecutive times with the same sample piece of sample, each time using a new portion of simulant. In this way, the determination of PAAs on the analyzed materials must be evaluated on the concentration determined in the simulant during the third trial. Nonetheless, it was necessary to choose the time conditions as well as the right stimulant. 2 cm2 of each sample and 3.3 mL of stimulant B (acetic acid 3%) were used and a temperature of 100 ºC for 2 hours to simulate the most extreme legislated conditions.

Among the analyzed samples, up to four PAAs were detected in three positive samples and they were quantified using external calibration method. Individual concentrations of PAAs have been expressed as aniline concentration, since

the legislation limit is fix for PAAs as µg of total PAAs (expressed as µg of aniline) per kg of sample (10 µg aniline/kg). Table 1 shows the obtained values for each positive sample and as it can be seen, in all cases it exceeds more than 100 time the maximum legislated limit.

Table 1. *Concentrations of PAAs found in positive samples*

Compound	**M1**	**M2**	**M3**
	Conc. (µg aniline/kg)		
ANL	743	795	1590
p-CLA	5	9	13
o-TUL	40	45	–
NAP	8	–	–
TOTAL	796	849	1603

For confirmatory purposes and for avoiding false positives, ion ratios between both quantitative and confirmatory transitions peak areas were compared with that obtained from the corresponding standards. For all the compounds detected in samples the ion ratio deviation ranged from 0.3 to 10% indicating the absence of false positive among the analyzed samples.

Conclusion

The performance of the UHPLC-ESI-MS/MS proposed method showed good performance and allowed the accurate determination of PAAS in plastic food contact materials. The selectivity and the interaction of phenylpropyl stationary phase made possible the use of an acidic mobile phase, which allows the protonation of PASs and the high responses in ESI positive ion mode, thus avoiding the use of ion-pair reagents. Among the samples analysed PAAS were identified and quantified in three samples imported from non-European countries, thus confirming the necessity to control the presence of these analytes in food commodities within the EU.

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P55

Proteomic characterization of kefir milk by two-dimensional electrophoresis followed by mass spectrometry

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Summary: *Kefir is a type of fermented milk rich in nutraceutical substances such as amino acids, vitamins and mineral salts. In this work a proteomic analysis, by 2DE and mass spectrometry, has been performed on kefir milk. As a result, milk-derived bioactive peptides with positive impact on human heath were identified.*

Keywords: *kefir, two-dimensional electrophoresis, mass spectrometry*

Introduction

Kefir is a type of fermented milk obtained thanks to the lactic and alcoholic fermentation of bacteria and yeasts provoked by kefir grains which are a set of bacteria and yeasts held together by a polysaccharide matrix called kefiran. Grains are a light, gelatinous and white mass containing proteins, lipids, and the kefiran, which surrounds bacteria and yeasts. The microbiological composition of kefir varies greatly depending on the composition of the grains, the milk used and the length of time in which the kefir grains are cultured. The chemical composition of kefir depends on the type of milk used. During the fermentation process, the amino acids valine, leucine, lysine and serine are formed as well as appreciable amounts of vitamin B6, vitamin B12, folic acid, and biotin. As regards the content of mineral salts, kefir is a good source of phosphorus, magnesium, and calcium. Kefir is also characterized by the presence of bioactive peptides which derive from the proteolytic processes of milk proteins (caseins and whey proteins) exerted by the
bacteria present in fermented food products and/or occurring naturally in the gastrointestinal tract. Bioactive peptides may have a positive impact on the health and functionality of the organism, in particular on the cardiovascular, digestive, immunological and nervous systems and this strictly depends on the sequence of amino acids and the length of the chain that normally ranges from 2 to 20 amino acids. Furthermore, kefir milk is suitable for lactose-intolerant individuals since the grains show a ß-galactosidase activity and a recent study performed in high-fat diet mice revealed that kefir consumption might help to prevent obesity and fatty liver disease by promoting fatty acid oxidation [1]. In light of the important role played by milk-derived proteins in kefir, in this study we performed a proteomic analysis by two-dimensional electrophoresis (2DE) followed by mass spectrometry (MS), in order to identify proteins and peptides that may have a positive impact on human health.

Experimental

Before 2DE, kefir milk was centrifuged (13000g for 20 min) and then filtered with 0.22µm filter to remove bacteria. The total protein present in the filtered kefir was concentrated by precipitation with 90% ammonium sulphate. The precipitated proteins were resuspended in 50mM Tris/HCl pH 7.5 and before 2-DE were treated with 2D-Clean-Up (GE Healthcare). 1.0 mg of total proteins were subjected to isoelectric focusing on an immobilized pH gradient 3-10 (Immobiline DryStrip gel, 18cm). The second dimension consisted of 15% SDS-PAGE. After electrophoresis, the proteins were stained by Coomassie blue. The gel was analyzed by the software PDquest (Bio-Rad) for spot quantitation, determination of isoelectric point and molecular mass. Target protein gel spots were in-gel digested with trypsin and extracted for MS identification. The tryptic peptides were injected into a reversed-phase chromatography (C18 Gemini-NX, 5 µm particle size, 110 Å pore size, 250 x 4.6 mm) connected to an HPLC Agilent Technologies 1100 Series. The column effluent was analyzed by MS using an electrospray ion trap mass spectrometer (Agilent Technologies LC/MSD Trap SL) operating in positive ion mode over the mass range 300-2200 amu (atomic mass units). MS operating conditions were: nebulizer pressure, 70 psi; draining gas flow, 12 L/min; drying gas temperature, 300°C; capillary voltage, 3.5 kV. Obtained spectra were extracted and analyzed by the MASCOT software (www.matrixscience.com).

Results

From the results shown in Figure 1, it can be seen that some of the low molecular weight spots, KA, KB, KC, KH, KI, KM are proteins derived from α- and κ-caseins and some of them contain specific amino acid sequences of the bioactive peptides derived from caseins.

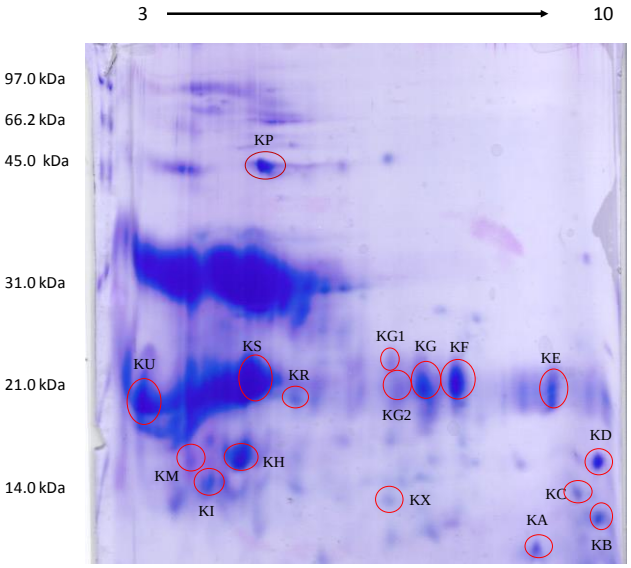

Fig. 1. *2DE map of proteins extracted from kefir milk*

In particular, the KA, KB and KC spots were identified as κ-casein (*Bos taurus*), that in kefir milk are present as fragments of κ-casein because of their low

molecular weight (10-19 kDa). The sequence obtained for the spots KA, KB and KC (YIPIQYVLSR) correspond to the casoxine C, a bioactive peptide that acts mainly as an opioid antagonist and is obtained precisely from the digestion of κ-casein.

The spots KH, KI, correspond to the α_{s1}-casein (*Bos taurus*), whereas the spot KM is a peptide derived from the α_{s2}-casein (*Bos taurus*) which contains the casocidin-1 sequence, a bioactive peptide with antimicrobial properties. This bioactive peptide is able to inhibit the growth of Gram-negative and Gram-positive bacteria *in vitro*. Spots KE, KF, KG, KG1, and KG2 correspond to the Glycosylation-dependent cell adhesion molecule 1 (*Bos taurus*) also known as Lactophorin, which is strongly expressed during lactation by the mammary epithelial cells. The expression of this protein is induced during pregnancy similarly to the hormonally induced milk proteins and the protein can be normally found in the milk of the secreting mammary gland [2]. It has been shown that Lactophorin exists in several molecular forms determined by different glycosylation levels [3]. Furthermore, in our study at least 5 forms of Lactophorin with similar molecular mass but different pIs were detected. Lactophorin shows several functions: emulsifier, inhibitor of lipoprotein lipase, and has a role in calcium absorption. Spot KR and KS were identified as □-lactoglobulin isoforms (*Bos taurus*) and, lastly, spot KP as enolase (*Streptococcus thermophilus*).

Conclusions

The results obtained from this study help to clarify the protein composition of kefir milk and also identify and characterize the bioactive peptides (of which this matrix is particularly rich) that could have an important nutraceutical function and hence a positive impact on human health.

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P56

Development of a multi-group screening method for the determination of over 270 mycotoxins, pesticides and veterinary drugs in food

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Summary: *This work describes a multi-group screening method to detect over 270 analytes in food of different origin using LC-Q-Orbitrap. The application of a hybrid high resolution mass analyser associated to a customised MS database allow to minimize the percentage of false positive results.*

Keywords: *LC-HRMS/MS, environmental contaminants, veterinary drugs, food*

Introduction

The enormous evolution of mass spectrometry analysers not only enables the improvement of the analytical performance of methods, but also the increase of the number of substances which can be reliably detected in only one analysis. In this context, it is now feasible the development of multi-group procedures encompassing, for example, environmental pollutants and veterinary drugs in food at one time. Since the sample preparation protocols of the generally complex food matrices must be generic, this approach could suffer of scarce selectivity causing high percentages of both false positive and false negative results. The aim of this work was to develop and validate a multi-group screening method using liquid chromatography coupled to a hybrid high resolution mass spectrometry (LC-Q-Orbitrap). A custom database including retention times, *m/z* and MS^2 spectra was implemented to detect and identify about 270 analytes. Preliminary validation experiments were carried out in food of different origin (bovine muscle and wheat flour).

