



Università
degli Studi di
Messina

DIPARTIMENTO DI SCIENZE BIOMEDICHE,
ODONTOIATRICHE E DELLE IMMAGINI
MORFOLOGICHE E FUNZIONALI



Società Chimica Italiana
Gruppo Interdivisionale
Chimica degli Alimenti



CHIMALI 2023

MARSALA



XIII CONGRESSO NAZIONALE DI CHIMICA DEGLI ALIMENTI

LIBRO DEGLI ABSTRACTS

www.chimali2023.it

29 – 31 maggio 2023

Hotel Resort Villa Favorita,
Marsala (TP)

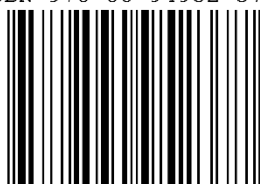
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XIII CONGRESSO NAZIONALE DI CHIMICA DEGLI ALIMENTI

29 – 31 maggio 2023
Hotel Resort Villa Favorita, Marsala (TP)

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XIII CONGRESSO NAZIONALE DI CHIMICA DEGLI ALIMENTI

PREFAZIONE

Le plenarie e le comunicazioni orali e poster presentate al XIII Congresso Nazionale di Chimica degli Alimenti, svoltosi a Marsala (TP) dal 29 al 31 maggio 2023, organizzato dall'Università degli Studi di Messina, dal Gruppo Interdivisionale di Chimica degli Alimenti della Società Chimica Italiana, dalla Società Italiana di Chimica degli Alimenti, in collaborazione con l'Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", sono tutte riportate in questo volume.

Anche questa edizione ha confermato che il Congresso Nazionale di Chimica degli Alimenti rappresenta un importante punto di riferimento ed un'occasione di incontro tra tutti gli studiosi delle strutture pubbliche e private, che declinano la *Food Science* e le realtà produttive che ruotano intorno all'intero settore agro-alimentare.

Il programma scientifico del Congresso comprende 3 plenarie, 92 comunicazioni orali e 106 poster con la partecipazione di quasi 250 ricercatori, che hanno illustrato i loro più recenti risultati conseguiti nell'ambito della Chimica degli Alimenti. Al riguardo tutti i contributi presentati al Congresso possono essere sottomessi a due Special Issue, Foods e Antioxidants.

Il Congresso ha registrato un'importante presenza di giovani a cui voglio augurare, specialmente a coloro i quali, spinti dalla passione per la ricerca e da una sana ambizione, continuano a lavorare in condizioni di precariato, la realizzazione dei loro sogni.

A questo proposito ringrazio il G.I.C.A., la ITACHEMFOOD, la S.I.S.S.G., la MDPI e la Prof.ssa P. Bambara per aver sostenuto la partecipazione di numerosi giovani.

Grazie ancora al C.U.M.O., all'E.R.S.U. Messina e al Polo di Innovazione della Calabria, Future Food Med, che hanno supportato finanziariamente la presenza di studenti dell'Ateneo di Messina e dell'Università Mediterranea di Reggio Calabria.

Voglio ringraziare tutti gli Autori, che con i loro interventi hanno contribuito alla riuscita e al successo del Congresso, l'Università degli Studi di Messina, le Istituzioni e i patrocini.

Ringrazio l'I.Z.S. Sicilia per aver accreditato il Congresso.

Non ultimi ringrazio gli sponsor per il loro prezioso supporto.

Infine, voglio esprimere la mia affettuosa gratitudine a tutti i Colleghi giovani e meno giovani coinvolti nell'organizzazione di questo Congresso e rivolgere un abbraccio a Giacomo.

Giuseppa Di Bella

XIII CONGRESSO NAZIONALE DI CHIMICA DEGLI ALIMENTI

MARSALA, 29 – 31 maggio 2023

PROGRAMMA

Domenica 28 Maggio (Complesso Monumentale di San Pietro, Marsala)

19.00 *EVENTO DI BENVENUTO presso lo storico COMPLESSO MONUMENTALE DI SAN PIETRO, sito nel centro della città di Marsala.*

Saluti istituzionali di:

Prof. Emerito Giacomo Dugo

Ass. Avv. Ignazio Massimo Bilardello

Dr. Nicola Catania, Deputato all'Assemblea Regionale Siciliana e Sindaco della città di Partanna

On. Raoul Russo, Senatore della Repubblica Italiana

Dr. Vincenzo Nicolì, Presidente Ordine Interprovinciale dei Chimici e dei Fisici della Sicilia

On. Avv. Girolamo Turano, Assessore dell'Istruzione e della Formazione Professionale

Degustazione di prodotti tipici.

Lunedì 29 Maggio

08.30 Registrazione Partecipanti (sala patio)

09.00 Apertura Congresso (sala centrale)

A cura di: *Proff. Alberto Angioni, Giuseppa Di Bella, Gianni Galaverna*

09.30 **Prolusione**

IL RUOLO DEL CHIMICO DEGLI ALIMENTI NELLA CERTIFICAZIONE DELLA FILIERA AGROALIMENTARE

Giacomo Dugo

I SESSIONE (sala centrale)

Sessione Parallela: Food composition and quality

Moderatore: Prof. Gianni Galaverna

10.00 **C01**

THE CHALLENGES OF CLIMATE CHANGE ON THE CONSISTENCY OF THE COCOA FLAVOUR QUALITY

Eloisa Bagnulo, Giulia Strocchi, Cristian Bortolini, Chiara Cordero, Erica Liberto

10.15 **C02**

NOVEL INSIGHTS ON CHEESE DEFECTS IN PECORINO ROMANO PDO CHEESE: A CASE STUDY

Giacomo L. Petretto, Giovanni Patta, Giacomo Zara, Paolo Urgeghe, Severino Zara, Francesco Fancello

10.30 **C03**

EVALUATION OF FOLATES IN CALABRIAN CITRUS FRUITS BY ORBITRAP MASS SPECTROMETRY

Lucia Bartella, Ines Rosita Talarico, Fabio Mazzotti, Ilaria Santoro, Melissa Cacciatore, Leonardo Di Donna

10.45 **C04**

GREEN COFFEE, BIOACTIVE COMPOUNDS' ANALYSIS OF EXPERIMENTAL CULTIVATION IN SICILY: A NEW FRONTIER IN THE SICILIAN FOOD SECTOR

Vita Di Stefano, Carla Buzzanca, Fortunato Ruvutuso, Giovanni Gugliuzza, Dario Scuderi, Eristanna Palazzolo, Vittorio Farina

I SESSIONE (sala convegni)

Sessione Parallela: Natural compounds and Nutraceuticals

Moderatore: Prof.ssa Maria Daglia

10.00 **C05**

NEUROPROTECTIVE AND ANTIOXIDANT EFFECTS OF UROLITHINS: QUANTUM MECHANICS (QM) AND MOLECULAR MECHANICS (MM) APPLICATIONS

Emanuela Marchese, Isabella Romeo, Giosuè Costa, Stefano Alcaro

- 10.15 C06**
EXPLOITING NEW SE-GLYCOCONJUGATES AS ANTIOXIDANT SUPPLEMENTS IN OXIDATIVE STRESS RELATED DISEASES
Giovanna Cimmino, Mauro De Nisco, Silvana Pedatella, Severina Pacifico
- 10.30 C07**
ULVA LACTUCA L. OF ORBETELLO LAGOON: MULTIVARIATE OPTIMIZATION OF THE EXTRACTION OF ULVAN POLYSACCHARIDES
Beatrice Zonfrillo, Maria Bellumori, Marzia Innocenti, Serena Orlandini, Sandra Furlanetto, Gianni Zoccatelli, Nadia Mulinacci
- 10.45 C08**
PHENOLIC EXTRACT FROM HAZELNUT SKIN: CHEMICAL COMPOSITION AND PROTECTIVE ROLE AGAINST ADVANCED GLYCATION END-PRODUCTS (AGES)-DAMAGE IN THP-1-DERIVED MACROPHAGES
Ludovica Spagnuolo, Laura Dugo, Laura De Gara

11.00 Pausa caffè

II SESSIONE (sala centrale)

Sessione Parallela: Food composition and quality

Moderatori: Prof.ssa Filomena Corbo, Prof. Pierluigi Plastina

- 11.30 C09**
QUALITY AND NUTRITIONAL PROFILE DETERMINATION OF THE HEMP ENRICHED FUNCTIONAL PASTA
Sonia Bonacci, Vita Di Stefano, Fabiola Sciacca, Carla Buzzanca, Nino Virzì, Antonio Procopio, Maria Grazia Melilli
- 11.45 C10**
AGEING AND PRODUCTION DISCRIMINATION OF PDO GRANA PADANO CHEESE WITH AN NMR-BASED METABOLOMIC APPROACH
Valentina Maestrello, Pavel Solovyev, Pietro Franceschi, Angelo Stroppa, Federica Camin, Luana Bontempo
- 12.00 C11**
UNDERSTANDING THE CHEMISTRY OF THE SICILIAN AMARENA WINE DURING BOTTLE AGING
Ambrogina Albergamo, Vincenzo Lo Turco, Angela Giorgia Potortì, Giuseppa Di Bella
- 12.15 C12**
DIRECT INJECTION MASS SPECTROMETRY FOR FOOD VOLATILOMICS: EMERGING GREEN APPROACHES FOR THE RAPID AND ONLINE SCREENING OF MICROBIAL RESOURCES
Iuliia Khomenko, Vittorio Capozzi, Antonia Corvino, Franco Biasioli
- 12.30 C13**
FOOD CHEMISTRY AS A USEFUL TOOL FOR BOTANICAL TAXONOMY: COFFEE DITERPENES AS MOLECULAR MARKERS
Elena Guercia, Paola Crisafulli, Silvia Colombari, Luciano Navarini

12.45 C14
CHARACTERIZATION OF COLOR, PHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY OF ITALIAN PIGMENTED RICE VARIETIES AFTER DIFFERENT TECHNOLOGICAL TREATMENTS

Corinne Bani, Francesca Colombo, Carola Cappa, Francesca Mercogliano, Patrizia Restani, Chiara Di Lorenzo

13.00 C15
ARTEMIDE PIGMENTED RICE: IMPACT OF COOKING ON CHEMICAL COMPOSITION, NUTRITIONAL PROFILE AND BIOACCESSIBILITY OF PHENOLIC COMPOUNDS

Antonio Colasanto, Marco Arlorio, Fabiano Travaglia, Matteo Bordiga, Vincenzo Disca, Yassine Jaouhari, Jean Daniel Coïsson, Ivana Rabbone, Monica Locatelli

II SESSIONE (sala convegni)

Sessione Parallela: Natural compounds and Nutraceuticals

Moderatori: Prof. Davide Bertelli, Prof.ssa Anna Lisa Picinelli

11.30 C16
CHEMICAL CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF *RHUS CORIARIA* L. EXTRACT: ANTIOXIDANT, ANTIGLYCATION AND DPP4 INHIBITORY EFFECTS

Laura Dugo, Elisa Pannucci, Ludovica Spagnuolo, Luca Santi

11.45 C17
A NEW MILLIFLUIDIC-BASED GASTROINTESTINAL PLATFORM TO INVESTIGATE ANTIGLYCATIVE AGENTS

Raffaella Colombo, Ilaria Frosi, Adele Papetti

12.00 C18
POLYPHENOLS IN LIVESTOCK NUTRITION: THE CASE OF RENEWABLE FAGACEAE LEAVES

Marialuisa Formato, Alessandro Vastolo, Simona Piccolella, Serena Calabrò, Monica Isabella Cutrignelli, Christian Zidorn, Severina Pacifico

12.15 C19
THERMAL DEGRADATION KINETICS OF RED CABBAGE (*BRASSICA OLERACEA* L. VAR *CAPITATA* F. *RUBRA*) ANTHOCYANINS

Laura De Marchi, Laura Salemi, Maria Bellumori, Federica Mainente, Ilaria Fierri, Roberto Chingola, Gianni Zoccatelli

12.30 C20
THE POTENTIAL OF COMMON DUCKWEED (*LEMNA MINOR* L.) AS A MEAT EXTENDER DURING STORAGE OF PACKAGED BEEF BURGERS

Gabriele Rocchetti, Annalisa Rebecchi, Leilei Zhang, Michele Dallolio, Daniele Del Buono, Giorgio Freschi, Luigi Lucini

12.45 C21
FLOW-BIOCATALYSIS FOR NATURAL FOOD BIOACTIVE AND NUTRACEUTICALS
Martina Letizia Contente, Lucia Tamborini, Sabrina Dallavalle, Francesco Molinari, Andrea Pinto

13.00 C22
ANTIOXIDANT AND ANTIGLYCATION EFFECTS OF MATCHA GREEN TEA EXTRACTS
Elisa Pannucci, Luca Santi, Laura Dugo

13.30 Colazione di lavoro

III SESSIONE (sala centrale)
Sessione Parallela: Food traceability
Moderatore: Prof. Luca Rastrelli

15.00 PL01
FRESHNESS OF FOOD PRODUCTS OF ANIMAL ORIGIN: EFFECTIVE METABOLOMICS APPROACHES FOR MILK & EGG
Michele Suman

15.30 C23
THE MOST REPRESENTATIVE TYPICAL DISH OF CATANZARO AS NEW MEDITERRANEAN DIET CASE STUDY
Stefano Alcaro, Francesco Bianco

15.45 C24
QUALITY AND AUTHENTICITY OF VACCINIUM CORYMBOSUM L.: A COMBINED CHEMICAL AND MOLECULAR APPROACH
Manuel Martoccia, Valeria Fochi, Fabiano Travaglia, Monica Locatelli, Matteo Bordiga, Jean Daniel Coïsson, Marco Arlorio

16.00 C25
PRELIMINARY CHARACTERIZATION OF SICILIAN BLACK PIG MEAT ACCORDING TO THE GEOGRAPHICAL AREA OF BREEDING
Federica Litrenta, Luigi Liotta, Alessandro Lazzara, Vincenzo Chiofalo, Antonino Iuculano, Giuseppa Di Bella

III SESSIONE (sala convegni)
Sessione Parallela: Natural compounds and Nutraceuticals
Moderatore: Prof. Antonello Santini

15.30 C26
N-TRANS-CAFFEOYLTYRAMINE AND CANNABISIN A: A (NUTRI)COSMECEUTICAL INNOVATION FROM HEMP SEED MEAL
Marialuisa Formato, Simona Piccolella, Severina Pacifico

15.45 C27
ENHANCEMENT OF OLIVE TREE CV. CAIAZZANA PRUNING/DEFOLIATION RESIDUES FOR INNOVATIVE FOOD FORMULATIONS
Hamid Mushtaq, Simona Piccolella, Marialuisa Formato, Severina Pacifico

16.00 C28
WASTE BY-PRODUCTS FROM *OLEA EUROPAEA* AS A POTENTIAL APPLICATION IN INFLAMMATORY BOWEL SYNDROME
Laura Beatrice Mattioli, Filomena Corbo, Maria Lisa Clodoveo, Roberta Budriesi

16.15 Pausa caffè

16.15 – 16.45 Sessione Poster (arcata giardino)

IV SESSIONE (sala centrale)
*Sessione Parallela: **Food traceability***
Moderatore: Prof.ssa Augusta Caligiani

16.45 C29
METABOLOMIC PROFILING OF MEDICINAL AND AROMATIC PLANTS THROUGH AMBIENT MASS SPECTROMETRY COMBINED WITH CHEMOMETRICS: A POWERFUL TOOL AGAINST FRAUDULENT ACTIVITIES
Francesca Rigano, Domenica Mangraviti, Paola Dugo, Luigi Mondello

17.00 C30
HOW TO GUARANTEE THE NATURAL ORIGIN OF NUTRACEUTICAL AND PHARMACEUTICAL PRODUCTS? THE POTENTIAL OF THE STABLE ISOTOPE RATIOS ANALYSIS
Matteo Perini, Silvia Pianezze

17.15 C31
SWATH-MS BASED PROTEOMICS AND CHEMOMETRICS TOOLS TO ASSESS CHICKEN MEAT AUTHENTICITY WITHIN DIVERGENT PRODUCTION SYSTEMS: ORGANIC VERSUS ANTIBIOTIC-FREE
Laura Alessandroni, Gianni Sagratini, Renzo Galli, Mohammed Gagaoua

IV SESSIONE (sala convegni)
*Sessione Parallela: **Food waste***
Moderatore: Prof.ssa Roberta Budriesi

16.45 C32
MOLECULAR CHANGES OF BLACK SOLDIER FLY LIPIDS WITH DIET, KILLING METHOD AND MICROBIAL FERMENTATION
Veronica Lollo, Andrea Fuso, Anna Valentina Luparelli, Francesca Bonzanini, Jasmine Hadj Saadoun, Camilla Lazzi, Stefano Sforza, Augusta Caligiani

17.00 C33

**THE FIRST 18 MONTHS OF THE H2020 “ECOEFSHENT” PROJECT:
SUSTAINABILITY AND EFFICIENCY IN THE FISH PROCESSING SIDE-
STREAMS**

Federica Turrini, Federica Grasso, Valentina Orlandi, Giulia De Negri Atanasio, Elena Grasselli, Raffaella Boggia

17.15 C34

**DEVELOPMENT OF A FERMENTATION PROCESS FOR THE PRODUCTION
OF LACTIC ACID FROM *OPUNTIA FICUS INDICA* BY-PRODUCTS**

Laura De Maria, Teresa Gervasi, Giovanna Lo Vecchio, Eleonora Di Salvo, Vincenzo Nava, Rossana Rando, Rita De Pasquale, Nicola Cicero

**17.30-18.30 Tavola rotonda (sala centrale)
ISTITUZIONI, ENTI DI CONTROLLO E SCIENZA A SOSTEGNO DELLE
FILIERE AGRO-ALIMENTARI**

Intervengono:

- | | |
|---------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Prof. Giacomo Dugo</i>
<i>(Moderatore)</i> | Emerito di Chimica degli alimenti, Università degli Studi di Messina |
| <i>Dr. Dario Cartabellotta</i> | Dirigente Generale del Dipartimento di Agricoltura della Regione Siciliana |
| <i>Dr. Fabio Ditta</i> | Vicepresidente di Bono&Ditta SpA |
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| <i>Dr. Luigi Liberatore</i> | Direttore Territoriale Dogane DT VII - Sicilia |
| <i>Dr. Pierluigi Reale</i> | Azienda Agricola Cielo Terra Mare |
| <i>Prof. Franco Roperto</i> | Ordinario di Patologia Generale Veterinaria e Presidente del Consiglio di Amministrazione dell'Istituto Zooprofilattico Sperimentale del Mezzogiorno |
| <i>Dr. Alessandro Spadaro</i> | Presidente di K2 Innovazione Srl e Vice Presidente del Comitato PMI Sicindustria |
| <i>On. Avv. Annalisa Tardino</i> | Deputata al Parlamento Europeo, Componente della Commissione per l'ambiente, la sanità pubblica e la sicurezza alimentare |
| <i>Arch. Vito Zichittella</i> | Vicepresidente del Consiglio di Amministrazione e Legale Rappresentante della torrefazione Zicaffè SpA |

21.00 APERICENA BORDO PISCINA

Martedì 30 Maggio

V SESSIONE (sala centrale)

Sessione Parallela: *Natural compounds and Nutraceuticals*

Moderatori: *Prof. Marco Arlorio, Prof.ssa Chiara Cordero*

09.00 PL02

TRENDS IN THE NOVEL FOOD AREA

Rosangela Marchelli

09.30 C35

SECOIRIDOIDS FROM EXTRA VIRGIN OLIVE OIL EXTRACTS: CHEMICAL CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITY IN OBESE CHILDREN

Filomena Corbo, Stefania De Santis, Laura Piacente, Anna Mestice, Pasquale Crupi, Paola Pontrelli, Antonio Moschetta, Maria Felicia Faienza, Maria Lisa Clodoveo

09.45 C36

CANNABIS SATIVA L. INFLORESCENCES AS POTENTIAL FUNCTIONAL FOOD: A PHYTOCHEMICAL CHARACTERIZATION

Cinzia Ingallina, Mattia Spano, Giacomo Di Matteo, Silvia Cammarrone, Francesca Ghirga, Bruno Botta, Enio Campiglia, Anatoly P. Sobolev, Luisa Mannina

10.00 C37

OPTIMIZATION OF URSOLIC ACID EXTRACTION IN OIL FROM ANNURCA APPLE TO OBTAIN OLEOLYTES WITH POTENTIAL NEUROPROTECTIVE APPLICATION

Maria Maisto, Paola Cuomo, Vincenzo Piccolo, Elisabetta Schiano, Fortuna Iannuzzo, Rosanna Capparelli, Gian Carlo Tenore

10.15 C38

SPECIALIZED METABOLITES FROM THE ENDEMIC SPECIES LAVANDULA AUSTROAPENNINA: A PROMISING BIORESOURCE TO BE EXPLOITED

Claudia Gravina, Simona Piccolella, Adriano Stinca, Severina Pacifico, Assunta Esposito

10.30 C39

EFFECT OF THE ADDITION OF JUJUBE (*ZIZIPHUS JUJUBA* MILL.) ON THE PRODUCTION OF VITAMINS AND PHENOLIC COMPOUNDS DURING KOMBUCHA FERMENTATION

Chiara La Torre, Alessia Fazio

10.45 C40

ARTICHOKE WASTE VALORIZATION IN READY-TO-USE (POLY)PHENOL FOOD SUPPLEMENTS

Simona Piccolella, Severina Pacifico

V SESSIONE (sala convegni)
Sessione Parallela: Innovative analytical techniques
Moderatori: *Prof.ssa Paola Dugo, Prof. Daniele Giuffrida*

- 09.30 C41**
REDUCED TIME AND ECO-FRIENDLY CHROMATOGRAPHIC ANALYSES FOR FAST QUALITY CONTROL OF OXYGEN HETEROCYCLIC COMPOUNDS IN FOODS
Marina Russo, Maria Rita Testa Camillo, Giovanna Cafeo, Paola Dugo, Luigi Mondello
- 09.45 C42**
COMPUTATIONAL METHODS IN FOOD CHEMISTRY
Giosuè Costa
- 10.00 C43**
VOLATILOME FINGERPRINTING FOR THE ASSESSMENT OF OLIVE OIL QUALITY AND AUTHENTICITY BY INNOVATIVE MULTI-CUMULATIVE TRAPPING EXTRACTION METHODOLOGY
Natasha Damiana Spadafora, Steven Mascrez, Laura McGregor, Alberto Cavazzini, Luisa Pasti, Giorgia Purcaro
- 10.15 C44**
USE OF AN HPLC-MS/MS METHOD COUPLED WITH LINEAR RETENTION INDEX SYSTEM TO CHARACTERIZE THE OXYGEN HETEROCYCLIC FRACTION IN CITRUS FLAVORED DRINKS
Giovanna Cafeo, Tania Maria Grazia Salerno, Marina Russo, Paola Dugo, Luigi Mondello
- 10.30 C45**
INTEGRATING VOLATILOME, PRIMARY AND SPECIALIZED METABOLOME BY DATA FUSION TECHNIQUES: A COMPREHENSIVE EVALUATION OF HAZELNUTS QUALITY
Simone Squara, Andrea Caratti, Angelica Fina, Carlo Bicchi, Chiara Cordero
- 10.45 C46**
INNOVATIVE GC-IMS ANALYSIS RECORDS THE DIGITAL FINGERPRINTS OF VOLATILES TO GAIN COMPLEMENTARY INSIGHT IN FLAVOUR COMPOSITIONS AS KEY ASPECT IN FOOD QUALITY AND AUTHENTICITY ASSESSMENTS
Cesare Rossini, Hansruedi Gyax, Thomas Wortelmann

11.00 Pausa caffè

11.00 – 11.30 Sessione Poster (arcata giardino)

VI SESSIONE (sala centrale)
Sessione Parallela: Food waste
Moderatori: *Prof. Nicola Cicero, Prof.ssa Marzia Innocenti*

11.30 C47

VALORIZATION OF APPLE POMACE AS A MULTIFUNCTIONAL INGREDIENT FOR THE DEVELOPMENT OF FUNCTIONAL FOODS

Lina Cossignani, Federica Ianni, Francesca Blasi

11.45 C48

GRAPE PRODUCTS AND BY-PRODUCTS: COMPARATIVE ANALYSIS OF PHENOLIC PROFILE AND IN VITRO BIOLOGICAL ACTIVITIES

Chiara Di Lorenzo, Corinne Bani, Enrico Sangiovanni, Francesca Mercogliano, Mario Dell'Agli, Patrizia Restani

12.00 C49

CHARACTERIZATION AND VALORIZATION OF PHENOLIC RICH EXTRACTS FROM MALUS DOMESTICA CV MELA ABBONDANZA ROSSA

Cinzia Mannozi, Diletta Piatti, Doaa Abouelenein, Laura Alessandroni, Gianni Sagratini, Sauro Vittori

12.15 C50

VALORIZATION OF LOMBARD CEREAL WASTES: EVALUATION OF CORN COB, RICE HUSK, AND WHEAT PROCESSING BY-PRODUCTS AS SOURCES OF POLYPHENOLS WITH ANTIGLYCATIVE CAPACITY

Ilaria Frosi, Anna Balduzzi, Raffaella Colombo, Chiara Milanese, Adele Papetti

12.30 C51

ANTHOCYANIN NANOENCAPSULATION THROUGH WASTE VALORISATION: WHEY PROTEIN/HIGH METHOXY APPLE PECTIN COMPLEX COACERVATION

Ilaria Fierri, Laura De Marchi, Giacomo Rossin, Federica Mainente, Anna Perbellini, Maria Bellumori, Ines Mancini, Gianni Zoccatelli

12.45 C52

'TULARE' WALNUT SUPPLY CHAIN WASTE: A NEW CULTIVAR AS POTENTIAL BIORESOURCE IN THE NUTRACEUTICAL SECTOR

Elvira Ferrara, Severina Pacifico, Simona Piccolella, Assunta Esposito, Milena Petriccione

13.00 C53

BIOCHEMICAL AND NUTRITIONAL CHARACTERIZATION OF RED AND PURPLE POTATOES PEEL

Debora Dessì, Giacomo Fais, Giorgia Sarais

VI SESSIONE (sala convegni)
Sessione Parallela: Food safety and contamination
Moderatori: *Prof. Alberto Angioni, Prof.ssa Raffaella Boggia*

- 11.30 C54**
DETERMINATION OF CHIRAL PESTICIDES IN HEMP SEEDS BY USING ON-LINE SFE-ENANTIOSELECTIVE SFC-QQQ/MS
Maria Rita Testa Camillo, Marina Russo, Paola Dugo, Luigi Mondello
- 11.45 C55**
PESTICIDE RESIDUE LEVELS IN SEVERAL FOOD SAMPLES COMING FROM THE FERMO AREA, MARCHE REGION
Annamaria Iannetta, Giovanni Angelozzi, Francesca Mazza, Lucia Coppola, Sabrina Tait, Enrica Fabbrizi, Lorella Ciferri, Cinzia La Rocca, Monia Perugini
- 12.00 C56**
THE FIGHT AGAINST WINE'S BIOGENIC AMINES BEGINS IN THE VINEYARD
Andrea Salvo, Cinzia Ingallina, Fabrizio Masciulli, Enrico Romano, Donatella Ambroselli, Federica Proietti, Giovanna Loredana La Torre, Archimede Rotondo
- 12.15 C57**
ORGANIC AND INORGANIC CONTAMINANTS IN MOROCCAN MONOFLORAL HONEYS
Vincenzo Nava, Angela Giorgia Potortì, Benedetta Sgrò, Vincenzo Lo Turco, Giuseppa Di Bella
- 12.30 C58**
BIOACCUMULATION OF DEHT IN MYTILUS GALLOPROVINCIALIS AND POTENTIAL IMPLICATIONS IN THE NUTRITIONAL VALUE
Miriam Porretti, Ambrogina Albergamo, Federica Litrenta, Caterina Faggio; Giuseppa Di Bella
- 12.45 C59**
MIGRATION OF MINERAL OIL HYDROCARBONS FROM RECYCLED PAPERBOARD UNDER ACCELERATED CONDITIONS
Laura Barp, Chiara Conchione, Michele Suman, Francesca Lambertini, Sabrina Moret
- 13.00 C60**
GREEN AND INNOVATIVE AGROCHEMICAL FORMULATIONS BASED ON LIPOSOMAL TECHNOLOGY
Francesco Corrias, Ines Castangia, Salvatore Marceddu, Arturo Cocco, Roberto Mannu, Maria Manconi, Ignazio Floris, Alberto Angioni

13.30 Colazione di lavoro

VII SESSIONE (sala centrale)