Experimental

Three hundred twenty-two compounds were tested at spiking concentrations depending on the level of interest [1-3]: 10 µg/kg for pesticides and permitted drugs, 1 µg/kg for banned drugs, except beta-agonists and chloramphenicol (0.2 µg/kg), 200 µg/kg for mycotoxins except aflatoxin B1 and ochratoxin A (2 µg/kg). Pesticides and mycotoxins (154 compounds) were spiked in wheat flour sample, whereas permitted and banned veterinary drugs (168 compounds) were added in bovine muscle. Six replicates were carried out for each matrix. The sample preparation scheme is reported in Figure 1. The analyses were carried out by LC-Q Orbitrap (Thermoscientific, San Jose, CA, USA) The instrumental conditions were described in Moretti et al. 2016 [4] with slight modifications. The acquisition was performed in a combined Full mass scan and data-dependent MS/MS mode (Full MS/dd-MS^2).

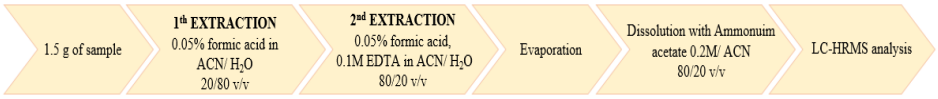

Fig. 1. *Sample preparation steps*

Results

The approach here proposed is based on a customized database, in which three different groups of substances are included: veterinary drugs (banned and authorized), mycotoxins and pesticides for a total of about 270 compounds. The database considers at first the accurate *m/z* value and retention time. In case of a suspect peak, its MS/MS spectrum was compared with the spectral library in order to confirm the identity of the found substance. Among the 154 pesticides and mycotoxins spiked in wheat flour, 139 were detected at the screening concentration levels detailed before, whereas, in meat, 137 out of the 168 added veterinary drugs were successfully revealed. No false positive results were observed. In Figure 2 the LC-HR MS chromatogram shows seven permitted veterinary drugs in bovine meat: 1) desacetylcephapirin, 3) sulfathiazole,) tulathromycin, 7) ciprofloxacin, 8) ampicillin, 9) demeclocycline and 11) albendazole). In the same chromatogram five banned drugs are also detected: 2) metronidazole, 4) chloramphenicol, 5) azaperol, 10) promazine and 12) 16-beta-hydroxystanozolol. In meat the majority of false negative results were observed for banned substances which were added at very low concentrations (1 or 0.2 µg/kg).

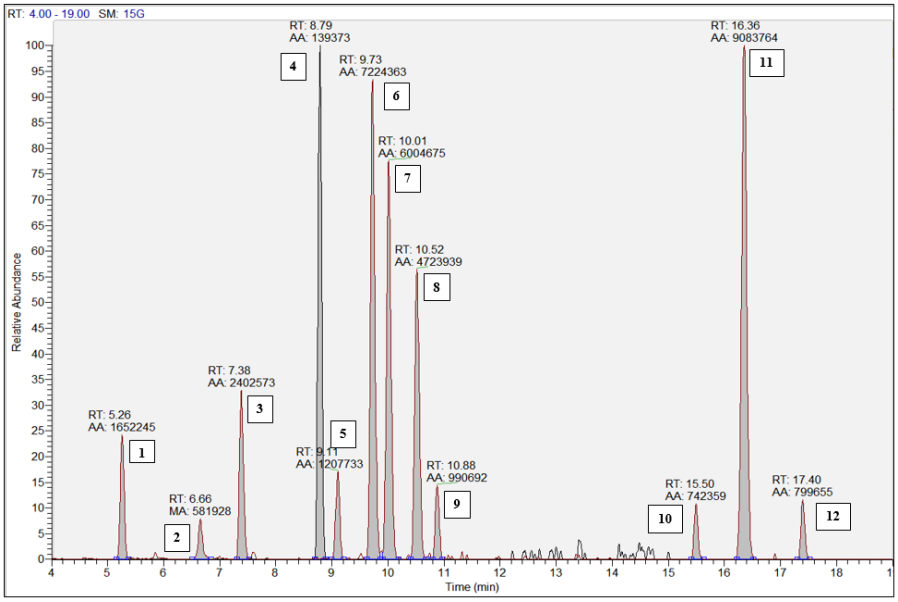

Fig. 2. *LC-HRMS/MS chromatogram of twelve veterinary drugs in bovine muscle*

Conclusions

The implementation of rapid screening tests able to detect both environmental contaminants and veterinary drug residues at the same time is an intriguing

chance both for the official laboratories and food industry since this approach can replace dozens of the single-class screening methods currently used. Further validation experiments are in progress also testing the method applicability to other commodities.

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P57

Proteomic characterization of olives from the Marche region cultivar *Piantone di Mogliano*

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Summary: *In this work a protein profile of the olives from Marche region Piantone di Mogliano cultivar was obtained using a proteomic approach based on 2DE and mass spectrometry. The results showed the presence of specific proteins that characterize this cultivar and gave an indication of the transformation processes effects.*

Keywords: *Table olives, two-dimensional electrophoresis, mass spectrometry*

Introduction

Piantone di Mogliano is a plant olive cultivar (*Olea europaea*) from Marche region, mainly located in Mogliano (Macerata). In general, olive drupe is composed by water, fat, carbohydrates, protein, fibre, pectin, biophenols, vitamins, organic acids and mineral elements which all together are responsible for the quality and aroma of the fruit. Moreover, in the olive pulp there are several enzymes involved in many metabolic processes and in the development of phenolic and aroma compounds. Therefore, olive fruit development is a combination of biochemical and physiological events that occur under strict genetic control and are influenced by several environmental conditions [1].

Because of their bitter taste due to the presence of oleuropein, raw olives are subjected to transformation processes before to be considered edible. In this work table olives from *Piantone di Mogliano* were produced by two different processing technologies: the Sevillian style and the Natural style introducing innovative techniques in order to improve the nutritional properties of this typical olive. The aim of the present work is to achieve a sort of "identity card" which is important for the traceability of olives from *Piantone di Mogliano* cultivar. At this purpose, a proteomic approach based on two-dimensional electrophoresis (2DE) followed by mass spectrometry (MS), was used for protein identification in the raw olive (RO) fruit. Furthermore, the RO proteome has been compared with that of the Sevillian style (SO) and the Natural style (NO) table olives in order to verify if the processing techniques used can affect the olive protein profile.

Experimental

In this work, a protein extraction protocol based on the modification of already published methods has been developed [2]. Briefly, the frozen olive pulp from RO, SO, and NO was ground using liquid nitrogen into a fine powder. The proteins were extracted from the olive powder through a first TCA/acetone extraction procedure, followed by a second phenol extraction procedure. The obtained protein pellet was resuspended in a rehydration buffer containing urea

and detergent and subjected to isolelectric focusing on an immobilized pH gradient 3-10 (Immobiline DryStrip gel, 18cm). The second dimension consisted of a 15% SDS-PAGE [3]. After staining with Coomassie blue, the gel was subjected to image analysis (PDquest software) for spot quantitation, and determination of pI and molecular mass. Selected spots were extracted from the gel (after trypsin digestion) and subjected to mass spectrometry for protein identification. The tryptic peptides were injected into a reversed-phase chromatography (C18 Gemini-NX, 5µm particle size, 110 Å pore size, 250 x 4.6 mm) connected to an HPLC Agilent Technologies 1100 Series. The eluent was analyzed by mass spectrometry using an electrospray ion trap mass spectrometer (Agilent Technologies LC/MSD Trap SL) operating in positive ion mode over the mass range 300-2200 amu (atomic mass units). MS operating conditions were: nebulizer pressure, 70 psi; draining gas flow, 12 L/min; drying gas temperature, 300°C; capillary voltage, 3.5 kV. Obtained spectra were extracted and analyzed by the MASCOT software (www.matrixscience.com).

Results

The protein expression profile of *Piantone di Mogliano* RO extracts was examined by 2-DE in the pH range 3-10 and was compared with the NO and SO samples. In figure 1A are shown the results obtained for the RO samples. In particular, Spot A (16.3 kDa; pI = 6.2) was identified as the Copper and zinc superoxide dismutase (Cu-ZnSOD) as well as the spots B and C (16.4 kDa, pI = 5.9; 16.1 kDa, pI = 5.6, respectively). Different isoforms of superoxide dismutase differing in the isoelectric point were found in several species and also in plants [4]. In higher plants, superoxide dismutase enzymes act as antioxidants and protect cellular components from being oxidized by reactive oxygen species.

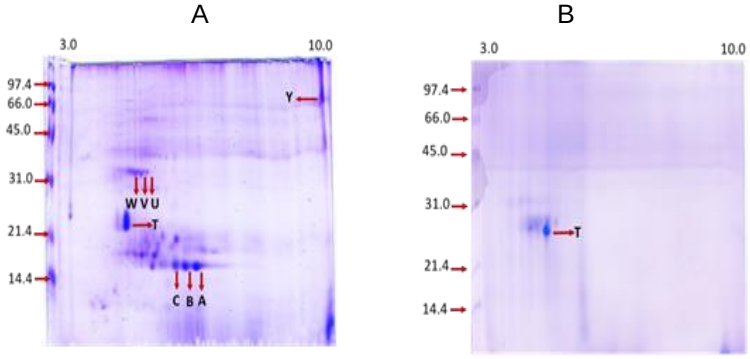

Fig. 1. *2DE maps of Piantone di Mogliano RO samples (A), and NO samples (B)*

Spot T (25.4 kDa; pI = 4.3) has been identified as the Thaumatin-like protein (TLP), a protein that belongs to a large and complex family involved in the defence of the host and in the processes of development in fungi, animals and plants. In particular, TLPs have a broad-spectrum antifungal activity determined by a series of interactions with the surface components of the pathogenic cells [6]. Recently have developed several transgenic plants that express a variety of TLPs genes in order to improve resistance against various pathogens or against environmental stress. Spots U, V, W (pI/Mr: 5.3/33.0; 5.1/32.7; 5.0/32.9, respectively) were assigned to the Phospholipid-transporting ATPase 1, a protein is involved in the transmembrane flipping of lipids. This enzyme

catalyses the hydrolysis of ATP coupled to the transport of phospholipids from the outer to the inner leaflet of various membranes ensuring the maintenance of asymmetric distribution of phospholipids. Spot Y (pI/Mr: 9.8/69.3) has been identified as the Microtubule-associated protein TORTIFOLIA1-like. The de-bittering treatment to which the SO and NO have been subjected, led to the loss of most of the proteins. In particular, from figure 1B it is evident that in the NO sampes was still observed the presence of the Thaumatin-like-protein, whereas, in the case of SO samples, the debittering process had caused a complete loss of the proteins (data not shown).

Conclusions

Thanks to this proteomic analysis, it has been possible to identify specific proteins that characterize the *Piantone di Mogliano* cultivar, a native species of the Marche region, such as the antioxidant enzyme Cu-ZnSOD and, among all, TLP that had never been found so far in a cultivar. In particular, TLP could be considered as a molecular marker of *Piantone di Mogliano* cultivar, although further studies will be needed to prove it. Furthermore, this study provided an insight into the changes that occur in the protein profile during the transformation processes.

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P58

Application of metabolomics methods on LC/GC-QTOF data to discriminate extra virgin olive oils from different Protected Designations of Origin

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Summary: *Combination of non-targeted and targeted approaches implying the use of powerful platforms (LC-ESI-Q TOF/GC-APCI-Q TOF) to identify potential "PDOs´ markers*

Keywords: *PDO extra vergin olive oil, target and untargeted analysis*

Introduction

The increasing popularity of extra virgin olive oil (EVOO) and the increasing problem of food fraud have provided the need for quality and authenticity control. Typical problems are mislabelling of protected designation of origin (PDO) or edible oil adulteration. Implementation of protected designations of origin (PDOs) and protected geographical indications (PGIs) is one of the most prominent differentiation strategies used in olive oil market. They are often perceived as valuable tools that promote specific attributes of the oil linked to its geographical provenance. Minor compounds of extra virgin olive oil, such as phenolic and triterpenic compounds, sterols and tocopherols, are highly influenced by agro-technological practices and can be used for olive oil authentication.

Methods

In this study 126 oil samples from 6 Mediterranean PDOs were analyzed by LC-MS and GC-MS combined to statistical methods. The extracts were eluted with a 15 min gradient including a flow gradient (0.4-0.6 mL/min) on an UHPLC using a C18 (2.1 x 100 mm, 1.8 µm) column, with acidified water and acetonitrile. The column oven temperature was 40° C. The derivatized extracts were injected in GC, using a BR-5 column with a 50 min T gradient from 150 to 320□C (4□C/min rate). Both systems were coupled to a Compact™ QTOF MS (Bruker) by an ESI and an APCI interface for LC and a GC-APCI source for GC.

Preliminary Data

Data acquired with all the platforms was processed with MetaboScape 3.0 (Bruker), which automatically extracts and combines isotopes, adducts and

fragments belonging to the same compound into one feature. The resulting bucket table was used for statistical analysis. Non-targeted and targeted approaches were used to offer maximum coverage of the olive oil metabolome's chemical space in a first step, and the possible validation of the identified markers afterwards. Statistical analysis (PCA, PLS-DA) led to a noticeable discrimination among the six evaluated PDOs considering the data coming from LC-MS and GC-MS. Several compounds such as elenolic acid, luteolin, oleuropein and ligstroside aglycones, and some other tentatively identified substances, were identified as possible PDOs distinctive markers. They enabled the discrimination among different PDOs. The combined use of non-targeted and targeted approaches enhanced the outcome of the study. GC-APCI-Q TOF preserves the pseudo-molecular ion information, which is a great advantage over the "classical" GC-EI-MS systems and facilitates the identification of unknown markers.

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Volatile organic compounds produced during cooking: development and application of an SPME-GC-MS method

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Summary: *In the present study an SPME-GC-MS method has been developed to investigate VOCs emission from cooking processes. The analytical method has been applied also to assess the efficiency of an aspiration system present in domestic kitchen.*

Keywords: *VOCs, cooking emissions, SPME-GC-MS.*

Introduction

In the last decades indoor air quality has become an important issue, because people tend to spend most of their time in closed buildings. Indoor the concentrations of some pollutants are often 2 to 5 times higher than typical outdoor concentrations [1]. The principal indoor pollutants are nitrogen oxides (NOx), carbon oxides (CO and CO_2), volatile organic compounds (VOCs) and particulate matter (PM), deriving from common domestic activities, such as cleaning, cooking, smoking or using fireplaces. Some VOCs are known to be irritant, toxic or carcinogenic and can negatively affect human health, according to the time of exposure and their level of concentrations [2]. Cooking processes are one of the main causes of VOCs development in domestic environments. In this study, a solid-phase microextraction coupled to gas chromatography with mass spectrometry (SPME-GC-MS) system has been developed to explore the profiles of VOCs produced from cooking. The air above the kitchen plate has been sampled using olfactometric bags and then analyzed. Health risks associated to VOCs resulted to increase when they tend to accumulate in poorly ventilated environments. Ventilation effectiveness (air exchange efficiency and pollutant removal efficiency) affect heavely indoor air quality. For example, several studies indicate that inadequate ventilation is a common denominator in buildings with sick building syndrome (SBS) problems [3]. Motivated by these reasons, the proposed method has been applied also to evaluate the efficiency of a hood aspiration filter present in domestic kitchens.

Experimental

For the sampling polyethylene terephthalate (PET) olfactometric bags were used, aspiring the air above the kitchen plate. Fries deep-fat frying was used as cooking model system. Then a divinyl-benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber assembly was exposed inside the bag for the extraction of the VOCs from the samples. Different extraction times (1h, 3h, 5h, 7h, 24h) were evaluated, finding that an extraction time of 24 hours allowed to quantitate and to detect a much higher number of analytes. Then VOCs were analyzed by GC-MS using a 6890N Network GC System coupled to a 5973

Network Mass Selective Detector (Agilent Technologies). A capillary column coated with polyethylene glycol (60 m x 0.25 mm x 0.25 µm film thickness, DB-WAX, Agilent Technologies) was used. The evaluation of the efficiency of the filter was made analyzing the air before and immediately after passing on the hood aspiration filter.

Results

The first analyses were made to evaluate the best extraction time. The shortest extraction times (1h, 3h, 5h and 7h) were not satisfactory, while an extraction time of 24 hours allowed to quantitate and to detect a much higher number of analytes. Sixty-seven compounds were identified and subdivided according to their chemical nature. Aldehydes are the predominant VOC species (50%), followed by alcohols (13%), aromatic hydrocarbons (12%), aliphatic hydrocarbons (12%), organic acids (7%), ketones (5%), other compounds (1%) and esters (<1%). Most of the detected VOCs are generated via chemical reactions occurring with the oil or the food matrix under high temperatures by three major pathways: 1) lipid oxidation and hydrolysis 2) Maillard reaction 3) secondary reactions of the intermediates or final products. Then the method was applied to assess the efficiency of the hood aspiration filter, determining the composition of VOCs in the air above the cooking plates before and after the passage through the filter (Fig. 1).

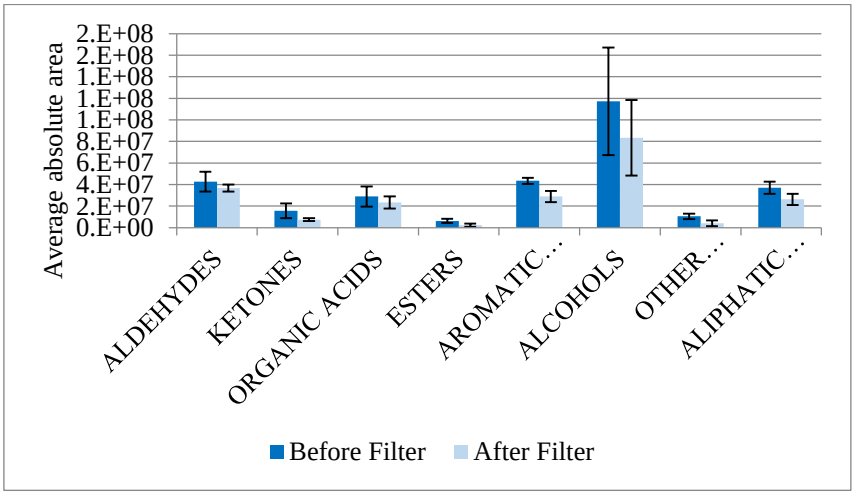

Fig. 1. *Amount (in terms of average absolute areas) of the different VOC classes before and after the passage through the filter. Bars indicate ± standard deviations.*

The amount of each analyte decreased by passing through the filter. The percentage of dejection was calculated for each class of VOCs; dejections were in the range 38-70%. Definitely, the analyzed filter resulted to be efficient towards all the detected VOCs even if with a certain selectivity for the different VOC classes.

Conclusions

The development of the proposed method has been useful for the study of

VOCs produced during cooking processes. This SPME-GC-MS system can successfully be exploited in several applications contributing to assess indoor air quality. Preliminary results on the evaluation of the efficiency of a hood aspiration filter have been presented. Further studies will be made also to monitor the development of VOCs during cooking processes and to define a pool of molecules indicating a state of food overcooking.

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P60

Characterization of Trentino maize flours for polenta-making using PTR-ToF-MS

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Summary: *Differentiation of Northern Italian maize flours and polenta were successfully performed using PTR-ToF-MS and multivariate data analysis. The different maize varieties and the place of origin could be both distinguished. Several markers of lipid oxidation and Maillard reactions were found both for raw maize flour and polenta.*

Keywords: *flint maize, polenta, PTR-MS*

Introduction

Maize is one of the most popular cereal grains in the world. In the food sector itself, maize has become part of staple food, which is widely known as maize porridge or, in Italy, as polenta. In northern Italy, up to 5.19% of the total European maize is produced [1]. For polenta preparation, a special maize category, the flint maize, is used. The aroma produced by volatile organic compounds (VOCs) is an important pre-determined factor for the quality of maize and consumer acceptance [2]. However, research about the aroma of Italian maize varieties and its link to geographical origin is still limited. This study aimed to characterize both raw maize flour and polenta of different variety and origin by physical (moisture content, water activity and texture) and volatile analysis through Proton Transfer Reaction Mass Spectrometry (PTR-MS).