Sessione Parallela: Innovative analytical techniques

Moderatori: Prof. Francesco Cacciola, Prof.ssa Chiara Di Lorenzo

15.00 PL03

CONTROLLI ANTIFRODE E QUALITÀ AGROALIMENTARE: ICQRF-MASAF

Stefania Carpino

15.30 C61

OPTIMIZATION OF GREEN EXTRACTION CONDITIONS TO RECOVER POLYPHENOLS FROM *SALICORNIA EUROPEA L.*

Francesco Limongelli, Marilena Muraglia, Roberta Tardugno, Pasquale Crupi, Sabrina Fiorentino, Maria Lisa Clodoveo, Filomena Corbo

15.45 C62

TOWARDS THE STANDARDIZATION OF A METHOD FOR THE DETERMINATION OF SELECTED VOLATILE COMPOUNDS IN VIRGIN OLIVE OILS

Tullia Gallina Toschi, Diego Luis García-González, Enrico Casadei, Ramón Aparicio-Ruiz, Maurizio Servili, Florence Lacoste, Stefania Vichi, Enrico Valli, Clemente Ortiz Romero, Roberto Selvaggini, Julien Escobessa, Beatriz Quintanilla-Casas, Alba Tres, Pierre-Alain Golay, Paolo Lucci, Erica Moret, Anastasios Koidis, Paul Brereton, Lanfranco Conte, Alessandra Bendini

16.00 C63

DEVELOPMENT OF A PRESSURIZED LIQUID EXTRACTION METHOD FOR GLUCOSINOLATES RECOVERY FROM BY-PRODUCTS OF *CAMELINA SATIVA (L.) CRANTZ SEED*

Stefania Pagliari, Ciro Cannavacciuolo, Chiara Maria Giustra, Matilde Forcella, Paola Fusi, Massimo Labra, Luca Campone

16.15 C64

QUANTITATIVE DETERMINATION OF THE LIPIDIC HYDROPEROXIDES IN VIRGIN OLIVE OILS BY USING A GREEN, EASY-TO-USE, AND SENSITIVE SPECTROPHOTOMETRIC METHOD

Francesco Longobardi, Vito Michele Paradiso

VII SESSIONE (sala convegni)
Sessione Parallela: Food composition and quality
Moderatori: *Prof. Giuseppe Avellone, Prof.ssa Mariateresa Russo*

- 15.30 C65**
THE WINE IS “NAKED”: FLINT GLASS BOTTLES CAUSE WINE AROMA IDENTITY DEGRADATION
Silvia Carlin, Fulvio Mattivi, Victoria Durantini, Stefano Dalledonne, Panagiotis Arapitsas
- 15.45 C66**
SAMPLE PREPARATION STRATEGIES FOLLOWED BY GC×GC BASED TECHNIQUES FOR FATTY ACIDS AND MINOR LIPID COMPONENTS INVESTIGATION IN FOOD-RELATED SAMPLES
Marco Beccaria, Angelica Fina, Chiara Cordero, Marco Piparo, Pierre Giusti, Pierre-Hugues Stefanuto, Luisa Pasti, Alberto Cavazzini, Jean-François Focant, Giorgia Purcaro
- 16.00 C67**
A MULTIMETHODOLOGICAL APPROACH FOR THE CHEMICAL CHARACTERIZATION OF EDIBLE INSECTS: THE CASE STUDY OF *ACHETA DOMESTICUS*
Mattia Spano, Giacomo Di Matteo, Alba Lasalvia, Carlotta Fila Totaro, Stefania Garzoli, Maria Elisa Crestoni, Luisa Mannina
- 16.15 C68**
COMPREHENSIVE METABOLOMIC INVESTIGATION OF FAUSTRIME FRUIT BY LC-MS AND GC-MS DATA FUSION COUPLED WITH MULTIVARIATE DATA ANALYSIS
Ciro Cannavacciuolo, Stefania Pagliari, Chiara Maria Giustra, Sonia Carabetta, Mariateresa Russo, Paola Branduardi, Massimo Labra, Luca Campone

16.30 Pausa caffè

16.30 – 17.00 Sessione Poster (arcata giardino)

17.00 RIUNIONE GICA

18.00 RIUNIONE ITACHEMFOOD

21.00 CENA SOCIALE presso Villa Favorita

Mercoledì 31 Maggio

VIII SESSIONE (sala centrale)

Sessione Parallela: *Innovative analytical techniques*

Moderatori: *Prof. Jean Daniel Coisson, Prof.ssa Marina Russo*

09.00 C69

ANALYSIS OF PESTICIDES IN CORN-BASED SAMPLES BY COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY

Francesco Cacciola, Katia Arena, Paola Dugo, Luigi Mondello

09.15 C70

APPLICATION OF DIRECT ANALYSIS IN REAL-TIME MASS SPECTROMETRY (DART-MS) AND MULTIVARIATE APPROACH FOR THE RAPID AND AUTOMATIC EVALUATION OF EDIBLE OILS

Domenica Mangraviti, Francesca Rigano, Cinzia Cafarella, Paola Dugo, Luigi Mondello

09.30 C71

DEVELOPMENT OF AUTHENTICATION MODELS BASED ON ¹H-NMR SPECTROSCOPY COUPLED WITH CHEMOMETRICS FOR CHOCOLATE PRODUCTS

Eleonora Truzzi, Davide Bertelli

09.45 C72

LABEL-FREE QUANTIFICATION OF THE KUNITZ INHIBITOR OF TRYPSIN KTI3 IN SOY PRODUCTS BY LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY

Barbara Prandi, Chiara Vacca, Stefano Sforza, Tullia Tedeschi

10.00 C73

QUALI-QUANTITATIVE SCREENING OF BIOACTIVE COMPOUNDS FROM HEALTH FOOD THROUGH COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY

Katia Arena, Francesco Cacciola, Paola Dugo, Luigi Mondello

10.15 C74

INVESTIGATING HAZELNUT ROASTING WITH A MULTI-ANALYTICAL TECHNIQUE APPROACH

Maria Mazzucotelli, Iulia Khomenko, Brian Farneti, Emanuela Betta, Elena Gabetti, Luca Falchero, Andrea Cavallero, Eugenio Aprea, Franco Biasioli

10.30 C75

FOOD METABOLOMICS BY GCXGC-TOF MS AND TANDEM IONIZATION: UNDERSTANDING THE IMPACT OF CLIMATE EVENTS ON EDIBLE CROPS QUALITY

Angelica Fina, Nemanja Koljančić, Simone Squara, Donatella Ferrara, Carlo Bicchi, Ivan Špáňik, Chiara Cordero

10.45 C76

COMPUTER VISION TO ANALYZE CHEMICAL SIGNATURES: A NOVEL WORKFLOW FOR RATIONALIZING RAW DATA EXPLORATION IN GC×GC

Andrea Caratti, Simone Squara, Angelica Fina, Stephen E. Reichenbach, Qingping Tao, Carlo Bicchi, Giorgio Borreani, Francesco Ferrero, Chiara Cordero

VIII SESSIONE (sala convegni)

Sessione Parallela: Food waste

Moderatori: Prof. Peter Quinto Tranchida, Prof. Gianni Zoccatelli

09.00 C77

GREEN EXTRACTION OF HYDROLYZED COLLAGEN PEPTIDES (HCPS) OBTAINED FROM TUNA YELLOWFIN SIDE-STREAMS AFTER INDUSTRIAL DEHYDRATION PROCESS

Valentina Orlandi, Federica Grasso, Lorenzo Dondero, Elena Grasselli, Federica Turrini, Raffaella Boggia

09.15 C78

INTEGRATED STRATEGIES AGAINST FOOD WASTE: BYPRODUCTS EXPLOITATION FOR SHELF-LIFE EXTENSION AND PACKAGING DEVELOPMENT

Antonella Cavazza, Maria Grimaldi, Edmondo Messinese, Daniel Milanese, Olimpia Pitirollo, Corrado Sciancalepore, Claudio Corradini

09.30 C79

COMPOSITION OF DISCARDED SICILIAN FRUITS OF *OPUNTIA FICUS INDICA* L.: PHENOLIC CONTENT, MINERAL PROFILE AND ANTIOXIDANT ACTIVITY IN PEEL, SEEDS AND WHOLE FRUIT

Maria Bellumori, Marzia Innocenti, Luisa Andrenelli, Fabrizio Melani, Lorenzo Cecchi, Gaetano Pandino, Giovanni Mauromicale, Stefano La Malfa, Nadia Mulinacci

09.45 C80

INTEGRATED AND SUSTAINABLE STRATEGY FOR THE INVESTIGATION AND VALORIZATION OF EXTRA VIRGIN OLIVE OIL EXTRACTS AND BY-PRODUCTS

Martina Bartolomei, Carlotta Bollati, Jianqiang Li, Carmen Lammi

10.00 C81

VALORISATION OF RESIDUAL ORANGE PEELS FROM PDO CULTIVARS OF THE RIBERA AREA, SICILY (ITALY): EXTRACTION, CHARACTERIZATION AND BIOACTIVITY ASSESSMENT OF ESSENTIAL OILS AND SECONDARY METABOLITES

Gregorio Peron, Sara Marcheluzzo, Giulia Bernabé, Gokhan Zengin, Kouadio Ibrahime Sinan, Michela Paccagnella, Ignazio Castagliuolo, Mirella Zancato, Stefano Dall'Acqua

10.15 C82
DEVELOPMENT OF SUSTAINABLE FOOD INGREDIENTS FROM AVOCADO WASTE AND BY-PRODUCTS. DRYING TECHNOLOGIES AND SENSORY CHARACTERISTICS

Maria Merlino, Fabrizio Cincotta, Anthea Miller, Martina Buda, Antonella Verzera, Concetta Condurso

10.30 C83
SURVEY ON THE PRESENCE OF ACRYLAMIDE IN STREET FOOD MARKETED AND PRODUCED IN ITALY AND APPLICATION OF POSSIBLE MITIGATION MEASURES

Francesco Giuseppe Galluzzo

11.00 Pausa caffè

11.00 – 11.30 Sessione Poster (arcata giardino)

IX SESSIONE (sala centrale)

Sessione parallela: Natural compounds and Nutraceuticals

Moderatori: Prof.ssa Vita Di Stefano, Prof. Gianni Sagratini

11.30 C84
CALENDULA ARVENSIS FLORETS: EFFECT OF FREEZE-DRYING ON THE ENCAPSULATION OF ITS SPECIALIZED METABOLITES AND ORAL BIOACCESSIBILITY

Marika Fiorentino, Simona Piccolella, Assunta Esposito, Severina Pacifico

11.45 C85
NMR AND HPLC CHARACTERIZATION OF GENTIANA LUTEA L. AERIAL PARTS: FLOWERS AS INGREDIENT FOR FUNCTIONAL FOODS WITH ANTI-MICOTOXINS ACTIVITY

Giacomo Di Matteo, Mattia Spano, Massimo Frangiamone, Alessandra Cimbalo, Lara Manyes, Luisa Mannina

12.00 C86
COMBINATION OF POMEGRANATE EXTRACT, B VITAMINS, AND VITAMIN C AGAINST PROLONGED FATIGUE: A MONOCENTRIC, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL

Lorenza Francesca De Lellis, Hammad Ullah, Daniele Giuseppe Buccato, Alessandra Baldi, Gaetano Piccinocchi, Roberto Piccinocchi, Alessandro Di Minno, Maria Daglia

12.15 C87
INVESTIGATION OF THE HYPOCHOLESTEROLEMIC ACTIVITY OF AN INNOVATIVE PLANT DERIVED EXTRACT

Lorenza d'Adduzio, Davide Marangon, Umberto Musazzi, Carlotta Bollati, Martina Bartolomei, Davide Lecca, Carmen Lammi

12.30 C88

COLD PRESSED SEED OILS AS A NEW SOURCE OF NUTRACEUTICALS – A COMPREHENSIVE CHEMICAL CHARACTERIZATION

Cinzia Cafarella, Francesca Rigano, Emanuela Trovato, Paola Dugo, Luigi Mondello

IX SESSIONE (sala convegni)

Sessione Parallela: Food waste and Food Composition

Moderatore: Prof.ssa Nadia Mulinacci

11.30 C89

SONCHUS ASPER (L.) HILL: REVALORIZATION OF WILD EDIBLE PLANT OF TRADITIONAL USE

Valentina Santoro, Valentina Parisi, Luigi Milella, Carla Caddeo, Antonio Nestico', Anna Lisa Piccinelli, Luca Rastrelli, Nunziatina De Tommasi

11.45 C90

FROM AGRO-FOOD WASTE TO ZERO-IMPACT SOURCE: THE POMEGRANATE AS A MODEL SYSTEM

Francesco Cairone, Chiara Salvitti, Irene Arpante, Caterina Frascchetti, Antonello Filippi, Stefania Cesa

12.00 C91

EVALUATION OF THE PISTACHIO'S AROMA COMPOUNDS BY HIGH-CAPACITY CONCENTRATION TOOLS COUPLED WITH MULTI-DIMENSIONAL GAS CHROMATOGRAPHY

Andrea Schincaglia, Giorgia Purcaro, Nicola Marchetti, Alberto Cavazzini, Marco Beccaria

12.15 C92

INNOVATIVE ACTIVE LAYER-BY-LAYER EDIBLE COATING FORMULATION TO PRESERVE THE QUALITATIVE AND BIOCHEMICAL TRAITS OF 'DELLA RECCA' SWEET CHERRIES

Anna Magri, Rosaria Cozzolino, Livia Malorni, Gianluca Picariello, Francesco Siano, Milena Petriccione

12.45 Premiazioni

13.30 Colazione di lavoro

15.00 Chiusura attività congressuali

17.00 Visita facoltativa alle CANTINE STORICHE DELLA FLORIO e degustazione di vini Marsala e fast food siciliano con spettacolo musicale.

PLENARIE

PL01

Freshness of food products of animal origin: effective metabolomics approaches for milk & egg products

Michele Suman^{1,2}, Daniele Cavanna^{1,3}, Cecilia Loffi^{1,3}, Giuseppe Sammarco^{1,3},
Dante Catellani¹, Chiara Dall'Asta³

¹Analytical Food Science, Barilla G. e R. Fratelli S.p.A., Via Mantova 166, 43122 Parma, Italy

²Department for Sustainable Food Process, Catholic University of the Sacred Heart, 29121 Piacenza, Italy

³Department of Food and Drugs, University of Parma, Parco Area delle Scienze 95/A, 43124 Parma, Italy

michele.suman@barilla.com

Milk & egg products freshness is an important parameter for both consumers' health and quality of their correspondent-based products.

Up to now there have been neither analytical methods nor specific parameters to uniquely define milk freshness from a complete and univocal chemical perspective.

At the same time, up to now, in the case of egg freshness, this parameter has been assessed with the quantification of few "late signal compounds", while the possibility to evaluate more early warning molecules simultaneously could help to provide robust results.

Metabolomics approaches are the winning key to solve this kind of needs.

In particular, exploiting sample lots collected directly from their arrival at the production plant sites and optimizing extraction procedures and ultrahigh-pressure liquid chromatography–high-resolution mass spectrometry (UHPLC-HRMS) analysis, different chemometric models were created to select gradually the most significant features that were finally extracted and identified through HRMS data.

In this way, relevant markers were identified as responsible for milk or egg products aging, complementing and successfully enhancing parallel evaluations obtainable through sensory measures.

References

[1] D. Cavanna, D. Catellani, C. Dall'Asta, M. Suman, *Journal of Mass Spectrometry*, **2018**, 53(9), 849.

[2] C. Loffi, D. Cavanna, G. Sammarco, D. Catellani, C. Dall'Asta, M. Suman, *Journal of Dairy Science*, **2021**, 104, 12.

PL02

Trends in the Novel Foods area

Rosangela Marchelli

European Food Safety Authority (EFSA), Working Group (WG) on Novel Foods and WG on Food Allergy
rosangela.marchelli@gmail.com

“Novel Food” defines a food or ingredient that has not been consumed to a significant degree by humans in the EU before 15 May 1997. The Union's rules on novel foods were established by Regulation (EC) No 258/97 of the European Parliament and of the Council and by Commission Regulation (EC) No 1852/2001, Reg. (EC) No 178/2002 and by the latest implemented Regulation (EU) 2015/2283. According to this Regulation, a NF falls in at least one of the following categories: food with a new or intentionally modified molecular structure, food from cell culture or tissue culture derived from animals, plants, microorganisms, fungi or algae, food from microorganisms, fungi or algae, food from material of mineral origin, food obtained with a new production process, food consisting of engineered nanomaterials, vitamins, minerals or other substances resulting from a production process not used for food production within the Union before 15 May 1997, and a food used before 15 May 1997 exclusively as, or in, a food supplement.

The lecture shall give an overview on the type of applications submitted to EFSA so far and will focus on the latest trends observed concerning novel foods from all the world and also traditional foods from third countries. The most numerous applications concern novel proteins from different sources, including insects, novel carbohydrates, identical to those present in human milk (HiMO) and as novel sweeteners and fibers, and extracts from plant and vegetables. Many NF are produced by chemical synthesis or by microbiological fermentations.

The lecture shall deal with the methods applied by EFSA for performing the risk assessment of NF. Risk assessment involves the identification of the NF, the detailed description of the production process, the characterisation of the NF and the specifications, which will be reported in the List of Novel Foods [1]. A particular attention is given to the physico-chemical properties of the NF and to the eventual presence of nano- or small-particles, which can have an impact on the solubility, bioavailability and toxicity of the product. The nutritional value, together with the adsorption, distribution, metabolism and excretion (ADME), toxicology and allergenicity are carefully evaluated. Finally, the lecture shall address in particular two of the most recent NFs, which are receiving a lot of attention by the media and the public opinion: insects and cannabinoids, in particular CBD (cannabidiol), either produced by synthesis or by extraction from different parts of *Cannabis sativa*.

Reference

[1] Commission Implementing regulation (EU) 2017/2470 of 20 December 2017, establishing the Union List of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods.

PL03

Anti-fraud controls and Agri-food quality: ICQRF-MASAF

Stefania Carpino

Ispettorato centrale della tutela della qualità e repressione frodi dei prodotti agroalimentari
Ministero dell'agricoltura, della sovranità alimentare e delle foreste
s.carpino@masaf.gov.it

Food safety and fraud prevention are of great importance in maintaining public health, safeguarding consumer trust, and ensuring the integrity of the import/export fluxes. The increasing complexity and globalization of the food industry have made it increasingly challenging to detect and fight against food fraud effectively. The ICQRF, with its daily action for the protection of “Made in Italy” products gives a significant contribution to consolidate the reputation of the quality of Italian products. Controls in the agro-food sector are more and more an active marketing factor, which can position Italian food as a high-end product. Fighting frauds and the ‘Italian Sounding’ phenomenon is therefore a priority. This work shows the activity carried out by the ICQRF against frauds, usurpations, and counterfeiting phenomenon, which harms Italian quality products, and consumers as well. The operational results confirm the quality of the Italian control system, where the ICQRF stands among the main enforcement Authorities worldwide. With six laboratories and about 100 laboratory technicians, ICQRF has an independent capacity for analytical verification of agri-food productions, a peculiarity that has few international comparisons. All laboratories operate in compliance with the UNI CEI EN ISO/IEC 17025:2018 standard "General criteria on the competence of testing and calibration laboratories", carrying out checks based on analytical determinations accredited by the national accreditation body ACCREDIA. The accreditation concerns a total of 240 tests, of which 13 managed in a "flexible field". ICQRF has also tasting panels, responsible for the evaluation and official control of the organoleptic characteristics of virgin and extra virgin olive oils, using the method defined at EU level. The tasting committees admitted pursuant to the Ministerial Decree of 18 June 2014, have obtained international recognition from the IOC. (International Olive Council). All ICQRF Laboratories participate in proficiency tests, i.e., ring tests organized by Providers, preferably accredited on the basis of the UNI CEI EN ISO/IEC 17043 standard, to evaluate their analytical performance, also for the purpose of maintenance of accreditation. The harmonization of the ICQRF Quality System, a priority objective for the uniform application of the specific sector standard and to guarantee the validity of the analytical data produced by the individual laboratories, is managed by the PREF IV Office with the active collaboration of a working group which involves all the Quality Assurance Managers of the Laboratories distributed throughout Italy. The ICQRF laboratories, as an institutional task, also carry out research activities with National and International Research Institutions and Universities, coordinated by the PREF IV Office. The various lines of research are in fact an essential tool for improving the action to combat fraud in the agri-food sector, as well as for enhancing the quality characteristics of foods. ICQRF develops new methods of analysis on agri-food matrices capable of highlighting any use of fraudulent production practices or identifying new parameters for the qualitative characterization of foods. ICQRF actively participate inside the DG AGRI EU Committee for several matrices (i.e., oil, wine, etc.). ICQRF appointed representatives actively also participate as expert members inside the DG AGRI EU Committee for several matrices (i.e., oil, wine, etc.)

COMUNICAZIONI

The challenges of climate change on the consistency of the cocoa flavour quality

Eloisa Bagnulo¹, Giulia Strocchi¹, Cristian Bortolini², Chiara Cordero¹, Erica Liberto¹

¹Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via Pietro Giuria 9, Turin, Italy

²Soremartec Italia S.r.l., P.le P. Ferrero 1, 12051 Alba (CN), Italy

erica.liberto@unito.it

Objective tools to trace sustainable cocoa production are necessary, especially with regard to climatic changes and the political situation in the producing countries. Fingerprinting is a good approach for monitoring and authenticating food [1-2]. Food authentication is often based on the degree of similarity of the fingerprint between the sample under investigation and a representative reference. This process is known as food identification [2-4] and its reliability depends on its correctness.

Cocoa (*Theobroma cacao* L.) is a tropical perennial plant and one of the most important economic factors in the countries where it is grown. It is also a raw material of great economic relevance for various market fields, of which confectionery and functional foods and beverages (cocoa and chocolate derivatives) account for more than 60% of the market. For chocolate products, flavour is one of the key characteristics associated with product quality, along with brand and price. Furthermore, flavour quality *identification* requires analytical platform able to produce detailed diagnostic profiles that can be correlated with sensory characteristics to be monitored for an objective evaluation in quality control (QC) specifications.

In this study, HS-SPME-GC-MS in combination with machine learning tools was applied to the identification of a range of cocoa samples from different origins. This approach was applied to one hundred and sixty samples of cocoa beans and cocoa liquors. Untargeted fingerprinting and profiling approaches were tested for the information, discrimination and classification capabilities given by the volatilome of incoming beans and liquors. Machine learning tools PCA, NEAR indices and PLS-DA modelling were applied to the data set for flavour identification of origins, to find flavour similarity of origins with the industry quality standard and to develop new blends to be compared with the flavour of standard references [2,5]. The results indicate a coherent, clear clustering of samples according to their origin with the two analytical strategies, both on raw beans and on cocoa liquors, albeit with differences at the molecular level. Predicting the classification of cocoa beans with the untargeted fingerprint on an external test set gave excellent results for beans with a classification rate of 100% and very good results for liquors (88%), despite the processing they underwent. Better results were obtained for targeted approaches with a classification rate above 92% (i.e., 92.86% for beans and 92.31% for liquors). The NEAR index, calculated using the OAVs of selected origin markers, showed Ecuador origin as to be the most similar to the reference in terms of taste. Through an OPLS-DA modelling of the chemical-specific origin marker and sensory similarity, blends with different proportions of Ecuador cocoa were created.

References

- [1] G.P. Danezis, A. S. Tsagkaris, V. Brusic, C. A. Georgiou, *Current Opinion in Food Science*, **2016**, *10*, 22.
- [2] F. Stilo, A. M. Jiménez-Carvelo, E. Liberto, C. Bicchì, S. E. Reichenbach, L. Cuadros-Rodríguez, C. Cordero, *Journal of Agricultural and Food Chemistry*, **2021**, *69*, 8889.
- [3] L. Cuadros-Rodríguez, C. Ruiz-Samblás, L. Valverde-Som, E. Pérez-Castaño, A. González-Casado, *Analytica Chimica Acta*, **2016**, *909*, 9.
- [4] S.D. Johanningsmeier, G. K. Harris, C. M. Klevorn, *Annual Review of Food Science and Technology*, **2016**, *7*, 413.
- [5] D. Bressanello, E. Liberto, C. Cordero, B. Sgorbini, P. Rubiolo, G. Pellegrino, M.R. Ruossi, C. Bricchi, *Journal of Agricultural and Food Chemistry*, **2018**, *66*, 7096.

Novel insights on cheese defects in Pecorino Romano PDO cheese: a case study

Giacomo L. Petretto¹, Giovanni Patta¹, Giacomo Zara², Paolo Urgeghe²,
Severino Zara², Francesco Fancello²

¹Departemnt of medical, Surgery and Pharmacy, University of Sassari

²Department of Agriculture, University of Sassari

gpetretto@uniss.it

The occurrence of defects in cheese is linked to a significant economic loss for dairy industry [1]. Defects in cheese could origin according to the starting milk quality, hygiene practices or to cheese technological process and they can appear at different steps of cheesemaking.

The microbial milk contamination still represents an unsolved problem for dairy productions as for example the contamination by bacterial producing spores such as *Clostridium* genus. Due to the high spore's resistance, these microorganisms can survive the treatments applied during milk pasteurization or cheese making, furthermore the spores can germinate to outgrowing vegetative cells all along the ripening period to giving rise to the so-called late blowing defect (LBD) [2]. Moreover, heterofermentative lactic acid bacteria as *Paucilactobacillus* spp., a component of the non starter lactobacilli in cheese, can cause LBD during aging step [3]. Although LBD has been detected also in ewe cheese, the main bulk of literature studies on LBD are focused on vaccine milk contamination.

Other cheese defect as split and secondary fermentation commonly occur in Swiss cheese, and it manifests as slits and cracks [4] while pink discoloration defect [5] is maybe the most common one studied for pecorino cheeses.

During spring 2021, a Sardinian dairy producing Pecorino Romano PDO cheese observed an unusual deformation and a slightly swelling of several cheese wheels. When cut, the wheel presented cracks, cheese discoloration and an unpleasant smell. With the aim of shed light on this defect the cheese main chemical composition has been evaluated and compared to control samples. The main physicochemical parameters were evaluated by literature methods, volatile organic compounds were determined by solid phase microextraction coupled with GC-MS analysis beside free- and total fatty acids were determined as fatty acid methyl esters (FAME). Furthermore, the obtained data were subjected to multivariate analysis to highlight any possible difference between the samples and try to identify any potential chemical marker linked to the observed defect.

References

- [1] D. Carminati, B. Bonvini, L. Rossetti, M. Zago, F. Tidona, G. Giraffa, *Food Control*, **2019**, *100*, 321.
- [2] D. Bassi, E. Puglisi, P.S. Cocconcelli, *Food Microbiology*, **2015**, *52*, 106.
- [3] F. Ortakci, J.R. Broadbent, C.J. Oberg, D.J. McMahon, *Journal of Dairy Science*, **2015**, *98*, 3645.
- [4] D.F.M. Daly, P.L.H. McSweeney, J.J. Sheean, *Dairy Science Technology*, **2010**, *90*, 3.
- [5] F. Martelli, E. Banclari, E. Neviani, B. Bottari, *International Dairy Journal*, **2020**, *111*, 104829.

Evaluation of folates in Calabrian citrus fruits by Orbitrap mass spectrometry

Lucia Bartella^{1,2}, Ines Rosita Talarico^{1,2}, Fabio Mazzotti^{1,2}, Ilaria Santoro², Melissa Cacciatore¹,
Leonardo Di Donna^{1,2}

¹Department of Chemistry and Chemical Technology, University of Calabria,
Via P. Bucci, Cubo 12/D, Rende, CS, 87036, Italy

²QUASIORA Laboratory, Agrinfra Research Net, University of Calabria,
Via P. Bucci, Cubo 12/D, Rende, CS, I-87036, Italy
lucia.bartella@unical.it

Folates belong to a class of essential water-soluble vitamins, naturally found in green vegetables, legumes, and citrus fruits. Their chemical structure consists of a pteridine ring with a *p*-aminobenzoate portion linked to polyglutamyl chains, and they can differ according to the reduction and substitution degree of the pterin cycle and to the glutamate chain length [1]. Folates are well known for their biochemical functions and the related benefits on human health, such as the normal fetal growth, normal blood formation, and the regular function of the immune system. These beneficial effects are also attested by the Regulation 432/2012 of the European Union, which permits some health claims on foods and beverages which contains a specific amount of folates (30 µg and 15 µg per 100 g, respectively) [2]. Citrus juices are an excellent source of folates, as they naturally contain high levels of these vitamins, especially in the form of 5-methyltetrahydrofolic acid (5-MTHFA) [3].