Experimental

Three different maize flour varieties from Trentino region: "Nostrano di Storo" (N=14), "Spin di Caldonazzo" (N=4) and "Dorotea di Primiero" (N=4) were collected from producers. They represent the local typical production form small field and plants. As a comparison, one variety from Veneto region: "Marano Vicentino" (N=5) and one instant polenta were sampled as well.

For the Nostrano variety (INT, GbM, GSNV, GS, C1-C4, P1-P4, BC and FM), some samples presented differences in the type of milling (cylinder mill *vs* stone mill). For the Spin variety (181227, 926, 120 and SP), different lots with different producing dates were obtained. The Dorotea variety (LM, FS, RC and LP) had a small scale production and different duration of the sun-drying exposure. In this case the different small-scale artisanal productions allow to have a better estimate of the variability among the producers. Dorotea samples comprised also different storage conditions: one of the Dorotea sample was

vacuum conditioned after milling (RC), one was stored in 12-17°C and the remaining sample were freshly grinded by the producers. The Marano samples (L283A, L270, L331, 414 and 9) had scattered production dates (range 0.5-4 months), and few of them were grown organically (L283A, 414 and 9). Pre-cooked or instant maize flour (VLC) was measured for comparison. All the flours were measured in triplicates for moisture content (1 gr flour for one hour at 130°C) and water activity (AquaLab Ser. 3 Water Activity Meter, USA) before conducting volatile analysis with a PTR-ToF-MS 8000 instrument (Ionicon Analytic GmbH, Austria) on five replicates.

After the measurement of raw maize flours, 100 g of flour were mixed with 400 mL of distillate water for 40 minutes at 100°C using Thermomixer (Bimby TM-31 Vorwerk, Italy) to make polenta. Texture analysis (hardness and stickiness) of polenta using 5 kg load cell and cylinder probe 25 mm (d) x 40 mm P/25 L (TA.XT plus, Stable MicroSystems, UK) was performed in triplicate on 55 mm (d) x 15 mm polenta samples after equilibration for one hour at 40°C. In parallel, PTR-MS analysis was run on 4 gr of polenta for 3 replicates.

For what concerns PTR-MS data analysis, firstly, analysis of variance (ANOVA) with Bonferroni correction was performed in order to select mass peaks of samples that were significantly higher than the blanks (p<0.01). On the reduced data set Principal Component Analysis (PCA) was run and further univariate data analysis (Tukey post hoc comparison) to find VOCs markers for each variety were performed. All data were calculated in mean and standard error and statistically analysed by R Studio Software.

Results

The majority of the maize flour samples presented moisture content between 10.9 and 13.9%. BC and FM showed the lowest moisture content, 8.62% and 9.56%, whereas FS showed the highest moisture content 18.29%, value higher than the CODEX limit of 15% [3]. The water activity is significantly correlated with humidity (r^2=0.943, p<0.05) while hardness of polenta was negatively correlated with stickiness (r2=0.815, p<0.05). The ranking for polenta hardness was from the highest to the lowest: the pre-cooked, Nostrano, Spin, Marano and lastly Dorotea. Differences in texture may be due to the different amylose content of the different flours. Further investigation regarding the maize composition is necessary to confirm this hypothesis.

For what concern PTR-MS analysis on VOCs, PCA (Figure 1) were performed on 60 mass peaks of maize flours and on 125 mass peaks of polenta obtained by the mass peaks selection procedure described previously. FS resulted as an outlier in both maize flour and polenta and was then removed from further analysis.

The PCA score plot in Figure 1 (a) explained 67% of the total variance in raw maize flours. The variance in the first principal component (PC1: 46.3%) related to moisture content, storage or processing condition, e.g., the lowest moisture content (FM and BC) or pre-treatment condition like in the pre-cooked sample (VLC). Meanwhile, the effect of variety and origin were better explained by the second principal component (PC2: 20.7%).

The post hoc test analysis found several significant markers for raw maize flour: Dorotea and Marano variety were characterized by low levels of the m/z = 75.044 tentatively identified as propanoic acid/ propanal/ acetone and resulted from lipid oxidation[4]–[8]. Nostrano variety was characterized by high levels of the m/z = 99.045 tentatively identified as furfuryl alcohol[9],[10]. Furfuryl alcohol is

a product from Maillard reaction and is known as a food contaminant that results from thermal processing like heating or roasting[11]. Spin variety and pre-cooked were characterized by high levels of the *m/z* = 103.076 tentatively identified as pentanoic acid/ 2,3-methylbutanoic acid/ propyl acetate, which is an outcome from lipid oxidation[4], [5], [10], [12].

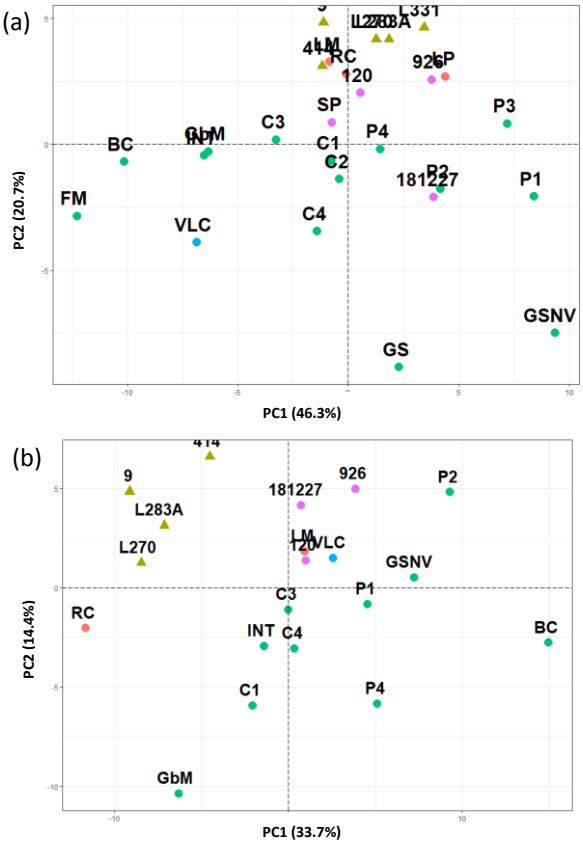

Fig 1. *PCA score plot obtained by the PTR-MS mass peaks for raw maize flour (a) and polenta (b), (ANOVA, p<0.01). Variety: Nostrano; Marano; Spin; Dorotea; Pre-cooked. Origin: Trentino; Veneto.*

The PTR-MS data on the polenta samples confirmed the previous findings: the PCA score plot in Figure 1 (b) explained 48.1% of the total variance in polenta. The first principal component (PC1: 33.7%) separates Marano variety and RC sample of Dorotea from the other samples. This could be related to the amount of lipid as higher lipid content increased the probability of lipid oxidation[13]. The nutritional facts reported on the packaging showed that Marano contained the lowest fat content (3g) compared to Nostrano (4.6g) and Spin (4.7g). Meanwhile, RC vacuum storage condition led to lower lipid oxidation. Besides, the effect of variety and origin were better explained by the second principal component (PC2: 14.4%). In general, the cooking process of maize flour into polenta increased the differences of the sample.

The post hoc test analysis found several significant markers in polenta as well: Nostrano variety was characterized by high levels of the *m/z* = 75.064 and

80.050 tentatively identified as propanoic acid/ propanal/ acetone and pyridine [4]–[9], [12]. Pyridine originates from Maillard-reaction and is a minor component in fresh product [14]. Dorotea and Marano were characterized by low levels of the *m/z* =121.067 and 125.056 tentatively identified as phenylacetaldehyde and guaiacol[5], [7], [9], [10]. Phenylacetaldehyde is a degradation product from phenylalanine and is a precursor for the formation of benzaldehyde, especially in wet condition[15]. The lower concentration of phenylacetaldehyde and guaiacol may be a quality marker since both compounds were associated with off-flavour/ food spoilage in other food matrixes [15], [16]. The pre-cooked polenta was characterized by high levels of *m/z* = 98.065 and 151.107 tentatively identified as pyrrole and decatrienal [4]. Pyrrole is one among the most common volatile found in heated food, which are produced from the Maillard reaction. The instant polenta had a higher amount of pyrrole compared to the other polenta samples because of the cooking process. Longer cooking process also increased the level of decatrienal, which is a decomposition product from linolenic acid[13].

Nostrano and Marano showed opposite aroma profile in both raw maize flour and polenta, even though some authors suggested the two varieties have related genetic traits as Nostrano was originated from Marano[17]. The aroma difference could be the result of open pollination seeding in Nostrano variety that might introduce some change factors [17].

Conclusions

Through volatilome analysis by PTR-MS it was possible to differentiate both raw flour and polenta for variety and production location despite the high variability deriving from small scale production. Other factors like moisture content, water activity, lipid oxidation, storage condition and production date may also give important contribution to VOCs profile. Several markers related to lipid oxidation (propanoic acid/ propanal/ acetone and pentanoic acid/ 2,3-methylbutanoic acid/ propyl acetate) and Maillard reaction (furfuryl alcohol, pyrrole, pyridine, phenylacetaldehyde and guaiacol) were found, thus further investigation related to the initial raw maize flour composition is important to understand the relation.

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P61

Hydrophilic interaction chromatography-tandem mass spectrometric method for the simultaneous determination of polar pesticides in fruits and vegetables

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Summary: *A simple high-throughput HILIC-MS/MS analysis method was developed for the simultaneous determination of chlorates, ethephon, fosetyl, glyphosate, perchlorate, phosphonic acid in fruits and vegetables. A single internal standard was used to correct for matrix suppression effect. The average recovery for all analytes ranged from 79-120%, with a coefficient of variation < 20%.*

Keywords: *Polar pesticides, QuPPe Method, Hydrophilic interaction chromatography*

Introduction

The analysis of polar pesticides is a difficult dare. Their polarity does not allow the direct analysis by reversed-phase HPLC, so alternative methods need to be applied such as QuPPe (Quick Polar Pesticides Method) [1]. The method involves acidified methanol extraction and LC-MS/MS analysis. The coupling between ion chromatography and mass spectrometry shows many advantages for the analysis of very polar substances [2]. Triple quadrupole MS/MS systems offer very low detection limits and high detection selectivity when operated in Multiple Reaction Monitoring (MRM) mode. The aim of this work was to develop and validate a Hydrophilic interaction chromatography tandem mass spectrometry (HILIC-MS/MS) method for simultaneous analysis of some polar ionic pesticides (chlorates, ethephon, fosetyl, glyphosate, perchlorate, phosphonic acid) in vegetable and fruit samples by matrix matched calibration in apple, orange, lettuce and chickpea.

Experimental

Instrumentation: The HPLC apparatus was an Agilent 1260 Infinity II LC equipped with a quaternary pump and an autosampler fitted with an autosampler thermostat. The LC included an Agilent 1260 Infinity thermostatted column compartment. The mass spectrometer was a 6470 triple quadrupole LC/MS.

HPLC Conditions: The chromatographic separations were run on Shodex HILICpak VT-50 2D column (strong anion exchange bound to polyvinyl alcohol), 2.0 × 100 mm, 5µm, at 55 °C. Injection volume was 10 µl and flow rate was 0.3 $ml \cdot min^{-1}$. The mobile phase consisted of Water : Ammonium bicarbonate 45 mM: Acetonitrile 68: 12: 20 (A) and Ammonium bicarbonate 50 mM in water (B). The following eluting conditions were used: 0 min 100 % A; 6.5 min 55 % A; 10 min 0 % A; 27 min 0 % A; 30 min 100% A for three minutes.

Calibration: Calibration curve was implemented in matrix extract solutions for several types of vegetables or fruits: acid (orange), rich water (apple), rich chlorophyll (lettuce) and dried (chickpea) matrices. Calibration curves were obtained by the addition of a single Internal Standard (Phosphonic acid $^{18}O_3$). The working range was from 5 to 1000 μg/Kg, with correlation coefficients >0.995.
Sample preparation: Samples were prepared using the QuPPe method for plant origin materials involving simultaneous extraction with acidified methanol (0.1% formic acid) [1].

Results
The slope of the calibration curve of polar pesticides changes in function of the matrix [3]. Matrix effects were assessed by comparing the slopes of matrix-matched calibration curves with the slopes of solvent-only calibration curves. The matrix effect percentage ($\%M_{eff}$) for each analyte was given by the following formula:

$$\%M_{eff} = 100 \cdot \left(1 - \frac{slope_{matrix}}{slope_{solvent}}\right)$$

$\%\ M_{eff}$ between ± 20% are not significant, because this variability is close to the Coefficient of Variation (CV). A value next to 0% means that no matrix effect is present; positive value means that there is matrix suppression and the negative value means that there is matrix enhancement.
Almost all compounds demonstrated severe matrix suppression ($\%M_{eff}$ >70%) except for glyphosate that showed the smallest $\%M_{eff}$ values with 30%, 39% and 49% in apple, orange, lettuce respectively. Based on these data, internal standards were needed for accurate quantification of these analytes. Phosphonic acid $^{18}O_3$ was chosen as the single internal standard for each target molecule. This simplified the Official Method [1] that employs the isotopically labelled analogues of each target analyte as internal standards.
The method performance was evaluated by spiking the analytes at 10, 200, and 700 μg/Kg (n = 10) in different matrices: orange, apple, lettuce and chickpea. The accuracy (recovery %) and precision (coefficient of variation or CV%) were determined using the calibration standard made in the respective matrix. The overall average recoveries were 85.3 − 119.9% with CV ≤ 10.8% for chlorates, 81.0 − 119.8% with CV ≤ 10.9% for ethephon, 74.1 − 118.0% with CV ≤ 6.3% for fosetyl, 83.7 − 119.0% with CV ≤ 14.1% for glyphosate, 78.5 − 119.5% with CV ≤ 11.4% for perchlorates and 78.5 − 119.5% with CV ≤ 11.4% for perchlorates and 78.7 - 112.7% with CV ≤13.2% for Phosphonic acid. The limit of quantification (LOQ) was estimated as the concentration of analyte in matrix that generates a signal 10 times the baseline noise. Because of matrix interferences and factor suppression unique to each food, the LOQs must be calculated for each matrix. Therefore, the estimated LOQs for all molecules ranged between 2 and 3 μg/Kg in apple, lettuce and orange. In chickpea the LOQ values were much lower, from 0.0029 μg/Kg for chlorates to 0.0131 μg/Kg for phosphonic acid. These LOQ levels were much lower than the tolerance levels (10,0 μg/Kg for biological products) therefore the method was suitable for regulatory work.
At least two product ions of each precursor ion were used in the study. Qualitative and quantitative ion ratios in the spiked samples did not differ >

20% from those of the calibration standard.

Conclusions

A method for the detection of polar pesticides (chlorates, ethephon, fosetyl, glyphosate, perchlorate, phosphonic acid) was developed and shown to provide limits of quantification <3 µg/Kg in vegetable and fruit samples establishing at 5.0 µg/Kg the lowest point useful for quantification in calibration curve. A strong anion exchange bound to polyvinyl alcohol column was employed for the chromatographic separation. Using the 1260 Infinity II LC coupled to the 6470 triple quadrupole LC/MS enables routine analysis. Several matrices were validated for the method. The use of a single internal standard added to the samples before extraction enabled accurate quantitation of each target molecule object of this study. Method validation was performed by matrix matched calibration curve in apple, orange, lettuce and chickpea.

The LC-MS-MS instrument used in this study was sensitive, selective and provided accurate identification and quantification with minimum sample cleanup.

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P62

Determination of main sterols in olive oil using Supported Liquid Extraction (SLE), Solid Phase Extraction (SPE) and GC-MS analysis

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Summary: *The sterols profile in olive oil is a very important parameter to check adulterations or authenticity, because it provides a fingerprint of the product. In this work we propose a simple and fast method for main sterols characterization in olive oil which involves two clean-up steps (Supported Liquid Extraction and Solid Phase Extraction) followed by GM-MS analysis.*

Keywords: *Sterols, Supported Liquid Extraction, Olive oil*

Introduction

Sterols are important functional components in fats and oils. The most abundant component of the sterol class in olive oil is beta-sitosterol which represents more than 90% of the total sterol compounds. These compounds contribute significantly to the nutritional value of olive oil; they show anti-inflammatory and anti-carcinogenic effects. The content of sterols in olive oil is regulated by the legislation of the European Union [1] and by the International Olive Oil Council (IOC) [2]. Each oil has its own peculiar steroid profile which is a real fingerprint useful for oil identification. Analysis and characterization of sterols can detect oil adulteration. For this reason it is important to determine and quantify sterols in extra virgin olive oil (EVOO). The current IOC method employs alkaline saponification of an oil sample, followed by liquid-liquid extraction (LLE) and clean up by thin-layer chromatography (TLC). The sterol fractions are scraped off the TLC plate and reconstituted, then derivatized and analysed by GC-FID. However, this procedure is long and employs techniques which are obsolete. In this work we propose an alternative, practical and fast method for the determination of main sterols in olive oil using Solid-Liquid Extraction (SLE) followed by Solid Phase Extraction (SPE) and GC-MS analysis.

Experimental

Internal Standard Preparation: Add 40 µL of 1 mg/mL cholestanol (Internal Standard) in chloroform to a 15 mL screw-top test tube and evaporate to dryness under a nitrogen flow.
Saponification: 1- Add 200 mg of olive oil sample to the test tube containing the internal standard; 2- Add 1.5 mL of 2M Potassium hydroxide in 95 % Ethanol; 3- Cap the tube and heat at 80 °C for 50 minutes, mix gently occasionally; 4- Add 13.5 mL of deionized water.
SLE (Supported Liquid Extraction) and SPE (Solid Phase Extraction): A Strata DE SLE 60 cc cartridge with a loading capacity of 20 mL was used for the SLE. Drying tubes were prepared from 20 mL disposable syringes hand-packed with anhydrous sodium sulfate (ca. 5 g) over a microglass fiber filter paper. Strata Si-1 was employed for the SPE cartridges (Tables 1 and 2).
Derivatization: Trimethylsilyl ether (TMS) sterol derivatives were obtained after

reaction with N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA).
Instrumental Condition: The GC-MS analysis was performed on an Agilent 7890A gas chromatograph directly coupled to an Agilent 5977E MSD mass spectrometer. A GC column Agilent HP-5MS UI (30 m, 0.25mm, 0.25um) was employed. The injector temperature was 250 °C with split injection (1:5) of 1 µL of sample. The oven operated initially at 190 °C. It was heated to 230 °C at a rate of 20 °C min^{-1} , then it was heated to 255 °C at a rate of 40 °C min^{-1} and was kept at this temperature for 8 min; finally it crewed to 290 °C at a rate of 40 °C min^{-1} with hold time of 18 min. Constant flow was 2.5 mL min^{-1}. Mass spectra were obtained in electron impact mode (EI) using 70 eV with a quadrupole analyzer in SIM mode. Monitored ions chosen for mass spectrometry analysis were M^+ and fragment $[M^+-90]$, generated by the loss of the hydroxytrimethylsilane (TMSOH) cluster [3]. Olive o il samples were provided by the Italian “Rete dei laboratori delle Camere di Commercio”.