Herein, we present a study concerning the assessment of folates in specific citrus fruits belonging to the Calabria region: *Bergamot*, *Citron* and *Clementine*. The determination was performed in different parts of the fruits investigated: juice, pulp and albedo. Vitamins were identified and characterized by high-resolution mass spectrometry experiments using an Orbitrap analyzer hyphenated with a HPLC system (HPLC-HR-ESI-MS and HR-ESI-MS/MS). High-resolution MS analysis highlighted that the main folate found in fruit sections and juices was 5-methyltetrahydrofolic acid (5-MTHFA), also present as polyglutamate.

In addition, a novel approach for an accurate quantification of 5-MTHFA was developed by means HPLC-Orbitrap-MS analysis and internal standard calibration method. After the evaluation of analytical parameters (accuracy, precision and matrix effect), the methodology was applied to real citrus samples, showing a high concentration of 5-MTHFA in all the fruit portions under investigation. The amount of 5-methyltetrahydrofolic acid ranged from 100 µg/L to 200 µg/L, proving that bergamot, citron and clementine fruits are excellent source of folates.

References

- [1] N. Delchier, A. L. Herbig, M. Rychlik, C. M. G. C. Renard, *Comprehensive Reviews in Food Science and Food Safety*, **2016**, *15*, 506.
- [2] European Union Regulation, N. 432/2012.
- [3] P. M. Thomas, V. P. Flanagan, *Journal of Agricultural and Food Chemistry*, **2003**, *51*, 1293.

Green coffee, bioactive compounds' analysis of experimental cultivation in Sicily: a new frontier in the Sicilian food sector

Vita Di Stefano¹, Carla Buzzanca¹, Fortunato Ruvutuso¹, Giovanni Gugliuzza², Dario Scuderi³, Eristanna Palazzolo³, Vittorio Farina³

¹Department of Biological, Chemical, and Pharmaceutical Science and Technology (STEBICEF), University of Palermo, via Archirafi 32, 90123 Palermo, Italy

²CREA—Research Centre for Plant Protection and Certification, 90128 Palermo, Italy

³Department of Agricultural, Food and Forest Science (SAAF) University of Palermo, Viale delle Scienze, 90128 Palermo, Italy

carla.buzzanca@gmail.com

Recently, climate change represents a new possibility for tropical cultivars fruit in Mediterranean areas. The focus of this work is the evaluation of the real possibility of coffee cultivation in Sicily, like coffee plants grown in tropical and subtropical regions. The objective was to evaluate plant adaptation to our climate and to study the chemical qualities of green coffee pulps and seeds: total phenolic content, antiradical capacity, fatty acids, amino acids, alkaloids, vitamins, proximate composition, polyphenolic profile and other bioactive compounds of cosmetic, pharmaceutical and agrary interest. Temperature, light and vegetative growth of *Coffea arabica* L. cv. “Caturra” plants were monitored. Our study highlighted how, by implementing small measures in terms of agronomic management, the crop adapts well to climate. Total phenolic content (TPC) using Folin-Ciocalteu method and antiradical capacity using DPPH and ABTS approach [1] were performed on dried pulps and green seeds samples. Data obtained show TPC values like coffee cultivars in Vietnam, Brazil and Kenya, instead, antiradical activity values were comparable to coffee grown in Central America, Indonesia, Uganda and Vietnam. Identification and quantification of fatty acids were carried out by GC-MS. In particular, the dried green coffee pulps show a low percentage of fatty acid (1,84 g/100g) of which 71,6% of SFA. Indeed, the green coffee seeds show a high level of fatty acid (13,85g/100g) of which 46,84% of SFA and 41,6% of PUFA. A study of proteins was carried out on pulps and seeds after acid hydrolysis; amino acids profile shows low concentrations of proteins in seeds (0,53 g/100g) and higher in dried pulp (10,45g/100g). The study of seed proteic fraction was extended to bound proteins. Hydrolysis of bound protein highlighted, by alkaline condition, higher level of released amino acids in seeds (10,56g/100g) [2]. The data obtained from the analysis of fatty acid and amino acids indicate similar values of chemical constituents like coffee cultivated in Brazil, Colombia, Honduras, Guatemala and Kenya. Quantification of caffeine was performed in GC-MS and expressed in g/100g. High levels of caffeine in seeds (2,34 g/100g) and in dried pulps (1,56 g/100g) were similar to Salvadoran, Brazilian and Mexican green coffee (*Coffea Arabica* var. Bourbon, Caturra and Mundo Novo). Quali-quantitative analysis of flavonoids, chlorogenic acids, anthocyanidins, trigonelline and other bioactive compounds was obtained with LC-MS [3]. The study revealed in the seeds a higher amount of vitamin C (0.40 mg%), vitamin B2 (0.18 mg%), vitamin B3 (12 mg%), K (2017 mg%), P (170 mg%), Mg (168 mg%) and Ca (133mg%). Our results indicate the possibility to cultivate coffee in Mediterranean climate obtaining seeds with interesting qualitative traits.

References

- [1] A. Ali, H. F. Zahid, J.J. Cottrell, F.R. Dunshea, *Molecules*, **2022**, *27*, 5126.
- [2] W. Dong, Q. Chen, C. Wei, R. Hu, Y. Long, Y. Zong, Z. Chu, *Ultrasonics Sonochemistry*, **2021**, *74*, 105578.
- [3] B. Nemzer, N. Abshiru, F. J. Al-Taher, *Journal of Agricultural and Food Chemistry*, **2021**, *69*, 11, 3430.

Neuroprotective and antioxidant effects of Urolithins: Quantum Mechanics (QM) and Molecular Mechanics (MM) applications

Emanuela Marchese¹, Isabella Romeo^{1,2}, Giosuè Costa^{1,2}, Stefano Alcaro^{1,2,3}

¹Dipartimento di Scienze della Salute, Università degli Studi “Magna Græcia” di Catanzaro, Campus “S. Venuta”,
Viale Europa, 88100 Catanzaro, Italy

²Net4Science Academic Spin-Off, Università degli Studi “Magna Græcia” di Catanzaro, Campus “S. Venuta”,
Viale Europa, 88100 Catanzaro, Italy

³Associazione CRISEA - Centro di Ricerca e Servizi Avanzati per l’Innovazione Rurale,
Località Condoleo, 88055 Belcastro, Italy
e.marchese@unicz.it

Urolithins (UROs) are secondary metabolites, produced in vivo by the human gut microbiota, after the intake of foods rich in polyphenols ellagitannins (ETs) and ellagic acid (EA), such as pomegranate. These *6H-dibenzo[b,d]pyran-6-one* derivatives exhibit various biological activities, including neuroprotective and antioxidant effects [1].

Starting with the consideration that EA demonstrated an efficiency comparable to the classic antidepressants and that the anti-depressant-like effect was nulled by co-administration of selective antagonists of the noradrenergic receptors, we investigated the involvement of EA *versus* presynaptic release-regulating α_2 autoreceptors (α_2 -ARs). Through theoretical studies, based on molecular mechanics (MM) calculations, we discuss how EA interacts with α_{2A} - and α_{2C} -ARs. In vitro experimental approach assessed that EA mimics clonidine in inhibiting noradrenaline exocytosis from hippocampal nerve endings in a yohimbine-sensitive fashion [2]. Considering that EA is significantly metabolized in UROs, we decided to also explore the behaviour of these secondary metabolites into α_2 -ARs catalytic pockets employing molecular docking and molecular dynamics simulations (MDs). In vitro assays are currently undergoing.

At the same time, we dealt with the antioxidant aspect of UROs. Through quantum mechanics (QM) methods [3], we elucidated the potential chemical routes related to the free radical scavenging activity, considering the environment’s factors, the physiological conditions, and the chemical nature of the generated free radicals. Our in-silico results reveal the mechanisms of antioxidant protection of UROs, in agreement with experimental assays [4].

References

- [1] R. García-Villalba, J.A. Giménez-Bastida, A. Cortés-Martín, M.A. Ávila-Gálvez, F.A. Tomás-Barberán, M.V. Selma, J.C. Espín, A. González-Sarrías, *Molecular Nutrition & Food Research*, **2022**, *66*, 2101019.
- [2] I. Romeo, G. Vallarino, F. Turrini, A. Roggeri, G. Olivero, R. Boggia, S. Alcaro, G. Costa, A. Pittaluga, *Antioxidants*, **2021**, *10*, 1759.
- [3] A. Galano, J.R. Alvarez-Idaboy, *Journal of Computational Chemistry*, **2013**, *34*, 2430.
- [4] E. Marchese, V. Orlandi, F. Turrini, I. Romeo, R. Boggia, S. Alcaro, G. Costa, *Antioxidants*, **2023**, *12*, 697.

Exploiting new Se-glycoconjugates as antioxidant supplements in oxidative stress related diseases

Giovanna Cimmino^{1,2}, Mauro De Nisco³, Silvana Pedatella², Severina Pacifico¹

¹Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania “Luigi Vanvitelli”, Via Vivaldi 43, 81100 Caserta, Italy

²Department of Chemical Sciences, University of Napoli Federico II, Via Cinthia 4, I-80126, Napoli, Italy

³Department of Sciences, University of Basilicata, Via dell’Ateneo Lucano 10, I-85100 Potenza, Italy
giovanna.cimmino@unicampania.it

Reactive oxygen species (ROS) overproduction, when unbalanced by endogenous antioxidants, favoured oxidative stress (OS), and a vicious cycle, which, associated with chronic inflammation, is involved in the onset of degenerative diseases, such as cancer, neurodegenerative, and digestive diseases [1]. Epidemiological studies and nutritional interventions daily emphasize that dietary antioxidants are an exogenous valuable strategy to counteract or slow-down OS preserving health and protecting from diseases [2, 3]. In particular, the literature is informative of natural products (in blend or pure form) or antioxidant micronutrients, the use of which is severely limited by crucial factors such as solubility, stability and availability. This is the case of phenols and polyphenols whose antioxidant goodness is often compromised by interactions with other constituents in the food matrix or by a loss of integrity during metabolic processes in the intestine, liver, etc. [4].

In the framework aimed at discovering new efficacious antioxidants, the synthesis of new selenoglycoconjugates is of our interest with the aim to overcome the poor (poly)phenol bioavailability and to provide a synergistic antioxidant effect at once. In fact, as selenium (Se) is physiologically related to cell homeostasis, body metabolism and antioxidant defence [1], and glycosylation modifies the bio-chemical and physical properties of (poly)phenols, enhancing their small intestine absorption [5], Se-based compounds featured by a sugar-type structure linked to (poly)phenols have been synthesized. The synthetic strategy proposed starts from D-ribonolactone derivative to obtain the donor, which is then used to produce glycoconjugates *via* a Mitsunobu reaction with well-known (poly)phenols [6].

Selenoglycoconjugates were evaluated for their antioxidant capability, and redox mitochondrial activity on human keratinocytes and neuroblastoma cells. Data acquired highlighted that phenolic moiety strongly affect both antioxidant and mitochondrial redox activity. The Se-sugar conjunction allowed the synthesized compounds not to exert cytotoxic effects, especially at the highest doses tested, which were differently observed from polyphenols when in the unconjugated form.

References

- [1] M. Sharifi-Rad, N.V. Anil Kumar, P. Zucca, E.M. Varoni, L. Dini, E. Panzarini, J. Rajkovic, P.E. Tsouh Fokou, E. Azzini, I. Peluso, A. Prakash Mishra, M. Nigam, Y. El Rayess, M. Beyrouthy, L. Polito, M. Iriti, N. Martins, M. Martorell, A.O. Docea, W.N. Setzer, D. Calina, W.C. Cho, J. Sharifi-Rad, *Frontiers in Physiology*, **2020**, *11*, 694.
- [2] G. Bjørklund, M. Shanaida, R. Lysiuk, H. Antonyak, I. Klishch, V. Shanaida, M. Peana, *Molecules*, **2022**, *27*, 6613.
- [3] S. Anand, N. Bharadvaja, *Revista Brasileira de Farmacognosia*, **2022**, *22*, 211.
- [4] J.M. Landete, *Critical Reviews in Food Science and Nutrition*, **2013**, *53*, 706.
- [5] S. Almeida, M.G. Alves, M. Sousa, P.F. Oliveira, B.M. Silva, *Neurotoxicity Research*, **2016**, *30*, 345.
- [6] L. Serpico, M. De Nisco, F. Cermola, M. Manfra, S. Pedatella, *Molecules*, **2021**, *26*, 2541.

***Ulva lactuca* L. of Orbetello lagoon: multivariate optimization of the extraction of ulvan polysaccharides**

Beatrice Zonfrillo¹, Maria Bellumori¹, Marzia Innocenti¹, Serena Orlandini², Sandra Furlanetto², Gianni Zoccatelli³, Nadia Mulinacci¹

¹Department of NEUROFARBA, Pharmaceutical and Nutraceutical Section, University of Florence; ²Department of Chemistry “Ugo Schiff”, University of Florence; ³Department of Biotechnology, University of Verona
beatrice.zonfrillo@unifi.it

Ulvan is a sulfated polysaccharide extracted from green algae of genus *Ulva* (family Ulvaceae) that has gelling properties and has shown anticoagulant, antiviral, anti-hyperlipidemic, immunomodulatory and antioxidant activities, offering great potential for the development of functional food [1]. Ulvan is mainly built on disaccharides repeating sequences composed of sulfated rhamnose and glucuronic acid, iduronic acid or xylose. The biological and chemo-physical properties of ulvan are directly related to its chemical structure; thus, it is crucial for its application to understand how the chemical composition of the extract is influenced by the extraction conditions [2]. This work involved *Ulva lactuca* L. samples collected in August 2022 in an aquaculture tank of Orbetello lagoon (GR, Italy), where the algae grow spontaneously and can be considered a by-product of fish farming. The aim of the study was the optimization of the extraction conditions for ulvan in order to maximize yields and purity of the polysaccharides extracted. A Free Wilson model [3] was used to determine the effects of the pH, extractant/sample ratio and time of extraction on yields, purity and sulfation degree. The coefficients of the model were estimated with a symmetrical screening matrix (pH: 2–3.5–5; extraction time: 30–105 – 180 min; extractant/sample ratio: 20–50–80 mL/g). Purity was evaluated by quantitative ¹H-NMR [4] as rhamnose percentage on dialyzed polysaccharides and turbidimetric assay was applied for the quantification of sulfate [5]; uronic acids and neutral sugars were determined by HPAEC-PAD [6]. Light Scattering techniques were applied to evaluate the hydrodynamic diameter, correlated to the size distribution of polysaccharides in water, and the zeta-potential, that provided an indirect measure of the surface charge [7]. Graphic analysis of effects showed that yields pre- and post-dialysis were not significantly affected by changing the pH. They were mainly influenced by the extractant/sample ratio, with the use of higher volumes of extractant leading to higher yields (up to 14.88%). The rhamnose content was dependent on the extraction time and on the extraction pH, with a higher degree of purity obtained at pH 2, confirming the higher selectivity for ulvan extraction at low pH values [2]. The sulfate percentage was in the range of 11–20% and was strongly influenced by the extraction time; an increase of the time corresponded to a decrease of the response. Considering the results of the screening phase, a Response Surface Methodology (RSM) study was carried out for obtaining a map of the predicted responses in the experimental domain under study. RSM has never been applied before for this species and enabled to identify the multivariate zone where high quality ulvan with high yields could be obtained. Further studies should be done to investigate how pH can modulate ulvan structure.

References

- [1] F.R. Cindana Mo'o, G. Wilar, H.P. Devkota, N. Wathoni, *Applied Sciences*, **2020**, *10*, 5488.
- [2] J. Kidgell, M. Magnusson, R. de Nys, C. Glasson, *Algal research*, **2019**, *39*, 101422.
- [3] G.A. Lewis, D. Mathieu, R. Phan-Tan-Luu, *Pharmaceutical Experimental Design*, *Marcel Dekker*, **1999**, New York.
- [4] M. Khatib, G. Pieraccini, M. Innocenti, F. Melani, N. Mulinacci, *Journal of Pharmaceutical and Biomedical Analysis*, **2016**, *123*, 53.
- [5] P.B. Torres, A. Nagai, C.E.P. Jara, J.P. Santos, F. Chow, D.Y.A.C. dos Santos, *Ocean and Coastal Research*, **2021**, *69*.
- [6] N. Wahlström, F. Nylander, E. Malmhäll-Bah, K. Sjökvold, U. Edlund, G. Westman, E. Albers, *Carbohydrate Polymers*, **2020**, *233*, 115852.
- [7] L. Yang, L. Zhang, *Carbohydrate Polymers*, **2009**, *79(3)*, 349.

Phenolic extract from hazelnut skin: chemical composition and protective role against Advanced Glycation End-products (AGEs)-damage in THP-1-derived macrophages

Ludovica Spagnuolo, Laura Dugo, Laura De Gara

Department of Science and Technology for Sustainable Development and One Health, University Campus Bio-Medico of Rome, Via Alvaro del Portillo 21, 00128 Roma, Italy

l.spagnuolo@unicampus.it

Polyphenols are a large group of secondary metabolites in plants and are, therefore, present in the human diet. These natural compounds are considered as source of bioactive molecules with beneficial effect on human health with a good anti-inflammatory property, and also antiglycation functions [1]. Glycation and the accumulation of advanced glycation end-products (AGEs) are known to occur during physiological aging, diabetes, chronic and neurodegenerative diseases and in the development of tumors [2]. AGEs are macromolecules derived from spontaneous non-enzymatic glycation (or Maillard reaction) between reducing sugars and proteins, nucleic acids or lipids [3]. In addition to endogenous AGEs formation, these compounds are also derived from foods high in lipids and proteins and are called dietary AGEs [3]. Hazelnut (*Corylus avellana* L.) belongs to the Betulaceae family and is one of the most popular tree nuts consumed worldwide due to its nutrients, fat-soluble bioactive components and phenols/phytochemicals [4]. Among hazelnut food waste, hazelnut skin has been investigated for its potential health effects.

The present study aims to investigate the protective effects of polyphenolic-rich extracts obtained by hazelnut skin (HSE) on AGEs damage, *in vitro* macrophages cell model. The polyphenolic composition of hazelnut skins by HPLC-PDA/ESI-MS revealed that this food waste represents a rich source of several polyphenolic compounds: seventeen phenolic compounds have been identified in the extract such as ten flavan-3-ols, two organic acids, four flavonols and one dihydrochalcone.

Data obtained showed that HSE inhibit BSA-MGO (AGEs model system) formation *in vitro* chemical assay. In THP-1 derived macrophages, HSE exert anti-inflammatory activity, evident by the reduction of the mRNA expression of tumor necrosis factor- α (TNF- α) and in protein secretion of both TNF- α and interleukin-1-beta (IL-1 β). Whereas, AGEs determine an increase in pro-inflammatory cytokines analyzed, indicating that these macromolecules can contribute to inflammation increment. We have also demonstrated the antioxidant capacity of HSE, which reduced ROS production in macrophages stimulated by AGEs. Data obtained show that polyphenols in hazelnut skin have protective effect in macrophages following AGEs stimulation. The use of natural bioactive molecules could represent an interesting new therapeutic strategy with positive effect on human health.

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References

- [1] W-J. Yeh, S-M. Hsia, W-H. Lee, C-H. Wu, *Journal of Food and Drug Analysis*, **2017**, 25, 84.
- [2] J. Chaudhuri, Y. Bains, S. Guha, A. Kahn, D. Hall, N. Bose, A. Gugliucci, P. Kapahi, *Cell Metabolism*, **2018**, 28, 337.
- [3] D. Hemmler, C. Roullier-Gall, J.W. Marshall, M. Rychlik, A.J. Taylor, P. Schmitt-Kopplin, *Scientific Reports*, **2017**, 7, 3227.
- [4] C. Alasalvar, B.W. Bolling, *British Journal of Nutrition*, **2015**, 113, S68.

Quality and nutritional profile determination of the hemp enriched functional pasta

Sonia Bonacci¹, Vita Di Stefano², Fabiola Sciacca³, Carla Buzzanca², Nino Virzì³, Antonio Procopio¹, Maria Grazia Melilli⁴

¹Department of Health Sciences, University Magna Græcia of Catanzaro, Italy

²Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

³CREA - Council for Agricultural Research and Economics –

Research Centre for Cereal and Industrial Crops of Acireale (CT), Italy

⁴National Council of Research, Institute of BioEconomy (CNR-IBE), Catania, Italy

s.bonacci@unicz.it

Functional foods are formulated to contain healthy components, which when consumed daily as part of the diet, can have beneficial health effects. Hemp seeds are gaining in the market with a growing interest in their usage for human nutrition as an excellent source of nutrients. In this study, chemical qualities and antioxidant capacity of fortified pasta samples, obtained by using a durum wheat cultivar "Ciclope" and fortified with different percentages of hemp flour (5%, 7.5% and 10%) of the cultivar "Futura 75", were evaluated. The influence of semolina replacement with two different hemp flours was evaluated highlighting the effect on chemical and nutritional characteristics of cooked pasta. The phenolic profile of hemp flour and pasta was investigated through an untargeted metabolomics-based approach using HPLC-ESI/QTOF-MS method. The antiradical activity of samples (flours and fortified pasta) was measured by using the DPPH assay. Cannabisin C, hydroxycinnamic and protocatechuic acids were the most abundant phenolic compounds flour samples. Total polyphenolic content in hemp flour was quantified in the range of 6.38 ± 0.002 - 6.35 ± 0.001 mg GAE/g and free radical-scavenging included in the range of 3.94 ± 0.0178 - 3.75 ± 0.0179 mmol TEAC/100g. Aminoacid contents were studied in raw materials and in pasta samples and isoleucine, glutamine, tyrosine, proline and lysine were the most abundant. Although the hemp seeds have been previously subjected to extraction of the oil, hemp flours retain oil percentages of around 8%; their lipid profile was evaluated by GC-MS analysis and the fatty acids present in largest amount were linoleic acid and α -linolenic acid. Minerals characterization was also performed. Data showed that macro and trace elements concentration increase according to fortification percentage. The present study concluded that the incorporation of hemp flours, with different particle size at various level of substitution, modulates the pasting and functional properties of pasta and improves nutritional and phytochemical profile. The addition of hemp flour into pasta effectively enhanced the antiradical potential to contribute prevention of chronic diseases related to oxidative stress. Hemp supplementation could be a potential option to produce high quality, nutritionally rich low-cost pasta with good organoleptic properties.

References

S. Bonacci, V. Di Stefano, F. Sciacca, C. Buzzanca, N. Virzì, S. Argento, M.G. Melilli, *Foods*, **2023**, *12*, 774.

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Ageing and production discrimination of PDO Grana Padano cheese with an NMR-based metabolomic approach

Valentina Maestrello^{1,2}, Pavel Solovyev¹, Pietro Franceschi¹, Angelo Stroppa³,
Federica Camin², Luana Bontempo¹

¹Fondazione Edmund Mach (FEM), via E. Mach 1, 38098, San Michele all'Adige (TN), Italy

²Center Agriculture Food Environment (C3A), University of Trento, Via Mach 1,
38098 San Michele all'Adige, (TN), Italy

³Consorzio Tutela Grana Padano, Via XXIV Giugno 8, 25010 San Martino Della Battaglia,
Desenzano del Garda (BS), Italy
valentina.maestrello@unitn.it

PDO Grana Padano cheese is one of the most renowned cheeses and because of its high nutritional values, the interest of consumers in it has been increased. It is a high-quality Italian product, which needs to be protected from possible imitations of lower-quality and, to preserve the high-quality standards, strict production specification rules need to be followed. In any case, a new step in the production process is under evaluation, that is, the bactofugation step. It is a centrifugation step which removes spores from milk, but it could affect the constituents of milk and thus, modify the final product. In this study the profile of PDO Grana Padano cheese produced with and without this step was studied with NMR spectroscopy, finding differences in the composition, supported by the statistical analysis. Another important aspect of Grana Padano is its ageing, which is one of the factors influencing its price: the higher the ageing, higher the price. In order to prevent mislabeling, the profile of various samples of Grana Padano with different ageing time were studied using an untargeted approach. After the analysis with ¹H NMR spectroscopy, multivariate statistical analysis was applied to identify the most discriminant compounds of the different ageing times, which, with further studies, could be used as potential markers of the ageing authenticity of Grana Padano.

References

A. Summer, P. Formaggioni, P. Franceschi, F. Di Frangia, F. Righi, M. Malacarne, *Food Technology and Biotechnology*, **2017**, *55*, 277.

Understanding the chemistry of the Sicilian Amarena wine during bottle aging

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

Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy
aalbergamo@unime.it

Traditional alcoholic beverages, such as distillates, liquors, aromatized and fortified wines, have always been part of the Mediterranean culture and, lately, they have been re-evaluated to valorize both the territory and local customs [1]. In Italy, most of the traditional beverages own an added value, as they are in the list of Traditional Agri-food Products (TAPs) set by the Italian Ministry of Agriculture, Food and Forestry [2]. Among others, Sicilian “Amarena” is a fortified wine prepared during the 1950s by rural communities from Valle del Nisi (province of Messina) through a peculiar and timeless ritual based on the use of local unselected red grapes and leaves of sour cherry. Given the scarce literature on TAP beverages [3,4], this study aims to explore the physicochemical, compositional, and sensory traits of Amarena wine aged the first year in an oak barrel and subsequently in bottles up to 25 years. Although production and aging of renowned fortified wines from the Mediterranean area, such as the “Fino” Sherry and the “Madeira”, typically occurs according to strict specifications, the proximity of the traditional Amarena beverage to these wines was also evaluated.

Amarena wine during aging was characterized by downward trends in the alcoholic strength, total dry extract, and total sugars. Similarly, the highest total phenol and total anthocyanin contents were recorded during the first years of aging and then decreased, reaching the lowest values in wines aged up to 25 years. Conversely, acetaldehyde, ethyl acetate and methanol increased during the aging of Amarena wine, along with fusel alcohols and furanic compounds, with conflicting effects on the aroma and its complexity in older wines. The level of minerals (i.e., Na, Mg and K) and trace elements (i.e., Mn, Fe, Cu, and Zn) also showed an upward trend with advancing bottle aging.

Younger wines were characterized by a purplish-red hue and a scarce luminosity which turned to a marked brownish-red hue and a greater transparency and brightness in older bottles. Fruity and floral odors and flavors marked younger beverages, while dried fruity, nutty, and spicy notes were displayed by older products both on the nose and palate. Based on the evolution of oenological parameters, the Sicilian Amarena showed similarities with the “Fino” Sherry aged according to the *Criaderas y Solera* dynamic system [5]. On a par with Sherry and Madeira wines studied during aging, acetaldehyde, ethyl acetate and methanol could be considered as aging markers also in Amarena wines [6]. Additionally, from a sensory perspective, older Amarena beverages were similar to the aged Sherry wine due to the perception of the valuable attribute of “oxidized” [6].

In the broader perspective of the valorization of traditional beverages, this study may encourage the production and commercialization of Amarena wine, thus, preserving the traditional ecological knowledge and cultural diversity of the Mediterranean area.

References

- [1] R. Motti, G. Bonanomi, B. de Falco, *European Food Research & Technology*, **2022**, 248, 1089.
- [2] Italian Ministry of Agriculture and Forestry, *Gazzetta Ufficiale della Repubblica Italiana*, **2000**, 194, 5.
- [3] F. Fratianni, A. De Giulio, A. Sada, F. Nazzaro, *Journal of Medicinal Food*, **2012**, 15, 18.
- [4] M. Pisani, P. Astolfi, S. Sabbatini, P. Carloni, *Foods*, **2021**, 10, 1953.
- [5] J. Moreno-García, R.M. Raposo, J. Moreno, *Food Research International*, **2013**, 54, 285.
- [6] J.A. Moreno, L. Zea, L. Moyano, M. Medina, *Food Control*, **2005**, 16, 333.

Direct injection mass spectrometry for food volatilomics: emerging green approaches for the rapid and online screening of microbial resources

Iuliia Khomenko¹, Vittorio Capozzi², Antonia Corvino^{1,3}, Franco Biasioli¹

¹Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

²Institute of Sciences of Food Production, National Research Council, Foggia (FG), Italy

³Center for Agriculture Food Environment C3A, University of Trento, S. Michele all'Adige (TN), Italy

iuliia.khomenko@fmach.it

Direct injection mass spectrometry (DIMS) techniques for volatile organic compounds (VOCs) form a subset within the field of mass spectrometry (MS) targeting real-time, online and non-destructive monitoring according to green analytical approaches [1]. These techniques are based on the direct injection of headspace with no sample treatment, VOCs extraction, or chromatographic separation. DIMS analytical approaches are versatile tools for understanding VOCs release in agri-food applications, combining low-cost analytical strategies, fast sample processing and good analytical performances. Proton Transfer Reaction Mass Spectrometry (PTR-MS) as a model of DIMS strategies provides interesting applications in the whole food chain, from high-throughput sample screening to process monitoring. In particular, PTR-MS suits for real-time monitoring of food fermentations, massive screening of microbial resources and fermented products, assisting food safety and quality assessment [2].