Table 1. *SLE (Supported Liquid Extraction) protocol.*

Cartridge:	Strata ® -DE SLE cartridge, 20 mL loading capacity, 60 cc Tube (Phenomenex)
Load:	Diluted sample plus 2 x 1 mL DI water rinse
Wait:	15 minutes
Extract:	3 x 15 mL Diethyl Ether, wait 1 minute before each addition
Evaporate	Dry by Rotating Evaporator, not exceed 40 °C
Reconstitute:	5 mL of Hexane

Table 2. *SPE (Solid Phase Extraction) protocol and derivatization*

Cartridge:	Strata Si-1 (1 g/6 mL) tube (Phenomenex)
Condition:	1. 2x 5 mL Hexane 2. 1 mL 0.2M Potassium hydroxide in 95 % ethanol
Equilibrate:	5 mL Hexane
Load:	Reconstituted SLE extract (5 mL) followed by 2x 1 mL. Hexane rinses
Wash:	5 mL Hexan
Extract:	10 mL Hexane/Diethyl ether (60:40)
Evaporate	Dry under N_2 at 40 °C. After evaporating to dryness, add 3-4 drops of acetone and then re-evaporate under N_2 to remove any occluded water. Place in 100 °C oven for 10 minutes.
Derivatization:	250 µL Pyridine/BSTFA (3:1) at 80 °C for 30 minutes

Results
The GC-MS analysis provided a clean and well resolved peaks. The identities of campesterol, cholesterol, b-sitosterol, stigmasterol and erythrodiol were confirmed by comparing retention times of their TMS derivatives with those prepared from commercial standard sterols. Uvaol was identified in chromatogram of samples by comparison with the NIST mass spectrum database. The method robustness was verified by changing the waiting time in SLE step (5, 10, 15 and 20 minutes), without significant differences in results. Different solvent washing volumes were tested during SPE step. It was verified

that the increase in washing volume led to a conspicuous loss of sterols at elution. Once a washing volume of 5 mL was established, the flow rate was varied; at high flows, the area of sterol peaks decreased although the percentage ratio among them remained unchanged. A gravitational flow was therefore chosen.
In order to verify the method repeatability, three different oil samples used for a Proficiency Testing (whose results have been released) were analysed. The results obtained with our method showed a good agreement with the expected ones, with a CV% in range 9.5-14.3% for Cholesterol, 3.2-4.9% for Campesterol, 3.5-4.8% for Stigmasterol and 0.2-1.0% for beta-Sitosterol. The analyses were performed in triplicate for each sample. As an example, the following results were obtained for one sample versus the expected data: 0.159±0.015% vs 0.147% for Cholesterol, 3.616±0.031% vs 3.976% for Campesterol, 1.094±0.035% vs 1.435% for Stigmasterol, 95.130±0.192% vs 92.599% for b-sitosterol and 1314±99 mg/kg vs 1321.54 mg/kg for total sterols.

Conclusion

A simple, rapid and reliable analytical method was developed for cholesterol, campesterol, stigmasterol and b-sitosterol analysis in olive oil by GC-MS. The method was found to be reproducible in terms of chromatographic profile and results. The use of a high selective method as mass spectrometry has allowed the employment of a fast clean up step with small solvent volumes. Erythrodiol and Uvaol were also detected for qualitative analysis.

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Analysis of polyphenols in Cocoa by means of LC-MS/MS during fermentation process

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Summary: *Cocoa and its derivatives, in particular chocolate, gained great popularity thanks both to its well-known taste and to the positive effects on health. In this work we wanted to evaluate how fermentation processes may affect the phenolic pattern in cocoa. A reliable and accurate method was developed and validated possible by means of UHPLC-MSMS system, which provided suitable performances.*

Keywords: *Cocoa, fermentation, polyphenols.*

Introduction

The popularity and widespread use of cocoa and its derivatives, in particular chocolate, can be attributed to its well-known taste and to the positive effects on health [1]. These effects are assumed to be associated in the presence of notable amounts of polyphenols. Cocoa is developed during the primary processing of cocoa beans, i.e. fermentation and drying. In this phase various microorganisms act in the cocoa pulp, while various enzymes have their action on carbohydrates, proteins and polyphenols, leading to the development of flavour precursors in cocoa beans. Its particular taste derives from the presence of sugars that come mainly from sucrose and its hydrolysis in glucose and fructose. Instead, polyphenolic compounds, in particular proanthocyanidins, flavan-3-ols (epicatechin and catechin) and other alkaloids give the bitterness and astringency in cocoa [2]. However, there is a significant reduction of the latter following fermentation, which tends to spread a part of the alkaloids and polyphenols from the beans.

The determination of these molecules is important for evaluation of quality of cocoa and its derivatives. LC-MS/MS a hyphenated techniques that provides good performances in terms of sensitivity and specificity; in this work LC-MS/MS was applied to a fermentation study of cocoa samples.

Experimental

In brief, extraction was performed as follow: 50 grams of cocoa were taken at each stage of the fermentation and the sample was dried in an oven at 60°C for 24 hours and triturated in an hammer mill (IKA M20); it was then subjected to a fat extraction process using three successive washes with hexane in a proportion of 5 ml of hexane per gram of sample, allowing the remaining hexane to evaporate for 12 hours. To one gram of defatted cacao, 5 ml of extraction solution (Acetone: Water: Formic acid 70: 29.5: 0.5) was added, stirred in vortex for one minute and ultrasound for 10 minutes at 20°C, the sample it was centrifuged at 4000 rpm for 10 minutes and the

supernatant was diluted 1:100 and filtered 0.22 µm to perform the analyses. LC−MS/MS analysis was performed by a Nexera XR system (Shimadzu, Tokyo, Japan) coupled to a 4500 Qtrap mass spectrometer (Sciex, Toronto, ON, Canada) equipped with a heated ESI source (V-source). An MRM acquisition mode, with two precursor ion/fragment ion transitions for each analyte, was carried out, in order to have the highest specificity. For the chromatographic separation, an Excel 2 C18-PFP column was used; the mobile phases were: (A) aqueous 0.1% formic acid and (B) acetonitrile with a total run time of 6 min. The quantitation of the analytes was achieved with the standard addition method and the peak areas of the selected ions were defined using Sciex MultiQuant software. Evaluation of matrix effect ion suppression was evaluated by a post-column infusion system coupled to the UHPLC system through a T connection.

Results

The results of analysis are shown in the following table.

Table 1. *Main polyphenols found during the fermentation of Colombian cacao.*

FERMENTATION PROGRESS (DAY)	Polyphenols (mg/g)		
	Catechin	Epicatechin	Isoquercetin
0	9,17 ± 0,57	11,63 ± 0,73	3,06 ± 0,28
1	9,61 ± 0,33	12,19 ± 0,41	3,50 ± 0,44
2	7,92 ± 0,66	10,04 ± 0,85	2,59 ± 0,04
3	7,38 ± 0,17	9,36 ± 0,22	2,81 ± 0,01
4	4,90 ± 0,35	6,22 ± 0,44	2,76 ± 0,22
5	0,63 ± 0,25	0,79 ± 0,31	2,78 ± 1,39
6	0,16 ± 0,00	0,21 ± 0,01	1,38 ± 0,03

Conclusions

During the fermentative cacao beans fermentation process, polyphenols diffuse with cell liquids from their storage cells and undergo oxidation to condensed high molecular mostly insoluble tannins. It is well known that these reactions could be formed by enzymatic and non-enzymatic reactions. In this work, concentrations of catechin (7.38 ± 0.17 mg g^{-1}) and epicatechin (9.36 ±0.22 mg g^{-1}) were reduced during fermentation. An unusual high concentration of this two compounds were recorded the fourth and five days of fermentation probably due to non-uniformity in the fermentation mass and/or in the sample of day 4 and 5. Similar behaviour of the chatechin was reported by Albertini [3] during the fermentation of natural fermented "fine flavour National cocoa". Reduction of the content of polyphenols appears to be one of the main phenomena involved during fermentation and it is associated with a decrease of astringency and bitterness in chocolate [4].

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Cannabinoids analysis as powerful tool for the identification of commercial marijuana

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Summary: *A HPLC-MS/MS method was developed for simultaneously analyzing the concentration of 6 neutral (THC, CBD, CBC, CBG, CBN, CBDV) and 3 acidic cannabinoids (THCA CBGA, CBDA). The hemp samples dataset was analyzed by the analysis of variance (ANOVA) to identify the Italian legal marijuana retailers.*

Keywords: *Cannabis sativa L.; HPLC-MS/MS analysis, Cannabinoids*

Introduction

During the past decades, companies, research organizations and institutions focused their interest on industrial hemp, *Cannabis sativa* L. species with low contents of the psychoactive substance THC (Δ^9-tetrahydrocannabinol) [1]. The secondary metabolism of *Cannabis sativa* L. produces interesting molecules with different biological properties. Among them, cannabinoids represent the most investigated ones thanks to the ability to interact with some receptors of the endocannabinoid system, related to the control and modulation several physiological and pathophysiological processes [2, 3]. Cannabinoid class is mainly made up of neutral cannabinoids as cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN), cannabidivarin (CBDV), and acidic cannabinoids as tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA) and cannabigerolic acid (CBGA).

Experimental

For the sample's treatment a fast and effective method was used, which consists in the first homogenization phase of the sample, by shredding with a chopper and sieving with a 1 mm sieve, followed by extraction in ethanol.
After suitable dilution, the analysis of cannabinoids was carried out by means of HPLC- MS/MS.

Results

The dataset of 161 hemp samples collected from four Italian retailers was analysed with ANOVA. To evaluate a correlation between the nine cannabinoids, Pearson coefficients were calculated using the dataset of the hemp samples. A partial positive correlation was found between decarboxylated cannabinoids (THC, CBD, and CBC) but no correlations between the 6 decarboxylated and 2 acidic cannabinoids THCA and CBGA. Tukey HSD multiple comparison test highlighted that the acidic cannabinoids had a lower capacity of discrimination than their neutral forms that showed a partial discrimination between the four hemp retailers, apart from CBDV.

Conclusions

In conclusion, the present study contributes to support the thesis that the concentrations of only THC and CBD are not sufficient to discriminate commercial marijuana, that can be identified by the synergic contribution of the nine cannabinoids investigated.

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P65

Contemporaneous determination nitrofurans and choramphenicol in muscle fish honey and feed by liquid chromatography tandem mass spectrometry

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Summary: *A specific and sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method for detection of chloramphenicol and nitrofurans metabolite in food and feed samples is presented. The present method was validated for muscle, fish, honey and feed according to Commission Decision 2002/657/EC and ISO 17025 requirements*.