In this presentation, we would like to present three case studies as examples of our experience in online monitoring of: i) microbial volatilome, ii) alcoholic fermentation in wine with different combinations of *Saccharomyces*/non-*Saccharomyces* [3], and iii) kefir-like cereal-based beverages using water and milk kefir starter cultures and a selected LAB strain [4]. Moreover, we would like to discuss the future perspectives in this field, like evaluation of the effect of selected biocontrol strains on the volatilome of target food matrices, VOCs analysis to evaluate the flavouring potential of microbial resources for food quality/safety improvement, and other applications of PTR-MS useful for accelerating innovation in the fermented products sector. Finally, we will underline the potential of rapid DIMS analysis with simultaneous portable sensors for better development and tuning of new industrial screening tools.

References

- [1] M. Mazzucotelli, B. Farneti, I. Khomenko, K. Gonzalez-Estanol, M. Pedrotti, M. Fragasso, V. Capozzi, F. Biasioli, *Green Analytical Chemistry*, **2022**, *3*, 100041.
- [2] A. Romano, V. Capozzi, G. Spano, F. Biasioli, *Applied Microbiology and Biotechnology*, **2015**, *99*, 3787.
- [3] C. Berbegal, I. Khomenko, P. Russo, G. Spano, M. Fragasso, F. Biasioli, V. Capozzi, *Fermentation*, **2020**, *6*, 55.
- [4] A. Yépez, P. Russo, G. Spano, I. Khomenko, F. Biasioli, V. Capozzi, R. Aznar, *Food Microbiology*, **2019**, *77*, 61.

Food chemistry as a useful tool for botanical taxonomy: coffee diterpenes as molecular markers

Elena Guercia, Paola Crisafulli, Silvia Colomban, Luciano Navarini

Illycaffè S.p.A., Via Flavia 110, 34147, Trieste, Italy
elena.guercia@illy.com

Coffee diterpenes, the major components of the unsaponifiable fraction of coffee oil, have always played an important role for the authenticity and the traceability of coffee products. Indeed, the content of coffee diterpenes such as cafestol, kahweol and 16-O-methylcafestol (16OMC) depends mainly on both botanical species and geographical origin. Specifically, 16OMC, for many years has been considered the ideal molecular marker for reliably detecting Robusta (*Coffea canephora*) in Arabica (*Coffea arabica*) coffee blends, as it was found only in Robusta coffee beans. However, quite recently, the Arabica diterpenes profile was revised and thanks to nuclear magnetic resonance combined with proper sample preparation the detection of very low levels of 16-O-methylated diterpenes was achieved [1]. For the quali-quantitative determination of extremely low levels of these compounds in Arabica green coffee, a new method via ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) was optimized and validated in our lab [2]. These findings, combined with the fragmentary and scarce information reported in the literature on botanical species other than Robusta and Arabica, led us to explore the potential of diterpenes as a useful tool for the discrimination of other coffee species. A very recent study, by characterizing diterpene profile of several seed samples from a single geographical origin (Reunion Island), added a piece of information on *Coffea liberica* varieties systematic [3]. *Coffea liberica*, the third commercially exploited coffee species, has not been extensively investigated and prior to the aforementioned study, very scarce data were available in the literature to clearly discriminate its varieties. We believe that diterpenes, not yet fully exploited from the chemo-taxonomical point of view, may also provide useful information on coffee species that are not currently of commercial interest, such as *C. pseudozanguebariae*. Further studies will be conducted.

References

- [1] Y. Gunning, M. Defomez, A.D. Watson, N. Beadmen, I.J. Colquhoun, G. Le Gall, M. Philo, H. Garwood, D. Williamson, A.P. Davis, E.K. Kemsley, *Food Chemistry*, **2018**, 248, 52.
- [2] E. Guercia, S. Colomban, L. Navarini, *Journal of Mass Spectrometry*, **2020**, 55, e4636.
- [3] P. Crisafulli, E. Guercia, L. Navarini, *Tropical Plant Biology*, **2022**, 15, 247.

C14

Characterization of color, phenolic profile, and antioxidant activity of Italian pigmented rice varieties after different technological treatments

Corinne Bani¹, Francesca Colombo¹, Carola Cappa², Francesca Mercogliano¹,
Patrizia Restani^{1,3}, Chiara Di Lorenzo¹

¹Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milano, Italy

²Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Italy

³Coordinating Research Center “Innovation for Well-Being and Environment” (CRC),
Università degli Studi di Milano, Italy

corinne.bani@unimi.it

Pigmented varieties are rich in different bioactive compounds, including anthocyanins, proanthocyanidins, carotenoids, and phenolic acid, with known antioxidant and anti-inflammatory activity [1,2,3]. Therefore, their consumption could exert beneficial effects on the health of the general population and in particular on that of people suffering from some chronic diseases such as celiac disease. Different pigmented rice varieties are present on Italian market; they are commercialized as brown rice or as a product after a technological treatment (e.g., parboiled, milling). Pigmented rice is commonly consumed as brown rice, but technological treatments could bring some advantages for both producers and consumers (higher milling yield, shorter cooking time, and longer shelf-life) [4] and have positive effects on rice nutritional values in terms of starch availability, glycemic index and/or vitamins and mineral contents. The objective of this study was the characterization of the samples in term of phenolic content and antioxidant capacity tested *in vitro*. In parallel the impact of two technological treatments (e.g., milling and parboiling) on their phytochemical composition was evaluated. Four pigmented (purple, black, orange, red) and one non-pigmented samples of Italian rice varieties, as such brown or processed with different technological treatments (parboiled and milled) were included in the study. Different spectrophotometric and chromatographic *in vitro* methods were applied: 1) Folin–Ciocalteu assay, 2) DPPH assay, 3) pH differential method, 4) High-Performance Thin Layer Chromatography (HPTLC), 5) HPLC-DAD method. According to our results, pigmented varieties represent a source of soluble polyphenols and anthocyanins with high antioxidant activity when compared to the non-pigmented ones. However, both technological processes affected the concentration of phenolic compounds and the relative antioxidant property. Milling reduced specifically the content of phenolic compounds and the antioxidant activity, while parboiling affected mainly the presence of anthocyanins.

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References

- [1] P.A. Rodríguez-Salinas, F. Zavala-García, V. Urías-Orona, D. Muy-Rangel, J.B. Heredia, G. Niño-Medina, *Arabian Journal for Science and Engineering*, **2020**, *45*, 95.
- [2] C. Di Lorenzo, F. Colombo, S. Biella, C. Stockley, P. Restani, *Nutrients*, **2021**, *13*, 273.
- [3] S. Piazza, F. Colombo, C. Bani, M. Fumagalli, O. Vincentini, E. Sangiovanni, G. Martinelli, S. Biella, M. Silano, P. Restani, M. Dell’Agli, C. Di Lorenzo, *Foods*, **2023**, *12*, 63.
- [4] B. Min, A. McClung, M.H. Chen, *Food Chemistry*, **2014**, *159*, 106.

C15

Artemide pigmented rice: impact of cooking on chemical composition, nutritional profile and bioaccessibility of phenolic compounds

Antonio Colasanto¹, Marco Arlorio¹, Fabiano Travaglia¹, Matteo Bordiga¹, Vincenzo Disca¹, Yassine Jaouhari¹, Jean Daniel Coïsson¹, Ivana Rabbone², Monica Locatelli¹

¹Dipartimento di Scienze del Farmaco - Food Chemistry, Biotechnology and Nutrition Unit – Università del Piemonte Orientale "A. Avogadro", Largo Donegani 2, 28100 Novara, Italia

²Dipartimento di Scienze della Salute - Università del Piemonte Orientale "A. Avogadro",
Via Solaroli 17, 28100 Novara, Italia

marco.arlorio@uniupo.it

Rice (*Oryza sativa* L.) is among the most highly consumed food worldwide. Even if the white type is usually consumed, the interest in pigmented varieties and its consumption have recently grown in Western countries. The black rice varieties can be considered a sort of “natural” functional food, due to their significant content of polyphenols, especially anthocyanins, and their antioxidant properties. Polyphenols are a huge heterogeneous group of molecules belonging to different chemical classes able to exert a beneficial action on the human organism, which depends not only on their quantity present in the diet, but also on their bioaccessibility and bioavailability. The impact of technological processing, but particularly the impact of cooking and digestive process could modify these healthy properties, modulating the bioaccessibility and bioavailability of nutrients and bioactive compounds [1,2].

Belonging the pigmented Italian varieties, the Artemide rice derives from the combination of Venere (medium grain, black pericarp) and Indica rice varieties (long, narrow grain and white pericarp). Artemide rice is aromatic and black in colour, having an intense and pleasant aroma.

The principal aim of this research was the evaluation of the impact of traditional (boiling, microwaves oven, under pressure pot, pilaf and risotto preparation) and alternative (“sous vide”) cooking methods on the chemical and nutritional composition of Italian Artemide black rice. The “sous vide” mode performed at 89 °C and the risotto mode resulted as the more efficient cooking methods to preserve anthocyanins, total polyphenols content and antioxidant capacity. The impact of the digestive process on the polyphenolic fraction of Artemide black rice, subjected to the risotto preparation, has been also evaluated. Our results allowed to highlight the main changes in the polyphenolic composition of Artemide black rice after cooking and following the simulated *in vitro* digestion. We also evaluated the impact of Artemide rice on the postprandial glycaemic trend in type 1 diabetic children and adolescents, comparing with that obtained eating the white variety “Gigante Vercelli”. Finally, a faecal *in vitro* batch fermentation of cooked and digested black and white rice varieties was performed, in order to evaluate their prebiotic activity and the rice impact on a healthy microbiota, through the quantification via GC-FID of SCFAs (Short Chain Fatty Acids) as desired indicators of the microbiota metabolism.

These studies confirmed a significant impact of cooking methods on nutrients and minor compounds of Artemide black rice, allowing to open new perspectives about the preservation of the antioxidant capacity in this commodity.

References

[1] D. Fracassetti, C. Pozzoli, S. Vitalini, A. Tirelli, M. Iriti, *Foods*, **2020**, *9*, 967.

[2] A. Colasanto, F. Travaglia, M. Bordiga, S. Monteduro, M. Arlorio, J.D. Coïsson, M. Locatelli, *Foods*, **2021**, *10*, 824.

Chemical characterization and biological activities of *Rhus coriaria* L. extract: antioxidant, antiglycation and dpp4 inhibitory effects

Laura Dugo¹, Elisa Pannucci¹, Ludovica Spagnuolo¹, Luca Santi²

¹Department of Science and Technology for Sustainable Development and One Health, University Campus Bio-Medico of Rome, Via Alvaro del Portillo 21, 00128 Roma, Italy

²Department of Agriculture and Forest Sciences, University of Tuscia,
Via San Camillo de Lellis snc, 01100 Viterbo, Italy

l.dugo@unicampus.it

Rhus coriaria L., commonly known as “sumac”, belongs to Anacardiaceae family. It is a wild edible plant growing in tropical and temperate regions worldwide. Several research highlighted the health properties of sumac drupes including antioxidant, antidiabetic, and anti-inflammatory activities; indeed, drupes are rich in phytochemicals which are responsible of the biological properties [1]. In this work a phytochemical characterization of an extract obtained from sumac drupes was carried out through HILICxRP-LC-PDA-ESI/MS. Furthermore, the total phenolic content (TPC) was determined using the Folin-Ciocalteu method (174,24 mgGAE/g) [2]. Antioxidant activity, one of the most prominent biological properties associated with sumac fruit, was explored through ORAC (225.836,14 $\mu\text{mol TE}/100\text{ g}$) [2], DPPH and ABTS (DPPH-IC₅₀: 0,41 mg/ml; ABTS-IC₅₀: 0,21 mg/ml) assays [3] and, also, ascorbate and glutathione levels were measured. Moreover, the inhibitory effects on DPP-IV, an enzyme secreted after a meal that degrades incretins, were evaluated by an *in vitro* enzyme activity inhibition assay. Results indicate a broad inhibition activity on DPP-IV, comparable with the sitagliptin reference compound. Besides, since AGEs and ROS formation has been considered as risk factors in the pathogenesis of diet-related disorders such as diabetes and insulin resistance, antiglycation effects of sumac extract were evaluated using the BSA-MGO model and the measurement of intracellular ROS generation was evaluated in THP-1 cell line through the H₂DCF-DA assay. Results indicate the ability of sumac extract to inhibit the *in vitro* AGEs formation in a dose-dependent behaviour and to inhibit ROS production in oxidative-stressed cells. In conclusion, results suggest that sumac extract has a marked antioxidant activity, can inhibit DPP-IV enzyme activity, can inhibit AGEs formation, and can protect cells from the oxidative stress. The overall results suggest that sumac extract represents a promising natural source for developing nutraceuticals or dietary supplements with antioxidant activity and boost its potential use in the food, nutraceutical, and pharmaceutical industries. Moreover, although it is still early to suggest the use of sumac in diabetes, this work would lay the basis to encourage further studies on antidiabetic activities of sumac.

References

- [1] H. Alsamri, K. Athamneh, G. Pintus, A. H. Eid, R. Iratni, *Antioxidants*, **2021**, *10*, 73.
- [2] K. Arena, E. Trovato, F. Cacciola, L. Spagnuolo, E. Pannucci, P. Guarnaccia, L. Santi, P. Dugo, L. Mondello, L. Dugo, *Molecules*, **2022**, *27*, 1727.
- [3] C. Isgrò, L. Spagnuolo, E. Pannucci, L. Mondello, L. Santi, L. Dugo, A.M. Sardanelli, *International Journal of Molecular Sciences*, **2022**, *23*, 12774.

A new millifluidic-based gastrointestinal platform to investigate antiglycative agents

Raffaella Colombo, Ilaria Frosi, Adele Papetti

Department of Drug Sciences, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy
raffaella.colombo@unipv.it

Methylglyoxal (MGO) is a highly reactive α -oxoaldehyde, produced both in food and endogenously in human organism; it is involved in the interaction with proteins and nucleic acids with the formation of toxic products, namely advanced glycation end products (AGEs). MGO and AGEs are potential glycotoxins in aging-correlated chronic diseases (diabetes, cardiovascular diseases, and neurological disorders), and the identification of antiglycative agents able to interfere with them became mandatory [1,2].

Many food-derived antiglycative agents have been reported in literature, but to investigate their bioaccessibility is fundamental to understand how the gastrointestinal process can affect their activity. Nowadays, *in vitro* static methods represent simple, rapid, and less expensive approaches to perform digestion studies, but their limits are well known. In fact, they are not able to reproduce some key factors of the digestion process, such as motility, dynamic circulation (physical/shear forces and mixing), and changes in biochemical parameters (hydration, pH, and electrolyte composition at different gastrointestinal tracts) [3]. Therefore, the search for dynamic platforms combined with cell cultures is very important to ensure models able to simulate a dynamic gastrointestinal system closer to *in vivo* models [4].

Our previous research investigated the effect of digestion on dietary MGO intakes, by setting-up a new dynamic multi-organ model (using LiveFlow® bioreactor) able to mimic gastric and intestinal absorption with two cellular models (human gastric stromal cells-GIST-882 and intestinal cancer cells-Caco-2). The results suggested that this new platform could be useful to study kinetic/metabolic profiles of different molecules, representing a very promising alternative to animal models, at least in preliminary investigations [5]. Recently, we applied this new gastrointestinal system to investigate the bioaccessibility of 5-caffeoylquinic acid (5-CQA), a food-derived antiglycative agent. In fact, 5-CQA, is a phenolic compound with a well-known trapping activity against MGO, thus inhibiting AGEs formation [2,6]. MGO and 5-CQA were co-digested and their concentrations at the different steps of digestion were monitored by a validated RP-HPLC-DAD method. Cell viability tests were also performed over time and the absorption/metabolization of the two compounds was compared with that obtained by using a static approach such as the InfoGest protocol, which received an international consensus.

References

- [1] A. Papetti, D. Mascherpa, G. Gazzani, *Food Chemistry*, **2014**, *164*, 259.
- [2] M. Maietta, R. Colombo, R. Lavecchia, M. Sorrenti, A. Zuurro, A. Papetti, *Food Research International*, **2017**, *100*, 780.
- [3] R. Colombo, L. Ferron, I. Frosi, A. Papetti, *Food & Function*, **2021**, *12*, 7619.
- [4] D. Mazzei, S. Giusti, T. Sbrana, A. Ahluwalia, *Bioreactors: Design, Properties and Applications*, Nova Science Publishers, Inc, **2011**, New York, 159.
- [5] R. Colombo, M. Paolillo, A. Papetti, *Food & Function*, **2019**, *10*, 4330.
- [6] R. Colombo, M. Paolillo, I. Frosi, L. Ferron, A. Papetti, *Food & Function*, **2023**, *14*, 541.

Polyphenols in livestock nutrition: the case of renewable *Fagaceae* leaves

Marialuisa Formato¹, Alessandro Vastolo², Simona Piccolella¹, Serena Calabrò²,
Monica Isabella Cutrignelli², Christian Zidorn³, Severina Pacifico¹

¹Dept of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania ‘Luigi Vanvitelli’, Via Vivaldi 43, 81100 Caserta, Italy; ²Dept of Veterinary Medicine and Animal Production, University of Naples Federico II, Via Federico Delpino 1, 80137 Napoli, Italy; ³Pharmazeutisches Institut, Abteilung Pharmazeutische Biologie, Christian-Albrechts-Universität zu Kiel, Gutenbergstraße 76, 24118 Kiel, Germany
marialuisa.formato@unicampania.it

Population growth and increased demand for food considerably augmented global livestock production, which was also massively affected through lifestyle changes. This led over time to an increase in diseases to which the animals are exposed, with impairment of the quality of the products brought to the consumers’ tables. In this context, the improvement of livestock nutrition is becoming a prerogative for producers and veterinarians in order to guarantee both well-being and product quality. Thus, alternative feedstuffs are a need to be explored for improving animal production since the banning of feed antibiotics by the EU in 2006. The innovative approach aimed at formulating animal feeds/supplements based on naturally occurring bioactive components with antimicrobial, antioxidant, anti-inflammatory and antitumor nutritional properties found wider consensus. Plants, dried or in the form of their extracts, or also pure specialised compounds, appear to be valuable candidates as innovative alternatives to preserve livestock and their productivity, also taking into account the renewed awareness of consumers for natural healthy foods [1]. Actually, also agri-food wastes or those from forest biomass biorefining are valid but still too unexplored resources.

Bioactive compounds, mainly polyphenols, appear valuable candidates as innovative alternatives to preserve livestock and its productivity with effects on ruminal fermentation, feed digestion, health, milk yield and chemical composition, with the main focus on the fatty acid profile. Although the scientific literature of the last twenty years is rich in studies concerning the effects of polyphenols on *in vitro* fermentation processes, there is still little attention regarding the qualitative-quantitative composition of the tested extracts and the possible synergistic effects of the various classes of compounds (i.e., flavonoids, tannins, hydroxycinnamic acids). In this context, leaves of *Fagus sylvatica* L., *Castanea sativa* Mill. and *Quercus robur* L., whose alcoholic extracts underwent fractionation obtaining two organic fractions differently enriched in bioactives, were of interest [2-4]. To confirm this, all fractions were preliminarily screened for their total phenol (TFC), flavonoid (TFC), lipid (TLC) and condensed tannin (TCT) content as well as for their antioxidant capability by means of DPPH• and ABTS•⁺ tests, and ferricyanide FRAP assay. Then, a deeper chemical investigation of the fractions was carried out through UV-Vis spectroscopy and UHPLC-HRMS/MS, proving a qualitative-quantitative difference between the three species taken into consideration. All the differently chemically constituted fractions were tested to evaluate their effects on *in vitro* ruminal fermentation (cumulative gas production; organic matter degradability; and end products, i.e., pH, volatile fatty acids, branched-chain fatty acid proportion and acetate/propionate ratio). The chemical diversity has led to different intra- and inter-species findings on the *in vitro* fermentation process. Dose-dependent effects were also recorded, in terms of fermentation rate and gas production.

References

- [1] M. Formato, G. Cimmino, N. Brahmi-Chendouh, S. Piccolella, S. Pacifico, *Molecules*, **2022**, *27*, 7752.
- [2] M. Formato, S. Piccolella, C. Zidorn, A. Vastolo, S. Calabrò, M.I. Cutrignelli, S. Pacifico, *Molecules*, **2022**, *27*, 2217.
- [3] M. Formato, A. Vastolo, S. Piccolella, S. Calabrò, M.I. Cutrignelli, C. Zidorn, S. Pacifico, *Molecules*, **2022**, *27*, 8662.
- [4] M. Formato, A. Vastolo, S. Piccolella, S. Calabrò, M.I. Cutrignelli, C. Zidorn, S. Pacifico, *Antioxidants*, **2022**, *11*, 2366.

Thermal degradation kinetics of red cabbage (*Brassica oleracea* L. var *capitata* f. *rubra*) anthocyanins

Laura De Marchi¹, Laura Salemi¹, Maria Bellumori², Federica Mainente¹, Ilaria Fierri¹,
Roberto Chingola¹, Gianni Zoccatelli¹

¹Dept of Biotechnology, University of Verona, Stara del Grazie 15, Verona, Italy

²NEUROFARBA Dept, University of Firenze, Via Ugo Schiff 6, Sesto Fiorentino, Firenze, Italy
gianni.zoccatelli@univr.it

Anthocyanins (ACNs) are flavonoids characterised by red colour and high solubility in water expressed in several fruits and vegetables. They are frequently used as natural pigments for food applications, though their importance in the last years increased due to their potent antioxidant and anti-inflammatory properties. These characteristics are believed to be the rationale for the capacity of ACNs to counteract degenerative diseases like type-2 diabetes, cardiovascular pathologies and cancer [1]. Red cabbage (*Brassica oleracea* L. var *capitata* f. *rubra*, RC) is acknowledged as one of the most important sources of ACNs. These are almost entirely represented by glycosylated cyanidins, most of which (generally >80%) are acylated with hydroxycinnamic acids (HCAs). Acylated ACNs (AACNs) are known to possess higher stability than ACNs, especially towards basic pH, due to their capacity of π -stacking of acyl groups with the pyrilium group (also named intramolecular co-pigmentation), which reduces the nucleophile attack of water and the subsequent formation of a pseudobase or a chalcone [2]. However, data about their thermal stability are controversial since Wiczkowski et al. [3] showed that the stewing process of RC leads to a higher degradation of AACNs than non-acylated ACNs. We aimed to study the thermal stability of ACNs, total phenolic content (TPC, by Folin Ciocalteu assays), and the antioxidant capacity (by ABTS and FRAP assays) of a model RC water extract (pH 3.3) subjected to an accelerated storage test (40°C x 30 days). HPLC-DAD-ESI-MS served to identify the single ACNs and to study their degradation kinetics. Free ACNs analysed by differential pH method showed a 1st-order degradation kinetics as described for other sources like cherry [4], with a $t_{1/2}$ of 17.4 days. On the contrary, the behaviour of TPC, ABTS and FRAP did not obey the same kinetics, showing time-dependent oscillations, especially in the case of FRAP. The trends are probably caused by the accumulation of degradation products characterised by antioxidant capacity during incubation. Kinetics study of the single molecules separated by HPLC (520 nm) confirmed a 1st order degradation kinetics for all ACNs ($R^2 > 0.988$, $P < 0.01$), even though different stability was observed depending on the bound HCAs ($t_{1/2}$: from 13.3 to 32.7 days). In contrast to previous results [2], some AACNs showed a faster degradation than the non-acylated form. In particular, as previously reported [3], mono- and di-acylated sinapoyl- AACNs were more susceptible than those bound to p-cumaric and ferulic acids.

The results suggest that the degradation pathway of RC ACNs/AACNs follows first an oxidation/cleavage of the flavonoid core instead of deacylation or deglycosylation steps since mono-acylated and non-acylated forms did not show fluctuations during storage nor cyanidin aglycone was detectable. The higher antioxidant capacity of sinapic acid could help explain the rapid oxidation of anthocyanins bound with this HCA. Indeed, its faster reactivity could lead to the formation of AACNs bound to oxidised sinapoyl moieties unable to adequately protect the flavonoid core by π -stacking.

References

- [1] A. Durazzo, M. Lucarini, E. B. Souto, C. Cicala, E. Caiazzo, A. Izzo, E. Novellino, A. Santini, *Phytotherapy Research*, **2019**, 33, 2221.
- [2] M. Moloney, R. J. Robbins, T. M. Collins, T. Kondo, K. Yoshida, O. Dangles, *Dyes and Pigments*, **2018**, 158, 342.
- [3] W. Wiczkowski, D. Szawara-Nowak, J. Topolska, *Food Chemistry*, **2015**, 167, 15.
- [4] J. Chen, J. Dua, M. Lia, C. Lia, *LWT*, **2020**, 128, 109448.

The potential of common duckweed (*Lemna minor* L.) as a meat extender during storage of packaged beef burgers

Gabriele Rocchetti¹, Annalisa Rebecchi¹, Leilei Zhang¹, Michele Dallolio², Daniele Del Buono³,
Giorgio Freschi⁴, Luigi Lucini¹

¹Università Cattolica del Sacro Cuore, Piacenza and Cremona, Italy

²General Partner G.D. Food&Technology sas, Castelluccio, Italy

³Università degli Studi di Perugia, Italy

⁴Agro Unit, Clever Bioscience srl, Pavia, Italy

gabriele.rocchetti@unicatt.it

In this work, beef burgers were formulated with an antioxidants-free control (CON), 1 g/kg sodium ascorbate (ASC), and three different levels of a duckweed extract (DE), i.e., 1, 5, and 10 g/kg, packaged under modified atmosphere (66% O₂, 25% CO₂, and 9% N₂) and stored at 4°C for 19 days. In the last years, the duckweed nutrient content and metabolite composition is gaining extensive attention, particularly in the fields of animal feed industry, aquaculture, health supplement, biofertilizer, biofuel, and as emerging food product for humans [1]. At the European level, the EFSA Panel on Nutrition, Novel Foods and Food Allergens stated that the duckweed powder is not nutritionally disadvantageous, being rich in proteins; however, more studies are necessary to exclude safety concern due to its manganese content. Given this background information, in this study, a metabolomics approach (based on UHPLC-Orbitrap mass spectrometry) followed by multivariate statistics, and coupled with microbiological and color analyses, were used to reveal the changes in meat quality and composition following the addition of DEs. These latter, previously evaluated by a response surface methodology study [2], were abundant in polyphenols and terpenoids, determining no issues with the hygienic status of the formulated burgers. Overall, DEs were ineffective in preserving linolenic acid from peroxidation, while after 19 days, the oxidative marker 2-nonenoic acid was down-accumulated in the DE10 sample. Coherently, a lower GSH:GSSG ratio imbalance was detected for DE10 sample. The GSH:GSSG ratio is an important bioindicator of cellular health, with a higher ratio indicating less oxidative stress [3]. Interestingly, the accumulation of the biomarker gamma-glutamyl semialdehyde (GGS) revealed the inefficiency of DEs in coping with protein oxidation during storage. The compound GGS is considered a suitable indicator of protein oxidation, accounting for up to 60% of the total carbonyl compounds in food systems [4]. However, DEs prevented the accumulation of biogenic amines arising from aromatic amino acids, likely due their antimicrobial effects. In light of the above, our findings suggested a potential pro-oxidant role and low chemical stability of DEs at the dosages applied, thus supporting further studies on their encapsulation.

References

- [1] N. Yahaya, N.H. Hamdan, A.R. Zabidi, A.M. Mohamad, M.K.H. Suhaimi, M.A.A.M. Johari, H.N. Yahya, H. Yahya, *Future Foods*, **2022**, *5*, 100128.
- [2] L. Zhang, G. Rocchetti, G. Zengin, D. Del Buono, M. Trevisan, L. Lucini, *Antioxidants*, **2023**, *12*, 313.
- [3] J. Lozano-Castellon, G. Rocchetti, A. Vallverdu-Queralt, F. Lucchini, G. Giuberti, X. Torrado-Prat, M. Illan, R.M. Lamuela-Raventos, L. Lucini, *Food Research International*, **2022**, *155*, 111030.
- [4] C. Guyon, A. Meynier, M. Lamballerie, *Trends in Food Science & Technology*, **2016**, *50*, 131.

Flow-biocatalysis for natural food bioactive and nutraceuticals

Martina Letizia Contente¹, Lucia Tamborini², Sabrina Dallavalle¹,
Francesco Molinari¹, Andrea Pinto¹

¹Dep. of Food, Environmental and Nutritional Science (DeFENS),
University of Milan, via Celoria 2, 20133, Milan, Italy

²Dep. of Pharmaceutical Sciences (DISFARM), University of Milan, via Mangiagalli 25, 20133, Milan, Italy
martina.contente@unimi.it

Preparation of food ingredients from natural substrates using biocatalysis is an appealing technique since the final product can be claimed as natural, but biocatalytic processes should be also characterized by high efficiency and productivity. Based on this idea three successful examples are reported:

i) **Preparation of aroma-compounds.** A straightforward one-step biocatalyzed synthesis of flavor-esters starting from primary alcohols and naturally available ethyl esters in water was accomplished firstly in batch [1], and subsequently in continuous mode employing the versatile and chemoselective acyltransferase from *Mycobacterium smegmatis* (MsAcT). Thanks to the improved stability obtained through immobilization techniques, MsAcT was incorporated in flow chemistry reactors where transesterification reactions occurred with very good yields (30->99%) and short residence time (5 min) even at high substrate concentrations (0.25 M) [2].

ii) **Flow synthesis of melatonin.** It was demonstrated that MsAcT has a very broad substrate scope and can be used for the preparation of *N*-acetyl derivatives in water starting from primary amines and using a variety of acyl donors [3]. Following the previous procedure an efficient, and sustainable enzymatic platform for the multi-gram synthesis of melatonin and its analogues was developed. The small packed-bed reactor (less than 2 mg of immobilized enzyme), was capable of handling high substrate loading (0.5M) in only 5 minute of residence time, and led to obtain excellent time-space-yield (up to 37 g/day). Here, aqueous phase and organic solutions have been recovered and reused, giving rise to a virtually zero waste reaction [4].

iii) **Hydrolysis of flavonoid glycosides.** Hesperidin (HES) and rutin (RT) represent widespread rutosyl flavonoids in citrus species both in fruits and their by-products which can be considered as an alternative natural source for their recovery. Although these compounds possess bioactive properties, much higher bioavailability is observed for aglycone [5]. Hydrolytic enzymes such as an α -rhamnosidase (RN) and an extremophilic β -glycosidase (HOR) have been co-immobilized on the same support to perform a one-pot two-step flow biotransformation for the direct obtainment of the corresponding aglycones from their glycosides with complete conversion in rapid residence time (5 min).

References

- [1] I. Chiarelli Perdomo, S. Gianolio, A. Pinto, D. Romano, M. L. Contente, F. Paradisi, F. Molinari, *Journal of Agricultural and Food Chemistry*, **2019**, 67, 6517.
- [2] M. L. Contente, L. Tamborini, F. Molinari, F. Paradisi, *Journal of Flow Chemistry*, **2020**, 10, 235.
- [3] M. L. Contente, A. Pinto, F. Molinari, F. Paradisi, *Advanced Synthesis and Catalysis*, **2018**, 360, 4814.
- [4] M. L. Contente, S. Farris, L. Tamborini, F. Molinari, F. Paradisi, *Green Chemistry*, **2019**, 21, 3263.
- [5] J. Xiao, *Critical Reviews in Food Science and Nutrition*, **2017**, 57, 1874.

Antioxidant and antiglycation effects of matcha green tea extracts

Elisa Pannucci¹, Luca Santi², Laura Dugo¹

¹Department of Science and Technology for Sustainable Development and One Health, University Campus Bio-Medico of Rome, Via Alvaro del Portillo 21, 00128 Roma, Italy

²Department of Agriculture and Forest Sciences, University of Tuscia,
Via San Camillo de Lellis snc, 01100 Viterbo, Italy
e.pannucci@unicampus.it

Oxidative stress and glucose metabolism alterations are significant risk factors for diabetes and its related complications. Diabetes is characterized by chronic hyperglycaemia. One of the consequences of hyperglycaemia is the excessive formation of advanced glycation end products (AGEs), which in turn generate ROS and contribute to diabetic complications; thus, great research efforts have been focused on the identification of AGEs inhibitors. Several data show that natural antioxidants represent a potential strategy to reduce AGEs formation [1]. Matcha green tea (*Camellia sinensis*) is a traditional Japanese tea particularly rich in bioactive compounds such as catechins [2, 3]. The aim of this research was to investigate the phytochemical composition of two types of matcha tea, the antioxidant potential, the antiglycation properties and the protective capacities, in THP-1 cell line, against oxidative stress. Matcha extracts (MEs) were prepared through an ultrasonic extraction procedure from two types of organic matcha tea: the grade 1, which derive only from the first harvest of the leaves, and the grade 4, which is a mix between sencha and matcha. The phytochemical profile was analysed through HPLC analysis, and the total phenolic content was determined by Folin–Ciocalteu method. The antioxidant potential was evaluated through ORAC and DPPH assays to investigate the nutraceutical relevance of MEs. Furthermore, the inhibitory activity against the formation of fluorescent AGEs in a BSA-MGO *in vitro* model was investigated. Finally, non-cytotoxic concentrations of MEs were utilized to evaluate intracellular ROS generation in THP-1 cell line through the H₂DCF-DA assay. Results obtained suggest that MEs have a broad antioxidant capacity and inhibit the *in vitro* AGEs formation in a concentration dependent way. Moreover, MEs were found to inhibit ROS production in oxidative-stressed cells. In conclusion, results suggest that MEs due to its antioxidant and antiglycative properties could be an interesting source of functional ingredients with a positive effect on human health, however, more studies are needed to understand the mechanism behind these activities.

References

- [1] D. Ramful, E. Tarnus, P. Rondeau, C. R. Da Silva, T. Baborun, E. Bourdon, *Journal of Agricultural and Food Chemistry*, **2010**, 58, 11119.
- [2] I. Sivanesan, J. Gopal, M. Muthu, S. Chun, J. W. Oh, *Applied Sciences*, **2021**, 1, 5087.
- [3] K. Jakubczyk, J. Kochman, A. Kwiatkowska, J. Kałduńska, K. Dec, D. Kawczuga, K. Janda, *Foods*, **2020**, 9, 483.

The most representative typical dish of Catanzaro as new Mediterranean Diet case study

Stefano Alcaro^{1,2,3,4}, Francesco Bianco⁴

¹Dipartimento di Scienze della Salute, Università Magna Græcia di Catanzaro, Viale Europa, 88100 Catanzaro (Italy)

²Net4Science srl, academic spinoff, Università Magna Græcia di Catanzaro, Viale Europa, 88100 Catanzaro (Italy)

³CRISEA - Centro di Ricerca e Servizi Avanzati per l'Innovazione Rurale, loc. Condoleo, 88056 Belcastro (CZ, Italy)

⁴Antica Congrega Tre Colli - No-profit Association, 88100 Catanzaro (Italy)

alcaro@unicz.it

The “Morzheddu” in local dialect of Catanzaro (“Morzello” in Italian language) is the official typical dish of the capital of Regione Calabria. It is a poor dish, relatively unknown at international level, that labels in extraordinary way the culinary identity of this City. Its tradition is not exactly determined. Historical evidence about its preparation were recovered starting from the Saracen domination, approximately between the end of the 9th and the first decades of the 10th centuries, probably related to the constitution of the “Principato Mussulmano in Calabria” founded in 906 and lasted beyond thirty years. After the discovery of the America, its preparation has been optimized and definitively fixed. Its recipe is, actually, based on veal, cow’s heart, tripe and lungs, tomato, peppers and hot peppers, ending up with a unique meat soup strictly based on the “fifth quarter”. Remarkably, no pork meet is used and, when all traditional ingredients are included in the complex and quite long preparation of this special dish, it can deserve to be named as “Illustrissimo” [1]. Another peculiar component always present when this meat soup is served is the “Pitta”, a special bread produced uniquely in Catanzaro too. Its etymology could be likely related to the Arabian tradition where the “Pita” bread is popular. Pitta’s round flat appearance resembles a sort of “bicycle wheel” and its typical rheological properties are definitively consequence of the double or triple levitation carried out to correctly prepare it.

In this communication an Illustrissimo’s story telling will be presented, pointing out the opportunity to study all peculiar properties of this unique dish in view of the circular economy related to the fifth quart [2], its food security [3], and the valorization of traditional Mediterranean Diet [4, 5].

References

[1] E. Zimatore, L’illustrissimo morzeddu, *Antica Congrega Tre Colli*, **2021**, Catanzaro.

[2] M. M. Henchion, A. P. Shirsath, *Journal of Cleaner Production*, **2022**, 280, 134845.

[3] P.O. Soladoye, M. Juárez, M. Estévez, Y. Fu, C. Álvarez, *Comprehensive Reviews in Food Science and Food Safety*, **2022**, 21, 1439.

[4] A. Maruca, R. Catalano, D. Bagetta, F. Mesiti, F.A. Ambrosio, I. Romeo, F. Moraca, R. Rocca, F. Ortuso, A. Artese, G. Costa, S. Alcaro, A. Lupia, *European Journal of Medicinal Chemistry*, **2019**, 181, 111579.

[5] D. Bagetta, A. Maruca, A. Lupia, F. Mesiti, R. Catalano, I. Romeo, F. Moraca, F.A. Ambrosio, G. Costa, A. Artese, F. Ortuso, S. Alcaro, R. Rocca, *European Journal of Medicinal Chemistry*, **2020**, 186, 111903.

Quality and authenticity of *Vaccinium corymbosum* L.: a combined chemical and molecular approach

Manuel Martoccia, Valeria Fochi, Fabiano Travaglia, Monica Locatelli, Matteo Bordiga, Jean Daniel Coisson, Marco Arlorio

Dipartimento di Scienze del Farmaco - Food Chemistry, Biotechnology and Nutrition Unit,
Università del Piemonte Orientale "A. Avogadro", Largo Donegani 2, 28100 Novara, Italia
manuel.martoccia@uniupo.it; valeria.fochi@uniupo.it

Berry fruits have been recognized to be a high-valued plant-based food as they are a good source of bioactive and phytochemicals, such as polyphenols and vitamins, able to contribute to a healthy diet [1]. In the last years the sector of berry fruits is become of great interest for the Piedmont Agro-food system, in relation to the growing market demand.

Blueberries (*Vaccinium corymbosum* L.) are one of the most appreciated berry fruits for their antioxidant and anti-inflammatory properties due to a high content of polyphenols such as anthocyanins, flavonoids and phenolic acids [2]. The genetic improvement of these small fruits has made it possible to develop many cultivars present on the market, which possess high quality, productivity and adaptability to different climatic zones. Thanks to pedo-climatic characteristics of the territory (acid soil and peculiar climate conditions), the cultivation of blueberries in the Piedmont region is rapidly increased and reached the 70% of the total Italian production (FAOSTAT, 2020). In this context, the need arises to valorize and protect the local product and to monitor the introduction of new cultivars of blueberry by an efficient authentication and traceability system also on related food products. DNA-based techniques and chemical-analytic approaches can be useful to solve the difficulty in identifying plant varieties and to characterize the chemical and nutritional profile of berries.

This work is part of the INNO.PI.FRUT project (leader partner 'Fondazione per la Ricerca, Innovazione e lo Sviluppo Tecnologico dell'Agricoltura Piemontese – Agrion) and its aim was the genetic and chemical characterization of different blueberry cultivars useful to develop a traceability system of blueberry-based products. Results obtained by chemical analysis confirmed different characteristics between blueberry cultivars based on their gross composition content and their polyphenols profile. In particular, *Cargo* showed the highest content of organic acids and *Elliot* has the highest total anthocyanins and polyphenol content. Genetic diversity between blueberry cultivars was then evaluated using traditional PCR, based on the application of specific molecular markers (SCoT, SSR). The characterization of the biological material will contribute to the creation of a chemical and genetic dataset relating to blueberry cultivars which will lead to new knowledge for strengthening the Piedmont production system.

References

- [1] J.S. Câmara, M. Locatelli, J.A. Pereira, H. Oliveira, M. Arlorio, I. Fernandes, R. Perestrelo, V. Freitas, M. Bordiga, *Nutrients*, **2022**, *14*, 5133.
- [2] Y. Duan, A. Tarafdar, D. Chaurasia, A. Singh, P.C. Bhargava, J. Yang, Z. Li, X. Ni, Y. Tian, H. Li, M.K. Awasthi, *International Journal of Food Microbiology*, **2022**, *381*, 109890.

Preliminary characterization of Sicilian black pig meat according to the geographical area of breeding

Federica Litrenta^{1,2}, Luigi Liotta², Alessandro Lazzara³, Vincenzo Chiofalo²,
Antonino Iuculano³, Giuseppa Di Bella¹

¹Department of Biomedical, Dental and Morphological and Functional Imaging Sciences (BIOMORF), University of Messina, Viale Palatucci, 13, 98168 Messina, Italy; ²Department of Veterinary Sciences, University of Messina, Viale Palatucci, 13, 98168 Messina, Italy; ³Department of Agriculture, Regione Siciliana
felitrenta@unime.it

The Sicilian Black pig is a Sicilian autochthonous breed highly appreciated for the high quality of its products, linked to traditional outdoor breeding system local gastronomic traditions.

A few years ago, the Sicilian Region's Agriculture Department set up a promotion committee to initiate the request to the Ministry of Agriculture, of Food Sovereignty and Forestry (Masaf) for the recognition of the Protected Designation of Origin (PDO) of fresh meat and its processed products, in particular raw ham [1]. In particular, the granting of a geographical indication mark certifies a high quality, typically Italian product, whose area of origin and the traditions used to produce it make it so distinctive and strictly linked to the territory that it must be protected against counterfeiting [2]. Food traceability studies are currently focusing on the correlation between the geographical origin of food and its chemical composition by means of chemiometric tools. In this study, moisture, proteins, lipids, total polyphenols, fatty acids, sterols, and mineral elements data, were correlated to the geographical origin of black pig meat produced in two different geographical areas of north-eastern Sicily in January 2023. Nine samples of fresh musculus *Longissimus dorsi* were from black pigs breeding in the Mirto Areale, inside the Nebrodi National Park, and nine from black pigs breeding in the Valle del Mela Areale, outside the Nebrodi National Park. The samples were analyzed for moisture content by AOAC official method 950.46; proteins by Kjeldahl method; lipids content by the Folch method; total polyphenols content by Folin-Ciocalteu method. Fatty acid and sterol profiles were evaluated by gas-chromatography with flame ionization detection; mineral elements content by inductively coupled plasma-mass spectrometry. The results were evaluated using the Mann-Whitney U test to analyze differences between two sites, and then Principal Component Analysis (PCA) was used for dimension reduction to find a more meaningful coordinate system for the data matrix results. The results showed significant differences ($p \leq 0.05$) between the Nebrodi and No-Nebrodi samples for 46 out of a total of 61 variables. The score plot for the three PCs showed an explained variance of 78.791% (67.197% for PC1, 6.874% for PC2 and 4.720% for PC3). Two groups were sufficiently differentiated by PCA: the Nebrodi samples were separated from the No-Nebrodi on the PC1. The former were placed in negative PC1 mean values and were characterised by higher values of protein, MUFA, brassicasterol, campesterol, campestanol, Δ -5,24-stigmastanol, Mg, and Ca, whereas the latter were placed in positive PC1 mean values and had the highest concentrations of moisture, SFA, PUFA, Na, Zn, and Pb. The Commission Regulation (EC) No 1881/2006 establishes the maximum residual value for Pb in meat of pig at 0.1 mg/Kg [3]. All samples from pigs breeding in Nebrodi Natural Park showed unquantifiable levels of this element, whereas samples bred outside the park showed levels above the legal limit. The results of this study show that the product aspiring to the PDO has a better nutritional quality. Furthermore, it has been shown that it is possible to link meat samples to their geographical origin, which is a necessary condition for the traceability of this peculiar product.

References

- [1] L. Flinzberger, Y. Zinngrebe, M. N. Bugalho, T. Plieninger, *Agronomy for Sustainable Development*, **2022**, 42, 43.
- [2] European Regulation (EU) N. 1151/2012.
- [3] Commission Regulation (EC) N. 1881/2006.

***N-trans*-caffeoyltyramine and Cannabisin A: a (nutri)cosmeceutical innovation from hemp seed meal**

Marialuisa Formato, Simona Piccolella, Severina Pacifico

Department of Environmental, Biological and Pharmaceutical Sciences and Technologies,
University of Campania 'Luigi Vanvitelli', Via Vivaldi 43, 81100 Caserta, Italy
severina.pacifico@unicampania.it

The reintroduction of hemp (*Cannabis sativa* L. subsp. *sativa*) into the cultivation systems requires the launch of research programs, which also aim to the full exploitation of its chemistry [1,2]. This is especially important when the plant has an edible application. Nowadays, hemp-based foods are expected to gain growing attention, although hemp seed oil, commonly obtained by cold pressing, is still a niche product, the benefits of which are only available to a small consumer audience. Similarly, the functional goodness of hemp flour, a by-product from the grinding and sieving of cold-pressing waste, is subject of little attention [2]. Indeed, both hemp oil and flour products enjoy, among their constituents, beyond essential and conditionally essential metabolites, some specialized metabolites (e.g., phenols and polyphenols) that contribute to benefit humans and animals [3,4]. In particular, the recovery of bioactive compounds from the processing and transformation of seed and other parts of the hemp plant could really provide the basis for the development of enriched and stable nutraceutical and/or cosmeceutical products. In this context, ultra-high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC-ESI-QTOF-MS) was applied to investigate phenol and polyphenol constituents, mainly phenylamides and lignanamides [5,6], in hemp seed meal. Based on metabolic profiling, fractionation and purification strategies were employed to isolate the most abundant compounds. Thus, defatted crushed hemp seeds underwent ultrasound assisted maceration in ethyl alcohol, and the extract preliminarily fractionated by liquid-liquid partition, and purified by RP-18 column chromatography. Thus, pure *N-trans*-caffeoyltyramine (N-Caf) and cannabisin A (CbA) were obtained, and following the assessment of their antioxidant value, which makes them preventive in slowing down oxidant species over-generation in normal cells, their cytotoxicity towards human HaCaT cells were profiled by means of MTT, SRB and LDH release assays. Cytotoxicity data highlight their inability to decrease cell viability or perturb cell integrity up to 50 μ M concentration, laying the foundation to evaluate the compounds efficacy to differentially modulate the release of proinflammatory cytokines and chemokines mediators by the Bio-Plex Pro Human Cytokine 27-plex Assay. The promising outcomes suggested the preparation of N-caf and/or CbA fortified hemp oils by spherification to create innovative alginate/hemp seed oil-based (nutri)cosmeceuticals able to provide benefits on impaired skin.

References

- [1] G. Crescente, S. Piccolella, A. Esposito, M. Scognamiglio, A. Fiorentino, S. Pacifico, *Phytochemistry Rev*, **2018**, *17*, 733.
- [2] M. Formato, M.T. Pecoraro, G. Crescente, S. Piccolella, S. Pacifico, Current Applications, Approaches and Potential Perspectives for Hemp: Crop Management, Industrial Usages, and Functional Purposes, *Elsevier (Amsterdam)*, **2022**, *281*.
- [3] S. Piccolella, M. Formato, M.T. Pecoraro, G. Crescente, S. Pacifico, *Journal of Pharmaceutical and Biomedical Analysis*, **2021**, *201*, 114125.
- [4] E. Nigro, M.T. Pecoraro, M. Formato, S. Piccolella, S. Ragucci, M. Mallardo, R. Russo, A. Di Maro, A. Daniele, S. Pacifico, *Molecules*, **2022**, *27*, 2566.
- [5] A. Di Palo, C. Siniscalchi, G. Crescente, I. De Leo, A. Fiorentino, S. Pacifico, A. Russo, N. Potenza, *Current Issues in Molecular Biology*, **2022**, *44*, 5106.
- [6] E. Nigro, G. Crescente, M. Formato, M.T. Pecoraro, M. Mallardo, S. Piccolella, A. Daniele, S. Pacifico, *Molecules*, **2020**, *25*, E1049.

Enhancement of olive tree cv. Caiazzana pruning/defoliation residues for innovative food formulations

Hamid Mushtaq, Simona Piccolella, Marialuisa Formato, Severina Pacifico

Department of Environmental, Biological and Pharmaceutical Sciences and Technologies,
University of Campania “Luigi Vanvitelli”, Via Vivaldi 43, 81100 Caserta, Italy
hamid.mushtaq@unicampania.it

Olives (*Olea europaea* L.) and olive oil have been widely studied for their flavor and health benefits, but the olive leaves and their chemical composition has only recently attracted interest [1]. This is because, olive leaves chemically contain a high number of specialized compounds mainly phenolic secoiridoids and flavonoids [1-3]. These compounds are worthy because of several uses as anti-bacterial, anti-inflammatory and antioxidant in nutraceutical and food sectors. Moreover, olive leaves by the pruning and harvesting of olive trees represent one of the by-products of olive oil industry with negative environmental impact. In this context, it is of interest to explore the potential resource of olive leaves, also taking into account cultivar biodiversity. Herein, the leaves from *Olea europaea* L. cv. Caiazzana, an autochthonous cultivar of Southern Italy, were collected after pruning in an orchard near Caiazzo (Caserta, Italy). The leaves first underwent ultrasound-assisted extraction (UAE) [4,5], using extractants with decreasing polarity, and extracts obtained differently fractionated by extractive and chromatographic techniques in order to massively remove photosynthetic pigments, small organic acids and sugars. Among the others, a polyphenol fraction and a pentacyclic triterpene fraction were obtained and quali-quantitatively profiled by UHPLC-ESI-QqTOF-MS/MS techniques. Both the fractions were evaluated for their antiradical capability, and Fe(III)reducing power, while an extensive cytotoxic screening was carried out towards normal-like and cancer cells. Based on data acquired, the development of starch-based edible films incorporated with Caiazzana olive leaf fractions was preliminarily pursued. FTIR spectroscopy allowed us to unravel the nature of the bonds established in films between the starch material and the investigated olive leaf fractions, while the evaluation of films antioxidant properties, as well as the antimicrobial activity against common food pathogens, suggest their promising applicability in food sector.

References

- [1] H.H. Orak, M. Karamác, R. Amarowicz, A. Orak, K. Penkacik, *Molecules*, **2019**, *24*, 1130.
- [2] D. Ryan, M. Antolovich, P. Prenzler, K. Robards, S. Lavee, *Scientia Horticulturae*, **2002**, *92*, 147.
- [3] A. Taamalli, D. Arráez Román, M. Zarrouk, A. Segura-Carretero, A. Fernández Gutiérrez, *Food Chemistry*, **2012**, *132*, 561.
- [4] A. Lama-Muñoz, M.D.M. Contreras, F. Espínola, M. Moya, I. Romero, E. Castro, *Energies*, **2019**, *12*, 2486.
- [5] S. Pacifico, P. Bláha, S. Faramarzi, F. Fede, K. Michaličková, S. Piccolella, V. Ricciardi, L. Manti, *Antioxidants*, **2022**, *11*, 1603.

Waste by-products from *Olea europaea* as a potential application in Inflammatory Bowel Syndrome

Laura Beatrice Mattioli¹, Filomena Corbo², Maria Lisa Clodoveo³, Roberta Budriesi¹

¹Dip. Farmacia e Biotecnologie, Università di Bologna Alma Mater Studiorum

²Dip. Farmacia – Scienze del farmaco, Università di Bari Aldo Moro

³Dip. interdisciplinare di Medicina, Università di Bari Aldo Moro

laura.mattioli13@unibo.it

The use of agri-food by-products represents an important resource in the nutraceutical field in a circular economy perspective oriented to the valorization of our territory products: among these, the olive tree (*Olea europaea*, Coratina cultivar in particular), is an excellent nutraceutical even though it derives from food waste. In this study we present a polyphenolic complex - derived from the mechanical filtration process of wastewater resulting from olive oil production - called MOMAST®. Based on the results obtained from the chemical composition analysis, we hypothesized a possible application of the phytocomplex as a food supplement in Irritable Bowel Syndrome (IBS) [1] [2]. After testing three different types of extracts (MOMAST Plus30, PW25, and HY100) on some IBS-related targets, we verified their antioxidant action and effects on spontaneous and induced intestinal contractility of ileum and colon [3, 4]. From the scientific evidence found, MOMAST® compounds have proved to be excellent candidates to become food supplements in the treatment of IBS [5]: in particular, Plus30 also showed an interesting action against some microorganisms due to its high concentration of polyphenols and oleuropein.

References

- [1] L. Recinella, A. Chiavaroli, G. Orlando, L. Menghini, C. Ferrante, L. Di Cesare Mannelli, C. Ghelardini, L. Brunetti, S. Leone, *Molecules*, **2019**, *24*, 3002.
- [2] T. Tian, Z. Wang, J. Zhang, *Oxidative Medicine and Cellular Longevity*, **2017**, *2017*, 4535194.
- [3] J.K. Triantafyllidis, A. Triantafyllidi, C. Vagianos, A. Papalois, *Annals of Gastroenterology*, **2016**, *29*, 268.
- [4] M. Micucci, M. Malaguti, T.G. Toschi, G. Di Lecce, R. Aldini, A. Angeletti, A. Chiarini, R. Budriesi, S. Hrelia, *Oxidative Medicine and Cellular Longevity*, **2015**, *2015*, 318125.
- [5] M.J. Oliveras-López, G. Berna, E.M. Carneiro, H.L.G. De La Serrana, F. Martin, M.C. López, *The Journal of Nutrition*, **2008**, *138*, 1074.

Metabolomic profiling of Medicinal and Aromatic Plants through Ambient Mass Spectrometry combined with chemometrics: a powerful tool against fraudulent activities

Francesca Rigano¹, Domenica Mangraviti¹, Paola Dugo^{1,2}, Luigi Mondello^{1,2,3}

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

²Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

³Unit of Food Science and Nutrition, Department of Medicine, University Campus Bio-Medico of Rome, Rome, Italy
frigano@unime.it

Medicinal and Aromatic Plants (MAPs) are a valuable source of functional compounds responsible of beneficial effects on human health and employed for therapeutic purposes in pharmaceutical field. On the other hand, as consequence of essential oils present in their extracts, aromatic plants are characterized by distinctive flavors useful in food preparation, and at industrial level to produce beverages, cosmetics, and perfumes.

Such properties may explain the economic interest around them, and make MAPs susceptible to illegal practices, involving mislabeling, adulteration with less valuable botanical species or addition of not declared ingredients (synthetic dyes, flavor enhancers).

Although chromatographic methods coupled to mass spectrometry (MS) are commonly employed to investigate the phytochemical components of plants, belonging to a specific chemical class, they are time consuming and often require considerable efforts of the researcher for method optimization and data processing. In addition, a laborious and tedious sample preparation is necessary for single chemical classes, thus making challenging a comprehensive and exhaustive chemical characterization. In order to face such drawbacks and introduce more competitive tools against food fraud, in the last decade different fast fingerprinting analytical methods were applied for the reliable identification of “precious” foodstuffs. Among them, Ambient Mass Spectrometry (AMS) attracted special interest due to the absent or minimal sample preparation, so that the sample is analyzed in its native form under ambient conditions. Moreover, AMS techniques often lead to a complete profile in a single analysis.

In the present work, two different AMS approaches were exploited for the differentiation of Moroccan plants belonging to the *Lamiaceae* family (*Mentha pulegium*, *Mentha suaveolens*, *Melissa officinalis*, *Calamintha nepeta*, *Thymus zizis*, *Sideritis incana*) and for the preservation of the authentic Taliouine saffron, protected by the slow-food association, which safeguards biodiversity and the economy of small territories. Specifically, Rapid Evaporative Ionization Mass Spectrometry and Direct Analysis in Real Time were explored to obtain the total metabolome of the plants under investigation. Then, classification models based on Principal Component Analysis and Linear Discriminant Analysis were employed for the differentiation of the *Lamiaceae* species and for the reliable identification of the Taliouine saffron against sample of different geographical origin.