Keywords: *nitrofurans, chloramphenicol, residue*

Introduction

Nitrofurans and Chloramphenicol (CAP) are a broad spectrum antibiotic drugs; due to concerns about their toxicity the use of these antibiotics on food sources and edible products is banned by EU Authority. The European Commission has established the minimum required performance level (MRPL) at 0.3 µg/kg and 1 µg/kg for CAP and NFs European Commission, 2003 [1], respectively.

These substances have often been found in imported aquaculture products in the past and are still found nowadays according to the Rapid Alert System for Feed and Food (RASFF) of the European Commission [2].

Detection of nitrofurans is made difficult by their rapid metabolism to protein-bound metabolites such as AMOZ, AOZ, SEM, DNSH and AHD. The nitrofuran antibacterials are rapidly biochemically transformed in still toxic metabolites which have the property to be highly bound to proteins and thus stable for longer periods (several weeks or even months) in the food producing animals. These metabolites are 3-amino-5-morpholinomethyl-1,3-oxazolidin-2-one (AMOZ) for furaltadone, 1-aminohydantoïne (AHD) for nitrofurantoine, semicarbazide (SEM) for nitrofurazone and 3-amino-2-oxazolidinone (AOZ) for furazolidone 3, 5-dinitrosalicylic acid hydrazide (DNSH) for nifursol. For their analysis by LC–MS/MS, they absolutely need to be derivatized for enhancing their detection.

In the vast majority of methods, liquid chromatography coupled to tandem quadrupole mass spectrometry (LC-MS/MS) was applied for the determination of Nitrofuran and cloranphenicol in different matrices but only separately. Kaufmann [3] reported the use of LC-HRMS for the detection of Nitrofurans and Chloramphenicol in animal food products. In the literature, no method is available for simultaneous determination of Nitrofurans and chloramphenicol in all matrices as meat, fish honey and feed. The aim of this study was to develop a rapid and reliable effective multi-analyte method coupling LC-MS/MS, for the simultaneous detection of Nitrofurans and Chloramphenicol in food and feed.

Experimental

Chloramphenicol was purchase from Sigma (St. Louis, MO, USA).3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-1,3-oxazolidin-2-one (AMOZ), semicarbazide (SEM), 1-aminohydantoin (AHD), 3-amino-2-oxazolidinone (-AOZ D4), Cloramphenicol D5, d5 3-amino-5-morpholinomethyl-1,3-oxazolidin-2-one (AMOZ D5) 3, 5-dinitrosalicylic acid hydrazide (DNSH) were obtained from Witega (Berlin, Germany) and Sigma (St. Louis, MO, USA). All the chemical and chromatographic reagents used were of HPLC or analytical grade. Methanol, ethyl acetate, hydrochloric acid, acetic acid, sodium hydroxide, tri-sodium phosphate dodecahydrate, 2 -nitrobenzaldehyde was purchased from Carlo Erba (Milan, Italy); Acid acetic 0.1% was prepared dissolving 1.0 mL of Acetic acid in 1000 mL of water. Ultrapure water was dispensed from a Milli-Q purification system (Millipore, Billerica, MA, USA).

An aliquot of 2.0 g of sample was spiked with 40 µL of an internal standard (IS) mixture of d4-AOZ µg/ml), d5-AMOZ (0.1 µg/ml) Cloramphenicol d5 (0.1 µg/ml). After sample equilibration were added with 5.0 ml of 0.1 M hydrochloric acid and 100 µl 0.1M 2-nitrobenzaldehyde in methanol. The sample is placed in ultrasonic bath and incubated overnight at 37°C. Following incubation, the samples were cooled to room temperature and neutralised by addition 0.5ml of 0.3 M trisodium phosphate and adjustment to pH 7 ± 0.5 with 1 M sodium hydroxide. Ethyl acetate 7 ml was added to the samples which were extracted in ultrasonic bath for 15 min. The samples were centrifuged at 3500 rpm for 10 min and the organic layer removed. The extraction was repeated with 5 ml ethyl acetate and the organic layers combined and evaporated to dryness under nitrogen stream at 40 °C. The extracts were finally reconstituted with 500 µL of mobile phase washed with hexane and automatically injected into the LC-MS/MS system.

Results

The validation procedure includes the determination of specificity, the decision limit, and precision. The specificity was verified by analyzing each matrix being considered, (muscle tissue of cattle, sheep and pigs, feed, fish, honey) at least 20 samples of different origin. In all the analyzed samples showed no interfering peaks at the retention time of the analytes. The present method was validated for muscle, fish, feed and honey. The precision (CV) ranged between 12.2 and 16.1% for muscle, 11.2 and 15.6% for honey and from 10.3 to 16.3% for feed. Linearity for the investigate nitrofurans and cloramphenicol were calculated from 0.2 to 2.0 µg kg^{-1}. The decision limit, calculated following the instructions given in Directive 2002/657/EC, were below 0.9 µg kg^{-1}. The accuracy of the method expressed in terms of % CV were below 19%, for all drugs analyzed and for all validated matrices.

A typical chromatogram of positive sample for SEM is shown in Fig.1.

The separation was obtained using a reversed -phase Zorbax C_{18} column with a mobile phase of methanol and acid acetic 0.1% at a flow rate of 0.300 ml/min^{-1}. Under the adopted conditions the analytes were fully separated in 15 min with symmetrical peaks.

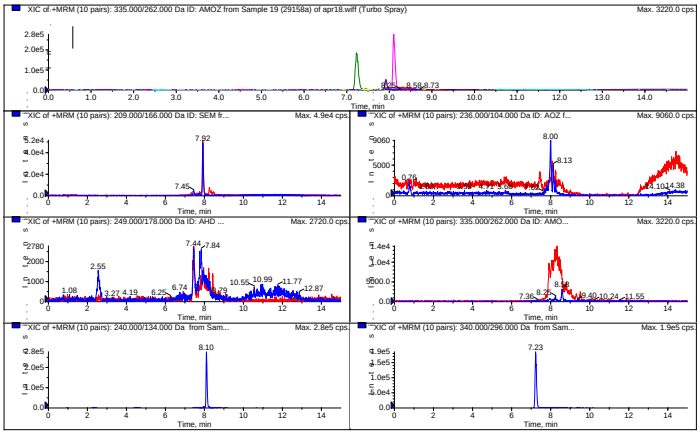

Fig. 1. *MRM chromatogram of a positive fish sample (SEM 7.92 min)*

Conclusions

Under the Official Control Plan activity, several samples were found positive for nitrofuran residues, such as SEM and AMOZ AOZ, and CAP, including those from non-European regions.

The method is used for confirmatory purposes in the research of nitrofurans and CAP residues in food and feed, therefore it is suitable for laboratories involved in official controls.

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P66

Detection of forbidden substances in dietary supplements by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS)

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Summary: *The aim of this study is to check for possible adulteration of different dietary supplements in order to assess the risk for consumers and acquire new scientific knowledge about chemical contamination of food supplements. A multiclass screening method to detect 124 forbidden substances by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) was developed and validated for this purpose*.

Keywords: *dietary supplements, LC-HRMS*

Introduction

Dietary supplements are used by the population in general for balancing the diet or to compensate for the lack of nutrients. They are concentrated sources of nutrients such as minerals, vitamins, amino acids, trace elements and other components whose purpose is to supplement the normal diet. They are sold in form of tablets, capsules or in powder for an oral consumption.

The increasing consumption of these products mainly through not-conventional channels such as internet websites, gym selling and "spice shops" requires more control to assure safety of consumers. The widespread diffusion supplements makes difficult the application of international and national directives regarding to the labeling and the controls on food supplements. Unscrupulous producers can falsify these products by the illegal addition of pharmaceutical substances or their analogs to enhance the activities claimed and to increase sales.

Illegal adulterants have been reported to be found in various functional foods without notification of their amounts and chemical identities. During recent years it has been reported that products marketed as dietary supplements could contain non-labeled substances, like erectile dysfunction drugs (sildenafil, vardenafil, tadalafil and analogs); anorectic drugs sibutramine; stimulants like synephrine, ephedrine, caffeine; diuretics, steroids and other illegal substances.

In 2018 the EU rapid communication system on food and feed (RASFF) notified that 22 food supplements imported from different countries (mainly China and the United

States) were contaminated with sildenafil and/or tadalafil; 4 dietetics food from USA contained synephrine and a high content of caffeine; unauthorized anabolic-androgenic steroids and 1,3-dimethylamylamine (DMAA) were found in a product traded online (RASFF 2018) [1].

Due to the increase of dietary supplements adulterated with undeclared chemicals, analysis of them, to exclude the presence of illegal drugs, is

therefore of fundamental importance.
In this study, we developed and validated a multiclass screening method covering 10 classes of compounds (β-agonists, corticosteroids, diuretic, NSAIDs, progestins, resorcylic acid lactones (RALs), steroids, stilbenes, stimulants and tranquilizer) in food supplements applying liquid-chromatography coupled to high-resolution mass spectrometry (LC-HRMS).

Experimental
Standard of β-agonists, corticosteroids, diuretic, NSAIDs, progestins, resorcylic acid lactones, steroids, stilbenes, stimulants and tranquilizer were purchase from Sigma (St. Louis, MO, USA) and Witega (Berlin, Germany). All the chemical and chromatographic reagents used were of HPLC or analytical grade. Methanol, acetonitrile, acetic acid, were purchased from Carlo Erba (Milan, Italy); Acid acetic 0.1% was prepared dissolving 1.0 mL of Acetic acid in 1000 mL of water. Ultrapure water was dispensed from a Milli-Q purification system (Millipore, Billerica, MA, USA).
For the extraction of analytes, 0.50 g of homogenized samples were spiked with 100 µL of IS. All samples were extract two time with 5 ml of methanol/water 80/20. After the addition of solvent, each sample was vortex-mixed for 30s, placed in an ultrasonic bath for 15 min and centrifuged for 10 min at 4000 rpm. After centrifugation, samples were filtered through pre-conditioned (3 ml of methanol) SPE cartridges and finally injected into the LC-HRMS system.
The LC–HRMS system was composed of a Thermo ULTIMATE 3000 system coupled to a Thermo single-stage Orbitrap (Exactive) MS system. Thermo Fisher Scientific (Milan, Italy) provided the whole equipment. Analytes were separated on a Kinetex XB-C18 column (3.0 mm × 100 mm, 2.5 µm particle size), purchased from Phenomenex (Torance, CA, USA) connect with a guard column Phenomenex C18 (40 mm × 2.1 mm).