How to guarantee the natural origin of nutraceutical and pharmaceutical products? The potential of the stable isotope ratios analysis

Matteo Perini, Silvia Pianezze

Fondazione Edmund Mach, via Edmund Mach n. 2, 38098 San Michele all'Adige (TN), Italy

matteo.perini@fmach.it

The analysis of the ratio between the heavy and light isotopes of the elements carbon ($^{13}\text{C}/^{12}\text{C}$), nitrogen ($^{15}\text{N}/^{14}\text{N}$), sulfur ($^{34}\text{S}/^{32}\text{S}$), oxygen ($^{18}\text{O}/^{16}\text{O}$) and hydrogen ($^2\text{H}/^1\text{H}$) is well known for its power to discriminate the geographical origin and guarantee the authenticity of many agri-food products [1]. In recent years, the field of application of this technique has expanded to include nutraceuticals and pharmaceuticals, in particular in order to guarantee their natural origin. Chemically identical molecules are significantly different from an isotopic point of view due to the isotopic fractionation that occurs in different processes and reactions (biological, biochemical, physical, chemical, etc.) which generates unique isotopic signatures in the product synthesized by plants compared to that produced in the laboratory usually starting from fossil sources. Thanks to the coupling of isotopic mass spectrometry to liquid chromatography (LC-IRMS) and gas chromatography (GC-IRMS) it is now possible to discriminate between natural and/or synthetic origin not only of the bulk product but also of its specific components. The "Compound specific analysis" makes it possible to identify much more sophisticated frauds than in the past such as, for example, the addition of a single synthetic component to a natural substrate in order to artificially increase its strength. In this context, the $\delta^{13}\text{C}$ analysis is a suitable tool to discriminate between Monacolin K (contained in red yeast rice-based dietary supplements) and the marketed statin [2] and between natural L-theanine (extracted from *Camellia Sinensis*) and the biosynthetically produced one [3]. The isotope ratios of hydrogen and, in some cases, carbon exhibit significantly different ranges of variability between natural extracts (such as curcuminoids [4] and cannabidiols [5]) and their synthetic adulterant, allowing for the identification of not only the two origins, but also the fraudulent additions of synthetic products to the natural complex (spiked samples). The combination of GC-MS/MS and GC-IRMS is potentially useful for botanical classification between lavender (*Lavandula angustifolia*) and lavandin (*Lavandula hybrida*) essential oils thus representing an additional powerful tool for assessing the authenticity of commercial essential oils [6].

References

- [1] A. Rossmann, *Food Reviews International*, **2001**, *17*, 347.
- [2] M. Perini, G. Carbone, F. Camin, *Talanta*, **2017**, *174*, 228.
- [3] M. Perini, S. Pianezze, L. Ziller, F. Camin, *Journal of Food and Drug Analysis*, **2021**, *29*, 312.
- [4] M. Perini, S. Pianezze, L. Ziller, R. Larcher, R. Pace, *Antioxidants*, **2023**, *accepted*.
- [5] M. Perini, S. Pianezze, L. Ziller, R. Larcher, *Journal of Food and Drug Analysis*, **2023**, *submitted*.
- [6] P. K. Khatri, M. Paolini, R. Larcher, L. Ziller, D.A. Magdas, O. Marincas, A. Roncone, L. Bontempo, *Microchemical Journal*, **2023**, *186*, 1.

SWATH-MS based proteomics and chemometrics tools to assess chicken meat authenticity within divergent production systems: organic *versus* antibiotic-free

Laura Alessandroni¹, Gianni Sagratini¹, Renzo Galli², Mohammed Gagaoua³

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

²Fileni S.p.A., Località Cerrete Collicelli 8, 62011 Cingoli, Italy

³PEGASE, INRAE, Institut Agro, 35590 Saint-Gilles, France

laura.alessandroni@unicam.it

Poultry meat is among the most consumed meats in the world. Many factors, such as the production systems and pre-slaughter stress, influence the physiological and metabolic functions of the animals, with consequent impact on final meat quality [1]. The ever-increasing concerns about food safety encouraged meat industries to develop various strategies to provide healthier, safe and sustainable products. Among the approaches, antibiotic-free and organic production systems are widely diffused in poultry sector [2]. However, and the best of our knowledge, there is a paucity of published literature about the application of high-throughput omics methods to characterize these production systems and their potential impacts on final meat quality. Thus, this research aims to study the impact on *post-mortem Pectoralis major* muscle at the proteomic level of organic farming over antibiotic-free production system. A total of 40 samples including 20 Ross 308 and 20 Ranger Classic chickens, served in this trial to perform an in-depth characterization of the early *post-mortem* muscle proteome of biopsies taken at Fileni® industry (Cingoli, Italy). From each chicken strain group, 10 were reared antibiotic-free inside ground farming and 10 according to European organic regulation (EC No 848/2018). All animals were slaughtered under standardised systems. *Pectoralis major* muscle (breast) biopsies were taken within 3 h and stored at -80°C until proteomics analysis. A shotgun proteomics approach was applied in this study [3]. The quantitative proteomics was performed with a TripleTOF 6600plus (Sciex, Redwood City, CA, USA) instrument using SWATH-MS (sequential window acquisition of all theoretical fragment ion spectra mass spectrometry) Data-Independent Acquisition mode [4]. The applied proteomics allowed the identification of 660 quantifiable proteins in chicken *Pectoralis major* muscle. The data were normalized and analysed by multivariate partial least squares discriminant analysis (PLS-DA) to discriminate the four groups of interest. The most significant protein markers were shortlisted using a variable importance in projection ≥ 1 and permutation diagnostics (1000 random permutations). A clear discrimination between the 4 groups based on their proteome was visualized. Notwithstanding, a slight overlap could be observed between the Ross 308 and Ranger Classic organic groups. A total of 48 putative biomarkers were identified. Pathway enrichment analyses (Gene Ontology, KEGG and Reactome terms) using Metascape® following the guidelines of Gagaoua *et al.* [3] was performed on the discriminatory identified proteins. The proteins were found to belong to 15 significantly enriched Gene Ontology (GO) terms. The “generation of precursor metabolites and energy (GO: 0006091)” was one of the top GO terms explaining the differences within the groups followed by others related to “muscle contraction”, “carbohydrate catabolic process”, “metabolic and biosynthetic processes”.

References

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

Molecular changes of black soldier fly lipids with diet, killing method and microbial fermentation

Veronica Lolli, Andrea Fuso, Anna Valentina Luparelli, Francesca Bonzanini,
Jasmine Hadj Saadoun, Camilla Lazzi, Stefano Sforza, Augusta Caligiani

Dipartimento di Scienze degli Alimenti e del Farmaco, Università di Parma
veronica.lolli@unipr.it

Insect-based bioconversion of agri-food waste constitutes an economic and sustainable way to valorise the residual biomasses, producing valuable biomolecules, such as lipids, proteins and chitin. However, because insects are mainly claimed as protein sources, insect oil is often under-valorised and frequently used for biodiesel production [1].

The Black Soldier Fly (BSF, *Hermetia Illucens*) is one of the insect species which are the richest in lipids (37% DM basis) [2] and most employed in bioconversion. The main fatty acid of BSF oil is lauric acid (49% total fatty acids), making BSF oil similar to tropical oils, thus suggesting being a potential more sustainable substitute of coconut and palm kernel oil for food and/or oleochemistry sectors. So, BSF was chosen as a case study to identify the optimal parameters, such as diet, storage/killing methods, pre-processing of insect biomasses (including microbial fermentation), in order to promote the exploitation of insect oil for foods and/or feeds.

Different leftovers combinations, based on seasonality, significantly affected the content of lipids and the nutritional composition of the oil (fatty acid profile) [3]. The killing procedures tested (i.e., freezing and blanching) only slightly influenced the general fatty acid and sterol profiles, while they mainly affected BSF lipid class distribution. Prepupae killed by freezing showed a relevant release of free fatty acids, probably due to the activation of lipases. On the contrary, prepupae killed by blanching showed a stable lipid fraction, which was mostly constituted by triacylglycerols [4].

Finally, in the optic of a biorefinery concept, the by-products of insect bioconversion processes, namely BSF adult flies, puparia, and excess of prepupae, were further valorised by fermentation [5]. Fermentation of BSF biomasses induced deep molecular changes of both insect protein and lipid fractions, significantly improving the BSF oil nutritional properties. Indeed, lipid fraction of fermented BSF biomasses resulted richer in monounsaturated and polyunsaturated fatty acids.

References

- [1] F. Cherubini, *Energy Conversion and Management*, **2010**, *51*, 1412.
- [2] A. Caligiani, A. Marseglia, G. Leni, S. Baldassarre S., L. Maistrello, A. Dossena, S. Sforza, *Food Research International*, **2018**, *105*, 812.
- [3] S. Barbi, L.I. Macavei, A. Fuso, A.V. Luparelli, A. Caligiani, A.M. Ferrari, L. Maistrello, M. Montorsi, *Science of the Total Environment*, **2020**, *709*, 136209.
- [4] A. Caligiani, A. Marseglia, A. Sorci, F. Bonzanini, V. Lolli, L. Maistrello, S. Sforza, *Food Research International*, **2019**, *116*, 276.
- [5] A.V. Luparelli, J.H. Saadoun, V. Lolli, C. Lazzi, S. Sforza, A. Caligiani, *Food Chemistry X*, **2022**, *14*, 100327.

The first 18 months of the H2020 “EcoFISHent” Project: sustainability and efficiency in the fish processing side-streams

Federica Turrini¹, Federica Grasso¹, Valentina Orlandi¹, Giulia De Negri Atanasio²,
Elena Grasselli², Raffaella Boggia¹

¹Department of Pharmacy, University of Genoa, Genoa, Italy

²Department of Earth, Environmental and Life Sciences, University of Genoa, Genoa, Italy
federica.turrini@unige.it

EcoFISHent (“Demonstrable and replicable cluster implementing systemic solutions through multilevel circular value chains for eco-efficient valorization of fishing and fish industries side-streams”) is a project of the Horizon 2020 Program - Green Deal (Innovation Action, Grant agreement ID: 101036428) dedicated to the circular economy in the fishing sector, designed to reconcile industrial development, socio-economic growth, and protection of the marine environment [1]. Recently, because of higher fish consumption, the consequent generation of side-streams by the fish supply chain is also increasing which significantly contribute to global food waste. Fish side-streams and processing by-products represent from 20 to 80% of the original fish weight including a wide range of materials like 'unwanted catches', and non-edible parts like viscera, fins, or skin [2]. The Food and Agriculture Organization (FAO) has estimated that up to 35% of global fish production is lost or wasted every year, representing a big environmental issue with a significant economic impact [3]. The project, answering to many of the Sustainable Development Goals (SDGs) of the 2030 Agenda, moved from the design and development of an innovative dehydration pre-treatment of this highly perishable biomass (currently under patent), coupled with specific extraction technologies allowing a sustainable and efficient use of fish processing side-streams in different fields including food, pharmaceutical, cosmetic and biomaterials.

The University of Genoa is the only academic partner of the project and participates with the involvement of researchers from the Department of Earth, Environmental and Life Sciences (DISTAV), and with the Food Chemistry research group of the Department of Pharmacy (DIFAR), with activities related to the recovery and valorisation of fish processing and their transformation into high value bioactive compounds, such as peptides (gelatine, collagen and hydrolysed collagen peptides, non-collagenous proteins), fatty acids (omega-3) and other bioactive metabolites to be exploited in different fields. Environmentally friendly techniques such as ultrasounds (UAE, Ultrasound-Assisted Extraction) and enzymatic treatments (EAE, Enzymatic-Assisted Extraction) have been proposed to process side-streams coming from canned Yellowfin tuna processing and side-streams deriving from aquaculture (sea bream and sea bass).

Innovative flowcharts have been proposed to obtain high value bioactive compounds, according to the principles of green extraction, recovering also the intermediate co-products which have been evaluated for their potential further exploitation. Gelatine has been extracted, qualitative and quantitative characterized, and its rheological properties have been studied. Concerning the lipid fraction isolation, different green solvents have been tested and compared in terms of ponderal yields and ToToX index. The Non-Collagenous proteins have been isolated and stabilized by spray-drying.

References

[1] <https://ecofishent.eu/>

[2] V.G. Alfio, C. Manzo, R. Micillo, *Molecules*, **2021**, 26, 1002.

[3] FAO 2022, The State of World Fisheries and Aquaculture, <https://www.fao.org/publications/sofia/2022/en/>.

Development of a fermentation process for the production of lactic acid from *Opuntia ficus indica* by-products

Laura De Maria¹, Teresa Gervasi², Giovanna Lo Vecchio², Eleonora Di Salvo¹, Vincenzo Nava², Rossana Rando², Rita De Pasquale³, Nicola Cicero^{2,3}

¹Department of Veterinary Sciences, University of Messina, Italy

²Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy

³Science4Life, Start-up of University of Messina, Messina, Italy

laura.demaria@studenti.unime.it

Although *Opuntia ficus indica* cladodes currently represent a by-product, they contain high amounts of fiber, including pectin, mucilage, lignin, cellulose, hemicellulose and bioactive and functional compounds. Given their high annual productivity per hectare (10–40 tones dry weight), cladodes represent a cheap and suitable substrate which could be for fermentation processes.

The aim of this research was to use *O. ficus indica* cladodes as substrate for the growth of *Lactobacillus acidophilus* LA-5. Lactic acid production was also measured. Methods: A separate hydrolysis and fermentation (SHF) procedure combining heat, diluted acid and enzymatic treatment, was performed in order to make *O. ficus indica* cladodes a suitable substrate for the growth of *Lactobacillus acidophilus* LA-5. The concentration of reducing sugars after hydrolysis was 28.45 g/L. Results: The lactobacillus count ranged from 6.03 log CFU/mL (time=0h) to 8.1 log CFU/mL (time=24h), whereas lactic acid yield and productivity were 0.63 g/g and 0.73 g/L/h, respectively. The maximum lactic acid concentration was found to be 17.5 g/L. Conclusion: This study reports the possibility of using the *O. ficus indica* cladode for lactic acid production by *Lactobacillus acidophilus* LA-5 aiming to reduce costs for a sustainable industrial production.

Secoiridoids from extra virgin olive oil extracts: chemical characterization and anti-inflammatory activity in obese children

Filomena Corbo¹, Stefania De Santis¹, Laura Piacente², Anna Mestice³, Pasquale Crupi⁴, Paola Pontrelli³, Antonio Moschetta⁴, Maria Felicia Faienza³, Maria Lisa Clodoveo⁴

¹Department of Pharmacy-Pharmaceutical Science, University of Bari Aldo Moro, Bari, Italy

²Department of Biomedical Sciences and Human Oncology, University of Bari Aldo Moro, Bari, Italy

³Department of Regenerative and Precision Medicine and Jonic area (DiMePRE-J),
University of Bari Aldo Moro, Bari, Italy

⁴Department of Interdisciplinary Medicine (DIM), University of Bari Aldo Moro, Bari, Italy

filomena.corbo@uniba.it

Extra virgin olive oil (EVOO) is a key functional food of the Mediterranean Diet exerting health-promoting effects. The presence of EVOO in the human diet can also improve different pathological conditions due to the presence of the saponifiable fraction as major constituents and the unsaponifiable fraction especially secoiridoids as emerging bioactive phenolic minor components. Several studies demonstrated the EVOO bioactivity of its phenolics to reduce inflammation, a typical feature of chronic disorders and obesity both in adults and children [1]. Nevertheless, the correlation between the chemical profile and the anti-inflammatory activity needs deeper investigation to better understand the roles of these unique phytochemicals. In light of the above, the aim of this work was to investigate the analytical qualitative-quantitative chemical phenolic profile of EVOO extracts and correlate them with anti-inflammatory activities *ex vivo*.

The characterization of the EVOO extracts was performed by HPLC-UV-MS/MS analyses which allowed the identification of several phenolic compounds including secoiridoids. The amounts of these compounds were expressed as $\mu\text{g/g}$ of oil.

Regarding the anti-inflammatory effects, peripheral blood mononuclear cells (PBMCs) collected from obese children were treated with phenolic-enriched EVOO and refined olive oil (OO) extracts, characterized by a low polyphenol content, in order to study the ability of secoiridoids to dampen the inflammatory response. A specific reduction of proinflammatory CD14⁺ CD16⁺ monocytes was detected by cytofluorimetric analysis when PBMCs were treated with EVOO as compared to OO extracts. According to this, a down-modulation of MIP-1 β and MCP-1 chemokines involved in the recruitment of inflammatory cells, such as monocytes and macrophages, was reported at the protein level in the supernatant of EVOO relative to OO extracts treated PBMCs. Moreover, the hierarchical clustering on real-time PCR gene expression data studying the PBMCs molecular profile in the context of the inflammatory pathways, showed distinct segregation between EVOO and OO when compared to untreated samples [2].

The results demonstrated a good correlation between the chemical characterization of EVOO extracts and their biological functions in terms of anti-inflammatory activity on obese children, thus highlighting the importance of polyphenols to dictate the beneficial effects for human health, as also indicated by the European Food Safety Authority (EFSA) Health Claim for the anti-oxidative effect of EVOO polyphenols [3].

References

- [1] R. Mallamaci, R. Budriesi, M.L. Clodoveo, G. Biotti, M. Micucci, A. Ragusa, F. Curci, M. Muraglia, F. Corbo, C. Franchini, *Molecules*, **2021**, *26*, 1072.
- [2] S. De Santis, M. Liso, G. Verna, F. Curci, G. Milani, M.F. Faienza, C. Franchini, A. Moschetta, M. Chieppa, M.L. Clodoveo, P. Crupi, F. Corbo, *Antioxidants*, **2021**, *10*, 1016.
- [3] S. De Santis, M.L. Clodoveo, F. Corbo, *Antioxidants*, **2022**, *11*, 258.
- [4] L. Roselli, M.L. Clodoveo, F. Corbo, B. De Gennaro, *Trends in Food Science & Technology*, **2017**, *68*, 176.

***Cannabis sativa* L. inflorescences as potential functional food: a phytochemical characterization**

Cinzia Ingallina¹, Mattia Spano¹, Giacomo Di Matteo¹, Silvia Cammarrone¹, Francesca Ghirga¹,
Bruno Botta¹, Enio Campiglia², Anatoly P. Sobolev³, Luisa Mannina¹

¹Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, P.le Aldo Moro 5, 00185 Rome

²Dipartimento di Scienze Agrarie e Forestali, Università degli Studi della Tuscia,
Via San Camillo de Lellis snc, 01100 Viterbo

³Istituto per i Sistemi Biologici, Laboratorio di Risonanza Magnetica “Segre-Capitani”,
CNR, Montelibretti, Km 29500 Via Salaria, Rome
cinzia.ingallina@uniroma1.it

The recent growing interest in *Cannabis sativa* L., better known as industrial hemp, lies in the chemical composition, in both primary and secondary metabolites, which may play pivotal roles in pharmaceutical field, as well as in cosmetics and food chemistry. After decades of oblivion (1960-2000), due to the association to the narcotic Cannabis, in 2000 this crop has been reintroduced in the European agronomical scenario [1]. The loss of cultivation has contributed to the expression of phenotypic features, despite the genomic properties. Therefore, a thorough investigation is needed to deeply characterize industrial hemp inflorescences, defining the authenticity of this matrix, and evaluating how several factors (e.g., genetics, harvesting period, pedoclimatic conditions, agronomical practices) may affect the phytochemical profile.

To this aim, the application of a combined untargeted and targeted analytical approach by means of nuclear magnetic resonance spectroscopy (NMR) and ultra-high performance liquid chromatography (UHPLC-DAD), and subsequent chemometric analysis allowed us to point out interesting results among three case-studies:

1. Investigation of genetics and harvesting period (June-September) on four monoecious cultivars (Ferimon, Uso-31, Felina 32 and Fedora 17) [2].
2. Investigation of genetics and harvesting period on seven dioecious cultivars (Carmagnola, Fibranova, Eletta Campana, Antal, Tiborszallasi, Kompolti, and Tisza) [3].
3. Investigation of agronomical practices (irrigation and fertilizers) on a single cultivar (Ferimon) over two years [4].

Results highlighted each cultivar presented a peculiar phytochemical profile affected by the harvesting stage and agronomical practices. However, in all the investigated cultivars, some metabolites showed a similar trend, not being influenced by pedoclimatic and agronomical conditions. All these findings suggest the importance to choose the proper cultivation conditions to obtain products with a determined phytochemical composition, thus offering the possibility to use inflorescence as ingredient in nutraceuticals and functional foods for humans.

References

- [1] Council Regulation (European Community) N.1251/1999.
- [2] C. Ingallina, A.P. Sobolev, S. Circi, M. Spano, C. Frascchetti, A. Filippi, A. Di Sotto, S. Di Giacomo, G. Mazzocanti, F. Gasparrini, D. Quaglio, E. Campiglia, S. Carradori, M. Locatelli, G. Vinci, M. Rapa, S. Ciano, A. M. Giusti, B. Botta, F. Ghirga, D. Capitani, L. Mannina, *Molecules*, **2020**, 25, 1908.
- [3] M. Spano, G. Di Matteo, C. Ingallina, B. Botta, D. Quaglio, F. Ghirga, S. Balducci, S. Cammarone, E. Campiglia, A. M. Giusti, G. Vinci, M. Rapa, S. Ciano, L. Mannina, A. P. Sobolev, *Molecules*, **2021**, 26, 2912.
- [4] M. Spano, G. Di Matteo, C. Ingallina, A. P. Sobolev, A. M. Giusti, G. Vinci, S. Cammarone, C. Tortora, L. Lamelza, S. A. Prencipe, L. Gobbi, B. Botta, F. Marini, E. Campiglia, L. Mannina *Foods*, **2022**, 11, 3658.

Optimization of Ursolic Acid extraction in oil from Annurca Apple to obtain Oleolytes with potential neuroprotective application

Maria Maisto¹, Paola Cuomo², Vincenzo Piccolo¹, Elisabetta Schiano¹, Fortuna Iannuzzo¹,
Rosanna Capparelli², Gian Carlo Tenore¹

¹Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy

²Department of Agriculture Sciences, University of Naples Federico II, 80055 Naples, Italy
maria.maisto@unina.it

Ursolic acid (UA) (3 β -hydroxy-urs-12-en-28-oic-acid) is a pentacyclic triterpenoid carboxylic molecule widely distributed in fruits, especially in apples. In recent years, UA has attracted considerable attention due to its functional properties, such as antioxidant, antitumor, anti-inflammatory, cosmetic, antibacterial, and especially for its neuroprotective activity [1].

The main aim of this study was the optimization of the UA extraction process from Annurca apple (AA), using sunflower oil as a lyophilic food-grade solvent, and applying a Response Surface Methodology (RSM) statistical approach. Then, we moved to the evaluation of the nutraceutical potential of the obtained extract for the management of neuropathic pain.

The results of RSM analysis showed that the maximum UA yield of 784.40 ± 7.579 ($\mu\text{g/mL}$) was achieved under the following optimized conditions: sunflower oil, as extraction solvent; 68.85 °C, as extraction temperature; 63 h, as extraction time. The HPLC-DAD-HESI-MS/MS analysis performed on the extract obtained under these optimized conditions, named Optimized Annurca Apple Oleolyte (OAAO), led to the identification of twenty-three phenolic and terpenoid molecules, and the quantification of eight of them.

Glutamate is an excitatory neurotransmitter, which plays a key role in the regulation of several physiological nervous system functions [2,3]. Interestingly, unregulated glutamate accumulation or high levels of glutamatergic neurotransmission in the central nervous system (CNS), have been positively correlated with several neurological disorders, including neuropathic pain [4]. Thus, the ability of OAAO to modulate the glutamatergic system was also evaluated in a neuroblastoma cell line model (SKNBE2), in order to investigate its potential capability to control neuropathic pain. Results indicated a significantly decreased glutamate-induced cell death after OAAO pre-incubation (5, 50, 200 $\mu\text{g/mL}$). Furthermore, a scratch test assay on the SKNBE2 monolayer cells was performed, in order to investigate the potential OAAO regenerative activity on damaged neurological tissue. Results showed that OAAO contributes to cell migration and wound healing, compared to untreated control ($p < 0.001$). Undoubtedly, further studies are needed in order to elucidate the mechanisms of action underlying the observed results.

References

- [1] L. López-Hortas, P. Pérez-Larrán, M.J. González-Muñoz, E. Falqué, H Domínguez, *Food Research International*, **2018**, *103*, 130.
- [2] M. Suzuki, A.D. Nelson, J.B. Eickstaedt, K. Wallace, L.S. Wright, C.N. Svendsen, *European Journal of Neuroscience*, **2006**, *24*, 645.
- [3] S.A. Shah, H.Y. Lee, R.A. Bressan, D.J. Yun, M.O. Kim, *Cell Death Disease*, **2014**, *5*, 1026.
- [4] K. Vincent, V.M. Cornea, Y.J.I. Jong, A. Laferriere, N. Kumar, A. Mickeviciute, J.S.T. Fung, P. Bandegi, A. Ribeiro-Da-Silva, K.L. O'Malley, T.J. Coderre, *Nature Communication*, **2016**, *7*, 10604.

Specialized metabolites from the endemic species *Lavandula austroapennina*: a promising bioresource to be exploited

Claudia Gravina, Simona Piccolella, Adriano Stinca, Severina Pacifico, Assunta Esposito

Department of Environmental, Biological and Pharmaceutical Sciences and Technologies,
University of Campania "Luigi Vanvitelli", Via Vivaldi 43, 81100 Caserta, Italy
claudia.gravina@unicampania.it

Medicinal and aromatic plants (MAPs) have been used for centuries for their medical, and healing properties, mainly due to their bioactive compounds, which represent an actual opportunity. In fact, traditional and innovative applications aim to exploit their not comparable chemical diversity, and specialized metabolites such as polyphenols, phenolic acids, triterpenes and fatty acids attract attention to sectors operating for health maintenance [1,2].

Among the traditional MAPs, species belonging to the *Lavandula* genus have recently gained a renewed interest in cosmetic, perfumery, and food sectors, and in aromatherapy thank to their phytochemicals, able to be differently employed as antimicrobial, anti-inflammatory, antioxidant and analgesic ingredients [3]. Indeed, of the more than 40 species belonging to the *Lavandula* genus, only a small number has been studied from a phytochemical and bioactivity point of view. Little or no knowledge is currently available for most of them, especially for those restricted to specific geographic areas (local endemic species), where they could provide a medical and economic value for local communities. This is the case of the endemic species *Lavandula austroapennina* N.G. Passal., Tundis & Upson which opens up to an in-depth investigation due to its long history of ethnobotanical use and the scarce knowledge available. "*Spicaddossa*" is its local name, as it is popularly used as a remedy, by rubbing the leaves, for disinfectant and soothing purposes [4].

In light of the above, our work focuses to the study of the chemical and biological profile of *Lavandula austroapennina* to promote mainly its cosmeceutical use. Therefore, after harvesting the plant in Cilento, Vallo di Diano and Alburni National Park on Cervati Mountains and dissected its various organs (corolla, calyx, leaf, stem and root), ultrasound-assisted maceration (UAM) was performed using first *n*-hexane and then methanol. Therefore, the extracts were chemically profiled by ultra-high pressure liquid chromatography hyphenated high-resolution mass spectrometry (UHPLC-Qq-TOF-MS/MS) and the bioactivity evaluated based on chemical analysis outcomes. In particular, *n*-hexane extracts, which mostly accounted by ursane-type and oleanane-type triterpenes, were screened on the Vero-CCL81 cell line for its cytotoxicity and the antiviral activity against *Herpes simplex virus 1* (HSV-1), while the alcoholic extracts, which diversely consisted in acylflavonoid glycosides (especially in corolla organ), and derivatives of phenylpropenoic and 8-OH phenylpropanoic acids, were evaluated for their antioxidant capability and wound healing properties on human keratinocytes. The data obtained show that the diversity in specialized metabolites of *L. austroapennina* is a resource to be explored, beyond the extraction of the essential oil from the inflorescences, for a fruitful use of all its organs in skincare applications.

References

- [1] T. Aftab, K. Rehman Hakeem, *Springer Nature Switzerland*, **2021**, 871.
- [2] M. Fiorentino, C. Gravina, S. Piccolella, M. T. Pecoraro, M. Formato, A. Stinca, S. Pacifico, A. Esposito, *Foods* **2022**, *11*, 247.
- [3] G.E. Batiha, J.O. Teibo, L. Wasef, H.M. Shaheen, A.P. Akomolafe, T.K.A. Teibo, H.M. Al-Kuraishy, A.I. Al-Garbeeb, A. Alexiou, M. Papadakis, *Naunyn-Schmiedeberg's Archives of Pharmacology*, **2023**.
- [4] E. De Falco, D. Rigano, V. Fico, A. Vitti, G. Barile, M. Pergola, *Plants*, **2023**, *12*, 465.

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Effect of the addition of jujube (*Ziziphus jujuba* Mill.) on the production of vitamins and phenolic compounds during kombucha fermentation

Chiara La Torre, Alessia Fazio

Department of Pharmacy, Health and Nutrition Sciences, University of Calabria, Via Alberto Savinio, Edificio Polifunzionale, 87036 Arcavacata di Rende (CS), Italy
chiara.latorre@unical.it

Kombucha is a fermented beverage which has become a highly commercialized drink for its perceived beneficial effects [1]. The aim of this study was to evaluate the biological activities of a new fermented kombucha based drink (KJ) by adding jujube powder to tea [2]. Variations in pH, protein, phenol (TPC), flavonoid (TFC), vitamin C and B12 content were monitored during a time period ranging from 24 h to 45 days. The results were compared with black tea (T), used as a control, and kombucha (K) without jujube powder. The protein content increases from 0.250 ± 0.08 mg 100 mL^{-1} in the control to 0.39 ± 0.02 mg 100 mL^{-1} in K while it doubles in KJ after 24 h. TPC increased by 38.2 % in K and more than 60 % in KJ compared to the control (T). TFC were doubled in K and increases by 35 % in KJ. The antioxidant activity of the samples evaluated by DPPH \cdot and ABTS $^{+\cdot}$ assays highlighted that they were able to inhibit both radicals by more than 90 % for up to 21 days. The vitamin C content of the black tea sample (0.25 ± 0.07 mg 100 mL^{-1}) quadrupled after 24 h of fermentation (1.3 ± 0.03 mg 100 mL^{-1}) remaining constant until 21 days. On the other hand the vitamin C content KJ after 24 h of fermentation (2 mg per 100 mL^{-1}), gradually increasing until the final value of 7.1 ± 0.3 mg 100 mL^{-1} on the 45th days of fermentation. Vitamin B12 was completely absent in the T sample and was produced after 4 days of fermentation (2.3 ± 0.01 mg 100 mL^{-1}) in K, while it was present in KJ sample after 24 h of fermentation and remained constant up to day 45 (2.3 ± 0.07 mg 100 mL^{-1}). These results showed that the fortification of kombucha with jujubes improved the biological activity of the final fermented beverage and the content of bioactive compounds.

References

- [1] C. La Torre, A. Fazio, P. Caputo, P. Plastina, M.C. Caroleo, R. Cannataro, E. Cione. *Molecules*, **2021**, 26, 5474.
[2] A. Fazio, C. La Torre, M.C. Caroleo, P. Caputo, R. Cannataro, P. Plastina, E. Cione. *Molecules*, **2020**, 25, 2706.

Artichoke waste valorization in ready-to-use (poly)phenol food supplements

Simona Piccolella, Severina Pacifico

Department of Environmental, Biological and Pharmaceutical Sciences and Technologies,
University of Campania 'Luigi Vanvitelli', Via Vivaldi 43, 81100 Caserta, Italy
simona.piccolella@unicampania.it

The wide distribution of polyphenols in plants ensures that they have become part of the diet through the consumption of edible plants and derived foodstuffs, contributing to multiple valuable effects on human health. However, food processing or even cooking procedures are responsible for qualitative and quantitative changes in (poly)phenol composition [1], resulting in a lower chance of exploiting their beneficial properties. Moreover, it has been demonstrated that their bioavailability after ingestion is poor. Thus, the possibility to develop food supplements aimed at increasing their content and protecting them from degradation must be pursued to counteract these matters of concern. In this context, taking into account that agro-waste and by-products represent a rich, but often undervalued, source of polyphenols [2], also plant parts commonly unsuitable for edible purposes could be exploited.

In light of the above, herein an innovative and green strategy is proposed, in which stems and leaves from Algerian globe artichokes have been valorized in the development of nutraceutical supplements, in which natural deep eutectic solvents were used as extracting solvents but not removed at the end of the process, resulting in ready-to-use jelly-like formulations. MTT test on the human colorectal adenocarcinoma (Caco-2) cell line, performed to obtain preliminary information about cell suffering due to cytotoxicity, highlighted that mitochondrial redox activity inhibition was absent below the 50 µg/mL tested dose. The chemical composition and bioaccessibility have been investigated by means of a joint approach of UHPLC-HRMS techniques and *in vitro* simulated digestion, and compared to products from capitula, which represent the edible part of the plant. Simple phenols characterized the stem sample, whose release was mainly in the gastric chyme, whereas capitula were enriched in flavonoids, especially luteolin and apigenin glycosides, beyond cynarin, which survived in the intestinal digesta, and were positively correlated to the antiradical efficacy [3].

References

- [1] M. Domínguez-Fernández, Á. Irigoyen, M. de los Angeles Vargas-Alvarez, I.A. Ludwig, M.P. De Peña, C. Cid, *International Journal of Gastronomy and Food Science*, **2021**, 25, 100389.
- [2] S. Piccolella, G. Crescente, L. Candela, S. Pacifico, *Journal of Pharmaceutical and Biomedical Analysis*, **2019**, 175, 112774.
- [3] N. Brahmi-Chendouh, S. Piccolella, C. Gravina, M. Fiorentino, M. Formato, N. Kheyar, S. Pacifico, *Foods*, **2022**, 11, 3955.

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Reduced time and eco-friendly chromatographic analyses for fast quality control of oxygen heterocyclic compounds in foods

Marina Russo¹, Maria Rita Testa Camillo¹, Giovanna Cafeo¹, Paola Dugo^{1,2}, Luigi Mondello^{1,2}

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

²Chromaleont S.R.L., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy ^{SEP}
marina.russo@unime.it

Coumarins, furocoumarins, and polymethoxyflavones are natural substances widely distributed in the plant world. They are secondary metabolites commonly named oxygen heterocyclic compounds (OHCs). These compounds possess numerous beneficial properties for human health. However, ingestion of large amounts of coumarins or the interaction of furocoumarins with UVA rays could be toxic to human health.

The present research is focused on the development of rapid analytical methods with a low impact on the environment, applying the latest generation HPLC columns based on the fused-core technology. The aim was to validate two analytical methods for routine analysis of OHCs to save time and solvent. The first method allowed the determination of OHCs through the use of supercritical fluid chromatography (SFC) coupled to triple quadrupole mass spectrometry detector (QqQ-MS). A fast separation has been achieved with a low consumption of methanol in less than 8 min. The second method has been developed with the purpose of performing very rapid screening of OHCs in foods, concluding the entire analysis in about 4 minutes and with the use of less than 3 mL of ethanol per analysis. At this purpose, a RP-HPLC-QqQ-MS method was validated.

All the validation parameters resulted satisfactory, with low LoQs, that could allow the quantification of these compounds even when they are contained at trace level in foods.

The developed RP-HPLC-QqQ-MS and SFC-QqQ-MS methods showed to be a valid and environmentally friendly analytical approach for the analysis of 28 selected OHCs in foods. These approaches were greener than the HPLC-MS/MS approach previously adopted.

Computational methods in Food Chemistry

Giosuè Costa^{1,2}

¹Dipartimento di Scienze della Salute, Università Magna Græcia di Catanzaro,
Viale Europa, 88100 Catanzaro (Italy)

²Net4Science srl, academic spinoff, Università Magna Græcia di Catanzaro,
Viale Europa, 88100 Catanzaro (Italy)

gcosta@unicz.it

Today, the contribution of computational methodologies in the discovery of bioactive agents is no longer in doubt and most academic and pharma/biotech companies worldwide use computational design tools. Computer-aided drug design includes computational methods and resources useful to facilitate the identification of new bioactive chemical entities, including natural compounds and food constituents with potentially nutraceutical activity.

The pivotal role of the computational tools came in 2003, when the Nobel prize for chemistry was awarded to Martin Karplus, Michael Levitt, and Arieh Warshel for “the development of multiscale models for complex chemical systems”. Thus, from this point of view, chemistry is an experimental science, and theoretical chemists are providing answers to questions about how to design bioactive agents to fit with their target molecules.

The advantages of these computational approaches can be in terms of time and/or resource savings in the development flowchart. Actually computational methods, such as Virtual Screening (VS) techniques, reduce the overall cost and the time associated with the discovery identification step [1-2]. In the last years (2021-2023), our research team has focused the attention on natural products and food chemistry [3-7] with the aim of identifying new active compounds from foods able to bind and modulate the activity of key targets involved in complex diseases.

Among the computational tools, the Structure-based VS approach provide a potent tool for the identification of potentially bioactive components contained in functional foods. Starting from the 3D structure of the target of interest, available into the Protein Data Bank (PDB) [16], *in silico* analysis were applied in order to selected from consistent chemical databases the most promising compounds basing on their theoretical binding affinity. In this talk a review about the potentialities of *in silico* methods and successful applications is presented.

References

- [1] D. J. Newman, G.M. Cragg, *Journal of Natural Products*, **2012**, 75, 311.
- [2] T. Langer, R.D. Hoffmann, *Current Pharmaceutical Design*, **2001**, 7, 509.
- [3] F. A. Ambrosio, A. Coricello, G. Costa, A. Lupia, M. Micaelli, N. Marchesi, F. Sala, A. Pascale, D. Rossi, F. Vasile, S. Alcaro, S. Collina, *Journal of Medicinal Chemistry*, **2021**, 64, 9989.
- [4] I. Romeo, G. Vallarino, F. Turrini, A. Roggeri, G. Olivero, R. Boggia, S. Alcaro, G. Costa, A. Pittaluga, *Antioxidants*, **2021**, 10, 1759.
- [5] A. Malacrida, V. Cavalloro, E. Martino, G. Costa, F.A. Ambrosio, S. Alcaro, R. Rigolio, A. Casseti, M. Miloso, S. Collina, *Molecules*, **2021**, 26, 6596.
- [6] D. Caracciolo, G. Juli, C. Riillo, A. Coricello, F. Vasile, S. Pollastri, R. Rocca, F. Scionti, N. Polerà, K. Grillone, M. Arbitrio, N. Staropoli, B. Caparello, D. Britti, G. Loprete, G. Costa, M. T. Di Martino, S. Alcaro, P. Tagliaferri, P. Tassone, *Journal of Translational Medicine*, **2022**, 20, 1.
- [7] E. Marchese, V. Orlandi, F. Turrini, I. Romeo, R. Boggia, S. Alcaro, G. Costa, *Antioxidants*, **2023**, 12, 697.
- [8] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, *Nucleic Acids Research*, **2000**, 28, 235.

Volatilome fingerprinting for the assessment of olive oil quality and authenticity by innovative multi-cumulative trapping extraction methodology

Natasha Damiana Spadafora¹, Steven Mascrez², Laura McGregor³, Alberto Cavazzini¹,
Luisa Pasti⁴, Giorgia Purcaro²

¹Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, 44121, Ferrara, Italy

²Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, Gembloux 5030, Belgium

³SepSolve Analytical, 4 Swan Court, Peterborough PE7 8GX, UK

⁴Department of Environment and Prevention Sciences, University of Ferrara, 44121, Ferrara, Italy

damiana.spadafora@unife.it

Virgin olive oil is a high-value food commodity and an important ingredient of the Mediterranean diet with characteristic health benefits and sensory quality. According to European Regulation No 2568/1991 and following modifications [1], physically extracted olive oil is classified into different commercial categories (i.e., extra virgin oil (EVO), virgin oil (VO), and lampante oil (LO)) based on physicochemical and sensory parameters.

Sensory perception is tightly correlated to the complex aroma profile of olive oil, which reflects several biological, geographical, and technological aspects (i.e., cultivar, geographical origin, fruit ripeness, processing practices, and storage). A strong effort has been dedicated to characterise and correlate the volatile profiles to the quality and authenticity attributes of the olive oil [2,3,4,5]. This study explores the potential of an innovative multi-cumulative trapping headspace solid-phase microextraction (MC-SPME) approach coupled with untargeted data analysis to enhance the information provided by aroma profiling of virgin olive oil. Sixty-nine samples of different olive oil commercial categories (EVO, VO and LO) and different geographical origins were analysed using MC-SPME coupled to gas chromatography–mass spectrometry and post-processing data analysis platform. The resulting data matrix allowed the classification of olive oil samples according to commercial category. Significant positive and negative correlations were shown between the most discriminatory volatile compounds and the sensory attributes describing VO and LO. Moreover, the volatile profile was clearly distinguished among different geographical regions, showing promising results on the possibility to create a “mixture scale” to highlight the presence of blended oil samples.

References

[1] European Commission Regulation N. 2568/1991.

[2] F. Angerosa, M. Servili, R. Selvaggini, A. Taticchi, S. Esposito, G. Montedoro, *Journal of Chromatography A*, **2004**, 1054, 17.

[3] G. Purcaro, C. Cordero, E. Liberto, C. Bicchi, L.S. Conte, *Journal of Chromatography A*, **2014**, 1334, 101.

[4] F. Stilo, E. Liberto, S. E. Reichenbach, Q. Tao, C. Bicchi, C. Cordero, *Journal of Agricultural and Food Chemistry*, **2019**, 67, 5289.

[5] N.D. Spadafora, S. Mascrez, L. McGregor, G. Purcaro, *Food Chemistry*, **2022**, 383, 132438.

Use of an HPLC-MS/MS method coupled with Linear Retention Index system to characterize the Oxygen Heterocyclic fraction in *Citrus* flavored drinks

Giovanna Cafeo¹, Tania Maria Grazia Salerno¹, Marina Russo¹, Paola Dugo^{1,2}, Luigi Mondello^{1,2}

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

²Chromaleont S.R.L., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy
giovanna.cafeo@studenti.unime.it

In the last years, consumers showed a growing interest in fruit flavored beverages. Thus, food industry adds flavors to beverages in order to enhance their taste and aroma and increase the market. In this context, *Citrus* beverages are particularly widespread. Flavored *Citrus* drinks can be produced by using the essential oil, the juice or the peel of the fruit as ingredient of the preparation. Citrus fruits contain, among other class of molecules, Oxygen Heterocyclic Compounds (OHCs), secondary plant metabolites, which include Coumarins (Cs), Furocoumarins (FCs) and Polimethoxyflavones (PMFs). The OHC profile is different according to the species and the part of the fruit considered. In fact, peels are the richest part of fruit in OHCs so, when peels or essential oils are added to beverages, the content of these molecules is higher than when the juice are used. Moreover, beverages flavored with orange or mandarin are rich in PMFs but do not contain FCs so the evaluation of OHC profile is important also to quality control. Several beneficial effects of OHCs have been demonstrated: in fact, they show antioxidant, anti-inflammatory and anticancer effects. However, recent studies are focused on the evaluation of the adverse effects as a consequence of their dietary intake. In particular, furocoumarins can seriously affect drug metabolism and show phototoxic activity. For this reason, the European Parliament and the European Food Safety Authority (EFSA) released regulations and opinions on the content of OHCs in several foods and beverages. Anyhow, only coumarin is subject to restriction by the Regulation (EC) No 1334/2008 of the European Parliament [1] and an official limit about the maximum content of FCs in food is still missing.

In this context, the HPLC-MS/MS method previously validated [2], applied in combination with the Linear Retention Index (LRI) system, represents an innovative analytical strategy for the characterization of 37 OHCs (21 FCs, 9 Cs, 7 PMFs) in food matrices. Thanks to the high sensitivity of the tandem mass spectrometry detection in Multiple Reaction Monitoring (MRM) mode, the Limits of Quantifications (LOQs) of the target compounds resulted very low.

This method is useful to quantify OHCs, especially FCs, contained also at trace levels in several food products in order to give informative data for new opinions and regulations in this field.

References

[1] European Commission. Regulation (EC) N. 1334/2008.

[2] A. Arigò, P. Dugo, F. Rigano, L. Mondello, *Journal of Chromatography A*, **2021**, 1649, 462183.

Integrating volatilome, primary and specialized metabolome by data fusion techniques: a comprehensive evaluation of hazelnuts quality

Simone Squara, Andrea Caratti, Angelica Fina, Carlo Bicchi, Chiara Cordero

Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, 10125 Torino, Italy
simone.squara@unito.it

Data fusion is the process of integrating information from multiple sources to produce more accurate and comprehensive data giving access to higher level information, i.e., understanding of complex phenomena. The practice has become increasingly important in recent years as the volume of data being generated by various sources continues to grow, as in the case of “omics” analytical approaches [1]. The process of data fusion involves several stages, including data acquisition, data cleaning and pre-processing, variable selection, and feature extraction, with each stage being critical in ensuring the accuracy and completeness of the fused data [2].

In this study, the data related to targeted and untargeted features generated by different analytical techniques (i.e., comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry - GC×GC-TOFMS, liquid chromatography coupled to high-resolution mass spectrometry – LC-HRMS etc.) were fused to reach a comprehensive understanding of hazelnuts quality along their shelf-life. Volatiles, primary metabolites, and selected specialized metabolites, collected as known and unknown features were investigated on a unique sample set that included different cultivars/geographical origins, post-harvest practices, and storage time/conditions.

The comprehensive dataset revealed the susceptibility of the various metabolites to shelf-life related phenomena, with the volatilome as more informative for storage impact and sensory quality, followed by specialized metabolites (mainly phenols and polyphenols) whose patterns are quite stable and more strongly related to cultivar and geographical origin. Last but not least, primary metabolites react to post-harvest practices influencing the aroma potential of raw fruits.

Regarding the effectiveness of the models, data fusion not only improves global classification/prediction capabilities but also lowers the level of uncertainty associated with each individual result and improves outlier detection in prediction.

References

- [1] E. Borrás J.F. R. Boque, M. Mestres, O. Busto, *Analytica Chimica Acta*, **2015**, 891, 1.
- [2] A. Bajoub, S. Medina-Rodríguez, M. Gómez-Romero, E.A. Ajal, M. G. Bagur-González, A. Fernández-Gutiérrez, A. Carrasco-Pancorbo, *Food Chemistry*, **2017**, 215, 245.

C46

Innovative GC-IMS analysis records the digital fingerprints of volatiles to gain complementary insight in flavour compositions as key aspect in Food Quality and Authenticity Assessments

Cesare Rossini¹, Hansruedi Gyax², Thomas Wortelmann³

¹LabService Analytica s.r.l Via Emilia 51/c 40011 Anzola Emilia (Bologna)

²Gygarome Consulting, Marktbündtenstrasse 8, CH-7310 Bad Ragaz

³G.A.S. Dortmund mbH, Otto-Hahnstr 13, D-44227 Dortmund

cesare.rossini@labservice.it

IMS has been used over decades as a very efficient, fast, reliable and affordable detection tool for VOC's.

As we enjoy food and flavours in our daily life, key VOC's – representative odour carrying molecules will be smelled by an individual within fractions of a second and produce behavioural responses which can be between rejection and addiction.

The responses are based on the individual perception and experiences with food quality, the cultural background and many more.

The presentation will introduce the principle of a GC-IMS-instrument, the broad application fields by providing a dedicated instrument configuration and an overview of case-studies/applications relevant for the Italian Food Market.

References

[1] M. Li, R. Yang, H. Zhang, S. Wang, D. Chen, S. Lin, *Food Chemistry*, **2019**, 290, 32.

[2] D. Cavanna, S. Zanardi, C. Dall'Asta, M. Suman, *Food Chemistry*, **2019**, 271, 691.

[3] N. Gerhardt, M. Birkenmeier, S. Schwolow, S. Rohn, P. Weller, *Analytical Chemistry*, **2018**, 90, 1777.

[4] N. Arroyo-Manzanares, A. Martín-Gómez, N. Jurado-Campos, R. Garrido-Delgado, C. Arce, L. Arce, *Food Chemistry*, **2018**, 246, 65.

Valorization of apple pomace as a multifunctional ingredient for the development of functional foods

Lina Cossignani, Federica Ianni, Francesca Blasi

Department of Pharmaceutical Sciences, University of Perugia
lina.cossignani@unipg.it

Food waste and by-products, produced in all the phases of the food supply chain, represent valuable sources of multifunctional ingredients to be exploited for the development of functional foods and nutraceuticals. In line with the priorities of the European Green Deal and the Farm to Fork strategy for a healthy and environmentally friendly food system, there is an urgent need of innovative processes and products to facilitate the transition towards a healthy and sustainable diet and favor the shift from a linear to a circular economy [1]. In this frame, the search for innovative and sustainable extraction methods based on the principles of green chemistry to isolate the bioactive compounds from agro-food waste is a challenge [2]. Among food by-products, apple pomace, the press cake resulting from apple juice production, contains plenty of compounds with nutritional and health properties, among which dietary fiber, minerals, and phenolic compounds [3].

Based on these premises, the results of research activities aimed to exploit the nutritional/health properties of apple pomace will be shown.

First, apple pomace phenolic fraction was investigated by developing and optimizing an ultrasound-assisted extraction procedure. The fractionation of apple pomace extract was also performed to obtain free and bound phenols, and hydrolyzed fractions in acid and alkaline conditions. The total extract and the different fractions have been characterized by UHPLC/q-TOF MS analysis and it was found that phloridzin and different glycosylated forms of quercetin were the most abundant phenolic compounds. In vitro antioxidant activity, measured by spectrophotometric assays, showed interesting antiradical and reducing properties of apple pomace extracts.

The subsequent steps of the research activity concerned with the design, formulation, and study of functional foods, enriched with apple pomace, based on a zero-waste approach. In particular, apple pomace-added meat products, such as beef burgers and swine salami, were developed and their quality attributes, including physicochemical, textural, nutritional, and sensory characteristics, were investigated. The outcome of these investigations is promising as the addition of apple pomace, rich in fiber and phenolic compounds, improves the nutritional and health properties of meat products. In fact, low-fat, fiber- and antioxidant/antimicrobial-rich products can be prepared, avoiding or reducing the addition of additives such as nitrites and nitrates.

The results of the recent investigation on the role of apple pomace in the formulation of an innovative healthy mayonnaise will also be presented. Besides the emulsifier and thickening capacities of this by-product, apple pomace-based innovative dressing showed relevant health properties. In fact, an egg- and cholesterol-free mayonnaise characterized by the presence of bioactive compounds can satisfy health-conscious consumers, also meeting the demand of those who prefer plant-based foods.

References

- [1] P. Tamasiga, T. Miri, H. Onyeaka, A. Hart, *Sustainability*, **2022**, *14*, 9896.
- [2] F. Chemat, M. Abert Vian, H.K. Ravi, B. Khadhraoui, S. Hilali, S. Perino, A.S. Fabiano Tixier, *Molecules*, **2019**, *24*, 3007.
- [3] B. Antonic, S. Jancikova, D. Dordevic, B. Tremlova, *Journal of Food Science*, **2020**, *85*, 2977.

Grape products and by-products: comparative analysis of phenolic profile and *in vitro* biological activities

Chiara Di Lorenzo^{1,2}, Corinne Bani¹, Enrico Sangiovanni¹, Francesca Mercogliano¹,
Mario Dell'Agli¹, Patrizia Restani^{1,2}

¹Department of Pharmacological and Biomolecular Sciences,
Università degli Studi di Milano, Milano, Italy

²Coordinating Research Center "Innovation for Well-Being and Environment" (CRC),
Università degli Studi di Milano, Italy
chiara.dilorenzo@unimi.it

Grapes (*Vitis vinifera* L.) are one of the most widely produced crops in the world, with approximately 75 million tons produced every year; about 41% are produced in Europe, 29% in Asia and 21% in the Americas. About 45% of grapes produced is used as such or as fresh derivatives, while the remaining 55% is fermented for wine production [1]. In the last years, in parallel with the wine industry, the interest in grape non-fermented derivatives or by-products, as a source of health-promoting compounds, has notably increased. This could be due, among other factors, to: 1- national/international campaigns intended to reduce the alcohol abuse/misuse, especially among adolescents and young people [2]; 2- the promotion of winery by-products valorization, with the aim of reducing the negative impact of winemaking practices on the environment [3]. As a consequence, grape products that received more attention are fresh table grapes, raisins, grape leaves and pomace, the latter deriving from wine industry. Grape products are consumed as such (table grapes), as ingredients of food products (raisins, winemaking by-products) or as extracts for food supplement formulation (mainly grape leaves and winemaking by-products). In addition to nutritional aspects, grapes derivatives are among the richest sources of polyphenols; among them, flavonoids are the most abundant, especially flavanols, flavonols, phenolic acids and anthocyanins (red varieties). A broad spectrum of beneficial properties for human health have been associated with these compounds, including the reduction of oxidative stress and inflammation, both factors contributing to the progression of different chronic diseases [4]. On this basis, the aim of the present work was the characterization of the phenolic pattern and the evaluation of some biological properties of different grape derivatives, including: thirteen table grapes varieties; four samples of raisins; one extract of *Vitis vinifera* leaves; nine grape pomaces deriving from different varieties of red wine grapes. Different spectrophotometric (Folin-Ciocalteu's assay, pH differential method, vanillin assay) and chromatographic techniques (HPTLC, HPLC-DAD and LC-MS) were applied for a quantitative measurement of the main classes of polyphenols. *In vitro* biological assays included: DPPH (1,1-diphenyl-2-picrylhydrazyl) test for antioxidant activity evaluation and NF- κ B assay for the evaluation of anti-inflammatory activity in an *in vitro* gastritis model.

The results obtained show that, generally speaking, flavonols, procyanidins, phenolic acids and anthocyanins (in red varieties) were the most representative compounds mainly in fresh grapes, raisins and grape pomace. Anthocyanins were the characterizing compounds in grape leaves. These classes of phenol compounds were also well correlated with antioxidant and anti-inflammatory activity, showing to be interesting and promising ingredients for functional foods or food supplement formulation.

References

- [1] FAO-OIV FOCUS, Focus on table and dried grapes, **2016**.
- [2] World Health Organization, World Health Statistics, *World Health Statistics*, **2009**, 1, 29.
- [3] International Organization of Vine and Wine. Managing by-products of vitivinicultural origin, **2018**.
- [4] Y. Y. Hsieh, C. H. Shen, W. S. Huang, C. C. Chin, Y. H. Kuo, M. C. Hsieh, H. R. Yu, T. S. Chang, T. H. Lin, Y. W. Chiu, C. N. Chen, H. C. Kuo, S. Y. Tung, *Journal of Biomedical Science*, **2014**, 21, 59.

Characterization and valorization of phenolic rich extracts from *Malus domestica* cv Mela Abbondanza rossa

Cinzia Mannozi, Diletta Piatti, Doaa Abouelenein, Laura Alessandrini,
Gianni Sagratini, Sauro Vittori

ChIP - Chemistry Interdisciplinary Project, School of Pharmacy, University of Camerino,
Via Madonna delle Carceri, 62032, Camerino (Italy)
cinzia.mannozi@unicam.it

Nowadays, the problem of food waste is recognized as a key challenge on the way to sustainable resource management. Losses and waste of raw materials and food products have a negative impact on the natural environment, the nutritional status of the population, and food security [1].

Particularly, much interest is devoted to fruit and vegetable processing, due to the abundance of valuable bioactive substances of health-promoting nature. For example, attempts have been made to use apple pomace to produce a powder rich in polyphenols and fiber, to obtain blackcurrant seed oil, or to replace part of the flour in cookies with dried raspberry pomace [2].

Characterization of phenolic compounds by using spectrophotometric assays (1,1-diphenyl-2-picrylhydrazyl reducing activity (DPPH), total polyphenol content (TPC) and total flavonoid content (TFC)) and HPLC-DAD-MS chromatography analysis of seeds, peel and flesh extracts from Mela Abbondanza rossa (MAr), Golden and Red delicious have been performed. This aims also at guaranteeing the authenticity, genuineness and quality of natural regional food, as well as at contributing to the valorization of its unknown nutritional value, compared to commercial cultivars.

The quali-quantitative analyses were done with 21 compounds belonging to 6 classes, namely flavan-3-ols/procyanidins (catechin, epicatechin, procyanidin A2, procyanidin B2), flavonols (rutin, quercetin, quercetin-3-D-galactoside, kaempferol, kaempferol-3-glucoside), anthocyanins (cyanidin-3-glucoside), phenolic acids (p-coumaric acid, neochlorogenic acid, chlorogenic acid, caffeic acid, gallic acid, trans-ferulic acid), dihydrochalcones (phloretin and phloridzin), and triterpenes (annurcoic acid, oleanolic acid, and ursolic acid).

The obtained results showed a noticeable enhanced extractability of polyphenols from MAr's seeds compared to the commercial cultivars, which were 12,8 and 7 mg gallic acid equivalent/g, respectively. Similarly, as regarding the peel, an increase of about two times in the polyphenol content was observed for the MAr, compared to the Red delicious. On the contrary, the flesh was poorer in bioactive compounds compared to the commercial cultivars. As regarding polar constituents, the peel samples exhibited higher levels of triterpenes and phenolic compounds, especially catechin, epicatechin, and procyanidin B2, than the pulp ones.

Therefore, it should be concluded that seeds and peel of MAr extract could be a valuable sources of phenolic compounds, particularly phenolic acids, flavan-3-ols/procyanidins and triterpens, which contributes to ensure the waste management as well as the valorization and promotion of the local products.

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References

- [1] D. Hoehn, I. Vázquez-Rowe, R. Kahhat, M. Margallo, J. Laso, A. Fernández-Ríos, I. Ruiz-Salmón, R. Aldaco, *Resources, Conservation and Recycling*, **2023**, 188, 106671.
- [2] M. Karwacka, A. Ciużyńska, S. Galus, M. Janowicz, *Innovative Food Science & Emerging Technologies*, **2022**, 77, 102949.