Results
The proposed method was applied to check the eventual presence of 124 illegal adulterants in 110 nutritional supplements, sold in websites or collected from police officers in Italy.
The identification of target compounds was based on the accurate mass and retention time. For positive samples the confirm was achieved by characteristic fragmentation pattern.
LC-HRMS analysis showed four non-compliant samples (Figure 1).
The amount of each compound detected was calculated from the calibration curve.
One sample resulted positive for paracetamol and sildenafil, present in quantities equal to 35.3 mg/g and 88.1 mg/g respectively.
In two plant food supplements progesterone was found at concentrations of 0.117 mg/g and 0.099 mg/g. Another sample contained DHEA at 180 mg/g.

Conclusions
The developed method was successfully applied to the analysis of 110 dietary samples, allowed the detection of illegal substance, 3.6% of food supplements analyzed were found to be non-compliant.

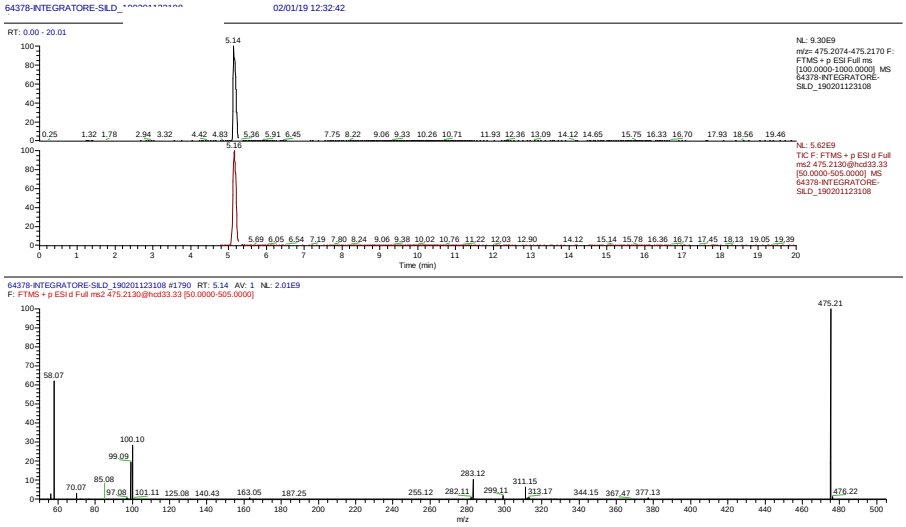

Fig. 1. *LC-HRMS chromatogram of a positive sample (Sildenafil)*

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Qualitative and quantitative determination of ethyl-2-hydroxy-4-methylpentanoate (ethyl leucate) in wine

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Summary: *In this work a GC-MS/MS method to qualify and quantify ethyl leucate in wine after solid phase extraction was developed and validated.*

Keywords: *Ethyl leucate, GC-MS/MS analysis, wine*

Ethyl-2-hydroxy-4-methylpentanoate, best known as ethyl leucate, was identified in red and white wine as a compound involved in blackberry and fresh-fruit aroma [1]. In literature was reported that its concentration was ranged between 50 and 700 µg/l, depending on type and vintage of wine, and that the perception thresholds of its racemic mixture (R and S) were 900 and 300 µg/l in dearomatized wine and model wine solution, respectively [2].
Sensory panel tests highlighted a clear impact on aroma of this compound and suggested a synergistic perceptive interaction with ethyl butanoate [1]. It is widely-known that, due to interactions, the perception of a mixture of many compounds cannot always be predicted from the sum of the individual compounds present [3]. In addition, there is also the possibility of a suppression effect [4].
The aim of the current work was to develop an analytical method able to identify and quantify the presence of ethyl leucate in wine samples.
Ethyl leucate was extracted from wine by adsorption on a SPE cartridge (ENV+), eluted with dichloromethane and finally the organic fraction was injected in a GC-MS/MS system [5] [6]. The analysis was carried out using an Agilent Intuvo 9000 GC system coupled with an Agilent 7000 Series Triple Quadrupole MS equipped with an EI source (70 eV, 50 µA) and injecting 2 µl in split mode (1:5) into a 20 m DB-Wax UI (0.18 mm i.d. × 0.18 µm film thickness) [7].
Validation of the method was performed in relation to the limit of detection (LOD), limit of quantification (LOQ), linearity range, repeatability, reproducibility, and recovery. Once validated, the method was tested on a selection of 32 red wine samples produced from 8 different varieties.
Moreover, using the same extraction process and the same chromatographic conditions, 50 volatile compounds of oenological interest belonging to the main chemical classes (acetates, acids, alcohols, aldehydes, esters, ketones, phenols, terpenes, and thiols) were identified and quantified for each sample of wine [7].
Ethyl leucate was quantified in a range of concentration between 70 and 300 µg/l with an average value of 120 µg/l, in accordance with literature [2].
Finally, a correlation matrix was applied to identify a possible relationship

between ethyl leucate and the other 50 aromatic compounds considered. A positive correlation was found between the amount of ethyl leucate and ethyl-2-methylbutyrate with a correlation coefficient of 0.55 in a values range comprising between -1.0 and 1.0.

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Discrimination of saffron samples using MS-based techniques (IRMS and GC-MS) and peptide gas sensors array (E-Nose)

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Summary: *In this work, the use of instrumental techniques (Isotope-ratio mass spectrometry-IRMS and solid-phase microextraction gas chromatography mass spectrometric, SPME-GC-MS) and peptide gas sensors array (Electronic Nose) were investigated to characterize and discriminate origin, drying and age of saffron samples.*

Keywords: *saffron, stable isotope ratio analysis, volatile pattern analysis*

Introduction

Saffron is a precious spice known as "red gold" obtained from the dried stigmas of the *Crocus Sativus* L., a plant of the Iridaceae family. Its importance is related to the peculiar quality and sensory properties, in particular aroma, colour and taste that are mainly associated to presence and concentration of safranal, crocins and picrocrocin, respectively [1].

Saffron, however, besides safranal, is characterized by a large number of volatile and aroma-yielding compounds that recent literature has indicated to be more than 150 [2]. Various factors contribute to define its overall composition and corresponding quality and among others, origin is the most acknowledged one. Process and storage conditions have been only sparingly investigated despite their important role in the development of non-volatile compounds and their presence in the spice [3].

Quality control of saffron is thus a critical aspect for both spice industries against adulteration and consumers upon purchase. Gas-chromatographic techniques are largely used in the evaluation of the volatile pattern while recently, the introduction of innovative screening tools like electronic noses into the area of food is envisaged for quality control of food and authenticity assessment [4].

The aim of this study, thus, was to combine MS-based techniques (GC-MS and IRMS) and peptide gas sensors array (E-nose) to characterize and differentiate saffron samples having different origin, process and age conditions.

Experimental

Thirty-five saffron samples with different origin (Italian and non-Italian) were collected during the harvesting period 2012-2016. All saffron samples were preliminarily classified by age and drying method and intensity that, in turn was based on the applied process temperature.

The IRMS analysis evaluated the stable ratios of the isotopes of $^{13}C/^{12}C$ and $^{15}N/^{14}N$. Aliquots of 1 mg of saffron powder were exactly weighted into a tin capsule, inserted in the autosampler for solids and introduced in the reactor for combustion of the elemental analyser.
For the volatile point of view, in particular GC-MS analysis, the DVB/CAR/PDMS fiber was exposed to the headspace of 10 ml vial containing saffron powder in H_20 MilliQ for a time of 5 min at the temperature of 40˚C under magnetic stirring at medium agitation speed. Identification of the volatile compounds in the chromatogram was made by using the NIST library.
The E-nose is based on peptide modified gold nanoparticles deposited onto 20MHz quartz crystal microbalances was used to determinate the volatile fingerprint of the saffron. The analyses have been carried out directly on the powdered saffron samples at 35˚C.
The data have been processed by Partial Least Squares Discriminant Analysis (PLS-DA) to evaluate the possibility to discriminate the saffron samples affected by different geographical origin, drying process and year of production.

Results

All techniques employed in this study were able to classify saffron based on origin of stigmas from *Crocus sativus* L., cultivated in Italy or other countries. The IRMS, with the analysis of the stable isotopes of carbon and nitrogen, has proved to be a very reliable method in discriminating the geographical origin of saffron. As regards the volatile compounds pattern, several compounds were detected and identified by the GC-MS analysis. In particular, under our conditions it was possible to identify sixteen aldehydes, thirteen alcohols, four ethers, twenty-six ketones and nineteen hydrocarbons. Amount of the volatile compounds was different in the saffron depending on origin and age despite, as commonly found, safranal resulted as prevalent volatile (95-98% of total GC area). This last technique revealed that, within all detected volatile compounds, aldehydes pattern could be used to classify the origin. As alternative way, also the peptide gas sensors array was applied as additional complementary analysis method to characterise complex aroma patterns, and it could be use as tool to recognize the traceability of saffron samples. No clear correlation between the detected volatile compounds and gas sensors was found. Each gas sensor had very similar response, influenced not only by the main volatile compound, safranal, but also by the total contribution of aldehydes. Instead, more difficult has been to find potential markers for the drying treatment and storage: only SPME-GC-MS analysis resulted suitable for these purposes. When the process was taken as criterion of classification, some saffron volatile compounds belonging from aldehyde group showed good values of classification, while the main volatile compounds discriminated the saffron age.

Conclusions

In conclusion, the present study contributes to routine food quality control, for the characterization and discrimination of saffron samples with different origin, process and age conditions using both MS-based techniques and peptide gas sensors array.

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