Valorization of Lombard cereal wastes: evaluation of corn cob, rice husk, and wheat processing by-products as sources of polyphenols with antiglycative capacity

Ilaria Frosi¹, Anna Balduzzi¹, Raffaella Colombo¹, Chiara Milanese², Adele Papetti¹

¹University of Pavia, Drug Sciences Department,
Viale Taramelli 12, 27100 Pavia, Italy

²University of Pavia, Chemistry Department,
Viale Taramelli 16, 27100 Pavia, Italy
ilaria.frosi01@universitadipavia.it

Nowadays, the minimization and reutilization of agrofood wastes are extremely relevant since they represent a good source of bioactive compounds with a potential positive impact on human health [1]. In order to follow the most recent guidelines of the European Green Deal [2], we focused our attention on the valorization of Lombard cereal processing by-products in order to support local waste management. Considering that several epidemiological studies have shown the correlation between advanced glycation end products (AGEs) and the development of chronic disorders [3] as well as the current increasing interest in the antiglycative agents from natural origin [4], the aim of our work (supported by Cariplo foundation) was to evaluate the potential antiglycative capacity of corn cob, rice husk, and wheat processing by-product extracts obtained with microwave assisted extraction (MAE) coupled with hydroalcoholic mixture. Different factors (such as ethanol percentage in the extraction mixture, time, temperature, and solid to solvent ratio) affecting MAE process were initially studied and optimized using design of experiments (DOE) approach. For each cereal, the richest extract in polyphenols was then tested for the antiglycative activity using different *in vitro* systems to monitor different stages of the glycation reaction. In particular, the inhibition of Amadori products and of advanced glycation end products (AGEs) formation was evaluated by NBT assay and bovine serum albumin (BSA)-glucose (GLU) or -methylglyoxal (MGO) based systems, respectively [5]. Results indicated that the optimal extraction conditions were 88 °C, 1:42.8 g/mL, 62.6% of ethanol, 5 min for corn cob (CBE); 90 °C, 1:35 g/mL, 80% of ethanol, 5 min for rice husk (RHE); 40 °C, 1:24.5 g/mL, 23.8% of ethanol, 5 min for wheat waste (WWE). Both CBE and RHE were able to inhibit 70-90% of AGEs generated in the used *in vitro* systems with their activity always higher than the aminoguanidine (used as reference standard), especially in BSA-MGO systems. Conversely, WWE had lower antiglycative activity in all the systems tested. In addition, a good capacity to directly trap glyoxal and methylglyoxal, well known AGEs precursors, was registered for all the cereal extracts. RHE was the most promising and the research is currently going on in order to investigate its bioaccessibility and bioavailability before stability investigation in order to reach the final goal consisting in a suitable ingredient for food supplement.

References

- [1] K. Kumar, A. N. Yadav, V. Kumar, P. Vyas, H. Singh Dhaliwal, *Bioresources and Bioprocess*, **2017**, *4*, 18.
- [2] Green Deal Europeo, COM/2019/640.
- [3] Q. Wei, T. Liu, D. W. Sun, *Trends in Food Science & Technology*, **2018**, *82*, 32.
- [4] S. Velinchkova, K. Foubert, L. Pieters, *Planta Medica*, **2021**, *87*, 780.
- [5] I. Frosi, D. Vallelonga, R. Colombo, C. Milanese, A. Papetti, *Foods*, **2023**, *12*, 529.

Anthocyanin nanoencapsulation through waste valorisation: whey protein / high methoxy apple pectin complex coacervation

Illaria Fierri¹, Laura De Marchi¹, Giacomo Rossin¹, Federica Mainente¹, Anna Perbellini¹, Maria Bellumori², Ines Mancini³, Gianni Zoccatelli¹

¹Department of Biotechnology, University of Verona, Strada Le Grazie 15, Verona, Italy

²Department NEUROFARBA, University of Florence, via Schiff, Sesto Fiorentino, Firenze, Italy

³Department of Physics, University of Trento, Via Sommarive, 14, Povo, Trento, Italy

ilaria.fierri@univr.it

Anthocyanins (ACNs) are a class of water-soluble polyphenols with remarkable antioxidant capacities. Their bright red colour and several health benefits, in particular for the prevention of many non-communicable diseases, raised considerable interest for potential employment as natural colourants and nutraceuticals [1]. In this scenario, red cabbage (*Brassica oleracea* L. var. capitata f. rubra, RC) is acknowledged as an important source of ACNs, which are mainly represented by glycosylated and acylated cyanidins. The amount and identity of the carbohydrate residues and their different acylation can positively increase the stability of ACNs, delaying their irreversible degradation [2]. However, these molecules are still inherently vulnerable to many environmental stresses, a condition that severely hampers their application [3] and that can be prevented through encapsulation. This represents an approach often employed to protect liable substances and simultaneously promote their functionality and vehiculation in living organisms [4]. Coacervation is a relatively simple encapsulation process involving the electrostatic interaction between two oppositely charged biopolymers, such as proteins and polysaccharides. This study aims to optimise a coacervation protocol involving whey proteins (WP) and high methoxy apple pectin (HMP), two food industry by-products, to encapsulate RC ACNs extracted from discarded stems and leaves. ACNs were quali/quantitatively characterised by HPLC-ESI-MS-DAD. Different blending methods were tested: the addition of 2% w/v HMP to a pre-heated solution (60°C, 40 mins) of 5% w/v WP (ratio 1:1) gave the best results in terms of size, polydispersity index and ζ -potential (371.0 ± 7.0 nm, PDI < 0.25, ζ -potential: -14 ± 0.31 mV). Increasing concentrations of RC extract (36 - 1041 mg/L ACNs) significantly modified the dimensions but not the superficial charge of the nanoparticles. Fourier-transform (FT) IR confirmed the anthocyanin inclusion into the coacervate. The total encapsulation efficiency (EE%) was $29,4\% \pm 0,5$, even though each ACN displayed different behaviour ($p < 0.05$), with a retention that seemed to be dependent on the acylated and glycosylated moieties. In particular, diacylated and/or triglycosylated ACNs were entrapped more efficiently compared to the non-acylated/monoacylated-diglycosylated forms, suggesting that the physicochemical characteristics of each ACN may affect the interaction with the protein-polysaccharide shell.

Even though further efforts are needed to elucidate the nature of the interaction between the different ACNs and the shell components and whether this could influence their bioaccessibility, these results outline a stable and cost-effective process to valorise agri-food by-products in a circular economy perspective and that could be potentially employed to encapsulate ACNs from other sources.

References

- [1] R. Mattioli, A. Francioso, L. Mosca, P. Silva, *Molecules*, **2020**, 25, 1.
- [2] J. E. Farr, G. T. Sigurdson, M. M. Giusti, *Food Chemistry*, **2019**, 278, 443.
- [3] B. Enaru, G. Dreţcanu, T. D. Pop, A. Stănilă, Z. Diaconeasa, *Antioxidants*, **2021**, 10, 1.
- [4] J. Grgić, G. Šelo, M. Planinić, M. Tišma, A. Bucić-Kojić, *Antioxidants*, **2020**, 9(10), 923.

'Tulare' walnut supply chain waste: a new cultivar as potential bioresource in the nutraceutical sector

Elvira Ferrara^{1,2}, Severina Pacifico¹, Simona Piccolella¹, Assunta Esposito¹, Milena Petriccione²

¹Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche,
Università degli Studi della Campania "Luigi Vanvitelli" Via Vivaldi, 43 81100 Caserta

²CREA-Centro di ricerca Olivicoltura, Frutticoltura e Agrumicoltura, Via Torrino, 3 81100 Caserta
elvira.ferrara@unicampania.it

Persian or English walnut, *Juglans regia* L. (*Fagales*, *Juglandaceae*), is a very ancient tree species native to Persia and widespread in Asia, Europe, and South America [1]. The walnut tree has been so far considered one of the most useful species for humankind: its fruits are able to provide food with a high nutritional value and long shelf life; its wood is one of the most valuable in term of color, hardness, and durability; its phenolic compounds held in leaves, bark, and seeds are used in pharmaceuticals, agrochemical and cosmetic industries. Nowadays, the walnut is cultivated in several temperate regions of the world, and the widespread consumption of its fruits is driven by the growing appreciation of the highly nutritional properties of the seed, also containing a lot of antioxidants.

Walnut production in Italy is about 22.300 tons [2] of which 37% in Campania region. In the last years, new walnut cultivars, obtained by Californian breeding programs, have been used to realize new commercial orchard in Italy. Among these, the 'Tulare' cv is appreciated for its agronomic traits, high yield and carpological features. Indeed, Italy is also the second walnut importing country in the world, oriented walnuts in-shell, which are shelled and further processed within the country, thus producing a large amount of waste. In fact, wastes and by-products of nut processing include: i) the hull, a rich source of bioactive compounds with antioxidant, anti-inflammatory and antibacterial properties; ii) the woody endocarp consisting mainly of cellulose, hemicellulose and lignin. The objective of the present work was to analyze the phytochemical profile and "bioactivity" of the Tulare cv extracts from the hull and endocarp processing by-products. For this purpose, woody parts were differently crushed and passed through sieves, and their green extraction was optimized using water as extractant [3]. Thus different extracts were obtained, and preliminarily investigated for their total phenolic, flavonoid, and condensed tannin content, as well as for their antiradical capacity and reducing power. Furthermore, to get insight into chemical constituents of the obtained extracts, and their relative quantitation within the extracts, UHPLC-ESI-QqTOF-HRMS/MS analyses were carried out. Data acquired highlight that the use of sieves with different mesh sizes, suitable for reducing the matrix to fine powder, is a necessary pre-treatment, to increase mass transfer guaranteeing an effective solvent diffusion inside the matrix and bioactive compounds recovery.

References

[1] A. Bernard, F. Lheureux, E. Dirlewanger, *Tree Genetics & Genomes*, **2018**, *14*, 1.

[2] ISTAT, **2022**.

[3] E. Ferrara, M.T. Pecoraro, D. Cice, S. Piccolella, M.L. Formato, A. Esposito, M. Petriccione, S. Pacifico, *Molecules*, **2022**, *27*, 8924.

Biochemical and nutritional characterization of red and purple potatoes peel

Debora Dessì¹, Giacomo Fais², Giorgia Sarais²

¹Department of Biomedical Sciences, University of Sassari, Sassari

²Department of Life and Environmental Sciences, University of Cagliari, Cagliari

d.dessi14@phd.uniss.it

As reported by the Food and Agriculture Organization of the United Nations (FAO) [1], in the last 50 years, the production and accumulation of food by-products have increased, and to contain this problem, researchers have been adopting strategies re-use based on a circular economic model to obtain innovative products with high added value, promoting a sustainable bioeconomy growth between industries [2]. Potatoes are one of the most consumed foods in the world [3] with global production of around 370 million tons/year [4], and their processing industry generates large amounts of waste, such as peel, fried products, screen solids, and wastewater. Particularly, peels represent up to 6-10% of the total potato waste [5], reaching 70-140 tons/year [6]. Some scientific studies report that peel keeps high content of bioactive molecules, such as polysaccharides, proteins, carbohydrates, vitamins, and in particular polyphenolic compounds that have several beneficial properties for human health representing, therefore, an exploitable source for food, pharmaceutical and nutraceutical industries [7]. Among cultivated potato varieties a considerable biodiversity exists, including an increasing number of red- and purple-coloured cultivars with a peculiar chemical composition because of anthocyanins content [8]. Recently, their consumption is continuously rising due to the crescent consumer's demand for nutritionally superior cultivars, which are high in antioxidant and phenolics compounds. On these grounds, this study focuses on the biochemical and nutritional characterization of peels of five purple and two red potato cultivars. For this purpose, total phenolic content, carbohydrates, proteins, and lipids were determined by UV-visible spectrophotometry. While the profile of phytochemical content was explored by High Pressure Liquid Chromatography-Diode-Array Detector (HPLC-DAD). Moreover, antioxidant and antiradical activity of all extracts analysed was determined by Ferric Reducing Antioxidant Power (FRAP) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assays, respectively. The results indicated that all parameters varied significantly among different cultivars. All samples resulted rich in carbohydrates, proteins and lipids, and showed the same phytochemical profile with the presence mainly of chlorogenic acid and its derivatives and caffeic acid. Contrary, anthocyanins profile was characteristic for each variety. All samples revealed high antioxidant and antiradical activity following the trend of total phenolic content.

In conclusion, the high nutritional value and the high concentration of phytochemicals show that they can be a promising candidate for health-promoting food supplements production.

References

- [1] L.L. Del Rio Osorio, E. Flórez-López, C.D. Grande-Tovar, *Molecules*, **2021**, *26*, 515.
- [2] N.J. O'Connor, S.A. Hoang, L. Bradney, S. Dutta, X. Xiong, D.C.W. Tsang, K. Ramadass, A. Vinu, M.B. Kirkham, N.S. Bolan, *Environmental Pollution*, **2021**, *272*, 115985.
- [3] H. Y. Gebrechristos, W. Chen, *Food Science and Nutrition*, **2018**, *6*, 1352.
- [4] K. Djaman, K. Koudahe, H.D. Koubodana, A. Saibou, S. Essah, *American Journal of Potato Research*, **2022**, *99*, 1.
- [5] S. Liang, A.G. McDonald, *Journal of Agricultural and Food Chemistry*, **2014**, *62*, 8421.
- [6] M.B. Hossain, B.K. Tiwari, N. Gangopadhyay, C.P. O'Donnell, N.P. Brunton, D. K. Rai, *Ultrasonics Sonochemistry*, **2014**, *21*, 1470.
- [7] J.P. Trigo, E.M.C. Alexandre, J.A. Saraiva, M.E. Pintado, *Critical Reviews in Food Science and Nutrition*, **2020**, *60*, 1388.
- [8] V. D'Amelia, G. Sarais, G. Fais, D. Dessì, V. Giannini, R. Garramone, D. Carputo, S. Melito, *Foods*, **2022**, *11*, 384.

Determination of chiral pesticides in hemp seeds by using ON-LINE SFE-enantioselective SFC-QqQ/MS

Maria Rita Testa Camillo¹, Marina Russo¹, Paola Dugo^{1,2}, Luigi Mondello^{1,2}

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina

²Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina
testacamillom@unime.it

Nowadays, a huge trend has been observed in the global marketplace toward the production and sale of hemp based food products. These are defined as “complete protein” foods because they contain all the essential amino acids. They also have a high content of vitamins, mineral salts and unsaturated fatty acids, such as omega 6 and omega 3.

As all derivatives of vegetable cultivation, hemp could potentially contain pesticides. Pesticides, are toxic substances, with wide differences in both molecular structure and chemical characteristics. A large variety of commercial pesticides (ca. 25%), are characterized by one or more chiral centres [1]. It is well known that enantiomers of a chiral molecule can possess different activities, and exhibiting a diverse toxicity.

The present research is focused on the determination of chiral pesticides in hemp seed, using an environmental-friendly online analytical method. A supercritical fluid extraction coupled with an enantioselective supercritical fluid chromatography-triple quadrupole mass spectrometry (SFE-SFC-QqQ MS) was employed to achieve this goal. To the best of authors' knowledge, this is the first description of an on-line approach for the extraction and determination of chiral pesticides by means of a sustainable analytical technique.

For this purpose, three chiral pesticides, namely Metalaxyl, Benalaxyl and Dimethenamid, were investigated in nine hemp seed samples belonging to three varieties of *Cannabis sativa*. Only in one case a pesticide was found at levels above the method limit of quantification (LoQ). The figures of merit determined were linearity, precision, limit of detection (LoD), and LoQ. Specifically, regression coefficients were between 0.9856 and 0.9973, the LoDs were in the 0.04-0.41 $\mu\text{g kg}^{-1}$ range, the LoQs were in the 0.12-1.38 $\mu\text{g kg}^{-1}$ range, while coefficients of variation were between 1 and 3% (at the 10 $\mu\text{g kg}^{-1}$ level).

References

[1] A.W. Garrison, *Environmental Science e Technology*, **2006**, *40*, 16.

Pesticide residue levels in several food samples coming from the Fermo area, Marche region

Annamaria Iannetta¹, Giovanni Angelozzi¹, Francesca Mazza¹, Lucia Coppola^{2,3}, Sabrina Tait³,
Enrica Fabbrizi⁴, Lorella Ciferri⁵, Cinzia La Rocca³, Monia Perugini¹

¹Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, 64100 Teramo, Italy, ²Department of Physiology and Pharmacology V. Erspamer, Sapienza University of Rome, 00185 Rome, Italy;

³Center for Gender-Specific Medicine, Italian National Institute of Health, 00161 Rome, Italy; ⁴Pediatric Departmental Simple Operative Unit, Civitanova Marche Hospital, AST 3 Marche, Italy; ⁵ AST 4 Marche, 63822 Porto San Giorgio (FM), Italy

mperugini@unite.it

In agriculture pesticides are used to control insects, weeds, fungi and rodents that can damage crops. Despite the beneficial effects on plant production, they can represent a risk for non-target organisms, including humans [1]. Several pesticides are recognized as endocrine disruptors (EDs) since they can interfere with the dysregulation of sexual, thyroid and neuro-endocrine hormones contributing to earlier pubertal onset. Exposure to pesticides can be considered an important factor associated with precocious puberty and premature thelarche in girls [2].

The main objective of the PEACH project was to evaluate the association between exposure to pesticides and idiopathic premature thelarche in girls, through the measurement of pesticides in urine and the dietary intake, by analysing locally produced foods. Girls (2-7 years old) living in an agricultural area of Marche region (Centre of Italy) with idiopathic premature thelarche, matched to healthy subjects (controls), were enrolled (N=60+60). They were asked to fill in the food frequency questionnaire and to deliver urine samples. Furthermore, sampling of locally produced foods was performed. Food and urine were analysed by LC or GC-MS/MS to detect the pesticide levels.

All the urine samples analysed (N=60 cases and N=60 controls) showed pesticide levels below the quantification limit (LOQ). Otherwise, several pesticides were detected in fruits and vegetables (N=13 cases and N=12 controls) sampled in the local farms. Small fruits and berries, in particular grape and strawberry, and stone fruits (apricots, peaches, cherries, and plums) reported the highest number of pesticides including carbamates, pyridinylethylbenzamide, benzamide, phenylpyrrole and triazole fungicides and insecticides as neonicotinoids and carbohydrazide. The pome fruit and cucurbits (melon and watermelon) reported only the presence of fluopyram, (fungicide) and imidacloprid (neonicotinoid). Leafy vegetables reported the presence of a systemic fungicide, the metalaxyl and boscalid. Differently, the great part of vegetables and fruit from private gardens reported pesticide levels below the LOQ except 3 samples of cases and 1 control that resulted contaminated by boscalid, cyprodinil and tebuconazol.

Fenazaquin, phosmet and deltamethrin were found in olive oil from private garden, while no pesticide was detected in all other commodity categories as meats (red and white), eggs and honey sampled in local and private gardens. Among all detected compounds, the triazole and pyrimidine fungicides were the most representative family in a large majority of the samples. Interestingly, the results of this study highlighted the presence of pesticides mixtures in several commodities groups, especially fruits and vegetables, confirming the importance to gather co-occurrence data to evaluate the human exposure to multiple pesticides and the associated risk for human health [3].

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References

- [1] M.W. Aktar, D. Sengupta, A. Chowdhury, *Interdisciplinary Toxicology*, **2009**, 2, 1.
 [2] G. Farelllo, C. Altieri, M. Cutini, G. Pozzobon, A. Verrotti, *Front Pediatrics*, **2019**, 7, 147.
 [3] S.K. Socianu, S.K. Bopp, E. Govarts, L. Gilles, J. Buekers, M. Kolossa-Gehring, T. Backhaus, A. Franco, *International Journal of Environmental Research and Public Health*, **2022**, 19, 612.

The fight against wine's biogenic amines begins in the vineyard

Andrea Salvo¹, Cinzia Ingallina¹, Fabrizio Masciulli¹, Enrico Romano¹, Donatella Ambroselli¹,
Federica Proietti¹, Giovanna Loredana La Torre², Archimede Rotondo²

¹Department of Chemistry and Drug Technology; University of Roma La Sapienza, via P. le A. Moro 5, 00185 Roma, Italy

²Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)

University of Messina, Viale G. Palatucci, 98168 Messina, Italy

andrea.salvo@uniroma1.it

Italian viticulture, with its historical and cultural heritage, represents an economic excellence that requires clever viti-viticultural practice to preserve the quality and the safety of the wine. Because of the critical safety burden constituted by biogenic amines (BAs), wine producers and institution are called, between innovation and safeguard of tradition, to explore new approaches with the aim of limiting the presence of BAs in wine as much as possible, thus establishing measures to be applied at each stage of production to reduce the risks associated with the presence of these substances in wines. BAs are bioactive nitrogen compounds that have long been recognized as responsible of physiological problems for humans, especially when ingested in high concentrations [1]. They are present in different type of foods (i.e.: cheese, meat, and fish) and are well represented in wine. The presence of BAs in must and wines is extensively documented in the literature and more than 20 BAs have been identified [2]. Generally, histamine, tyramine, and 2-phenylethylamine are endowed with greater toxicity, although polyamines may enhance the effects of the other BAs [3]. The occurrence of BAs in the wine has been attributed to numerous factors, and two main sources have been identified: a) endogenous, since are synthesized naturally by the grape, b) or exogenous, as BAs can be formed by enzymatic reactions of free amino acids because of the action of specific living organism, mainly of microbial origin, during winemaking practice and aging condition. In addition, the BAs' formation is also influenced by countless factors that can exert a combined and synergic effect on the final concentration of BAs in wine [4]. Currently it was evidenced that vineyard management can influence some sensory properties of the wine [5]; thus, to assure high levels of wine quality and safety, this study considered the impact of two different horticultural cropping methods (with pruning procedure, and without pruning procedure) and three breeding techniques (namely: *Cortina*, *Spalliera* or *Controspalliera* and *Alberello*) on the BAs' concentrations in 21 experimental Italian red wines produced in the same region of west Sicily from 8 different grape varieties (both allochthonous and autochthonous) and under identical vinification procedures. The determination and quantification of eight BAs in experimental not aged red wines was carried out by ultra-high performance liquid chromatography (UHPLC) coupled to a photodiode array (PDA) detector on dansylated amines. The results of the multivariate analysis evidenced that a correlation does exist between the BAs' content in wine and the relative vine management. The data evidenced that the development of BAs is linked to characteristics inherent in the grape variety used, but the cultivation technologies are not extraneous to their presence.

References

- [1] C. Ruiz-Capillas, A.M. Herrero, *Foods*, **2019**, *8*, 62.
- [2] I. Mitar, I. Ljubenkov, N. Rohtek, A. Prkić, A., I. Anđelić, I., N. Vuletić, *Molecules*, **2018**, *23*, 2570.
- [3] L. Filipe-Ribeiro, J. Milheiro, L.C. Ferreira, E. Correia, F. Cosme, F.M. Nunes, *LWT - Food Science and Technology*, **2019**, *115*, 108488.
- [4] H. Vasconcelos, J.M.M.M. de Almeida, A. Matias, C. Saraiva, P.A.S. Jorge, L.C.C. Coelho, *Trends in Food Science and Technology*, **2021**, *113*, 86.
- [5] Y. Bouzas-Cid, E. Trigo-Córdoba, I. Orriols, E. Falqué, J.M. Mirás-Avalos, *Beverages*, **2018**, *4*, 76.

Organic and inorganic contaminants in Moroccan monofloral honeys

Vincenzo Nava, Angela Giorgia Potortì, Benedetta Sgrò, Vincenzo Lo Turco,
Giuseppa Di Bella

Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy
vnav@unime.it

Honey is a product with valuable health and therapeutic properties due to the presence of substances such as enzymes, vitamins, phenols, and some minerals [1]. However, honey is a matrix easily susceptible to chemical contamination [2,3].

This study focuses on monitoring of 108 pesticides, 18 polychlorinated biphenyls (PCBs), 13 polycyclic aromatic hydrocarbons (PAHs), 10 plasticizers, 9 bisphenols (BPs) and 4 potentially toxic elements (Al, Pb, Cd, As) in four different types of monofloral honeys (jujube [*Ziziphus lotus*], sweet orange [*Citrus sinensis*], PGI Euphorbia [*Euphorbia resinifera*] and *Globularia alypum*) from the Moroccan Béni Mellal-Khénifra region.

Analyses were carried out by gas chromatography coupled mass spectrometry (GC-MS), high performance liquid chromatography-mass spectrometry (HPLC-MS) and inductively coupled plasma mass spectrometry (ICP-MS).

Jujube, sweet orange, and GPI Euphorbia honeys showed levels of acephate, dimethoate, diazinon, alachlor, carbofuran and fenthion sulfoxide, which were above the maximum residue levels reported by the European Union (Regulation EC No. 396/2005 and subsequent amendments).

Among PCBs, the PCB118 was quantified in all jujube samples, whereas PCB180 in all jujube, orange and Euphorbia samples. Regarding to PAHs, jujube honey showed 5 of 13 residues, with higher values for chrysene; sweet orange only 3 with higher content of benzo[a]anthracene, while Euphorbia and *Globularia* did not show any residue.

All samples showed plasticizer residues, in particular dibutyl phthalate (DBP) showed higher levels when compared (improperly) to the respective specific migration limit (SML). Furthermore, 3 bisphenol residues were determined: bisphenol B (BPB) in all samples, bisphenol AF (BPAF) in 75% (all jujube, orange, and Euphorbia); bisphenol A (BPA) in 50% (all orange and Euphorbia).

Relative to potentially toxic elements, Pb showed contents above EU maximum levels (0.1 mg/Kg) in sweet orange, Euphorbia and *G. alypum* honeys.

In conclusion, all types of honey had widespread contamination, both organic and inorganic. Considering these findings, it is hoped that Moroccan government agencies will find appropriate measures to mitigate this contamination.

References

- [1] A. Massous, T. Ouchbani, V. Lo Turco, F. Litrenta, V. Nava, A. Albergamo, A.G. Potortì, G. Di Bella, *Foods*, **2023**, *12*, 969.
- [2] G. Di Bella, P. Licata, A.G. Potortì, R. Crupi, V. Nava, B. Qada, V. Lo Turco, *Natural Product Research*, **2022**, *36*, 636.
- [3] G. Di Bella, A.G. Potortì, A. Beltifa, H. Ben Mansour, V. Nava, V. Lo Turco, *Foods*, **2021**, *10*, 724.

Bioaccumulation of DEHT in *Mytilus galloprovincialis* and potential implications in its nutritional value

Miriam Porretti¹, Ambrogina Albergamo², Federica Litrenta², Caterina Faggio¹, Giuseppa Di Bella²

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, 98166 Messina, Italy

²Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy
miriam.porretti@studenti.unime.it

The accumulation of plastic in marine systems and its degradation into microplastics has become a global issue not only for its environmental ubiquity, but also for the release of intrinsic additives, such as plasticizers, which may negatively affect marine biota [1]. While phthalates are the plasticizers that have been present and accumulated in marine environments for the longest time, alternative non-phthalate plasticizers, such as di(2-ethylhexyl) terephthalate (DEHT), have been increasingly employed in the last decades, because of their lower migration frequency and the absence of use restriction. The mussel *Mytilus galloprovincialis* is an organism of dual value, being considered both as a seafood of great economic importance, and as a sentinel organism in biomonitoring programmes. This species can bioaccumulate a wide variety of contaminants and, consequently, it can provide valuable insights into the pollution scenario of the environment from which it comes [2]. Very few studies have explored the extent to which these plasticizers, including DEHT, accumulate in *M. galloprovincialis* and the resulting metabolic changes. The aim of this study was therefore to assess the possible effects of exposure of *M. galloprovincialis* to environmentally relevant doses of the non-phthalate plasticiser DEHT. To this purpose, two groups of adult mussels in duplicate were separately exposed to a concentration of DEHT that commonly occurs in the sea [3] (DEHT1:1 mg/L), and to a concentration 100 times higher (DEHT100:100 mg/L). Also, a pool of mussels not exposed to DEHT was considered as the experimental control. Then, the content of such plasticizer was analytically determined in mussel tissues and total lipids, fatty acid (FA) composition, and crud protein were assessed. The pools of mussels significantly bioaccumulated DEHT (123.69 mg/Kg and 595.13 mg/Kg respectively), the control group was considered DEHT-free. Both DEHT1 and DEHT100 groups showed a strong increase in lipid content than control mussels (respectively 5.52%, 8.75% and 8.41%, with $p < 0.05$). Considering the FA composition, a decrease of SFA (i.e., C16:0, C17:0), MUFA, and nutritionally relevant PUFAs, such as C20:5 ω -3 and C22:6 ω -3, and a concomitant increase in C16:1 ω -7, other PUFAs, such as C18:3 ω -4, C20:3 ω -6, was observed in the exposed organisms compared to the control ones. Total protein also shows a decreasing trend in the experimental groups, as DEHT1 and DEHT100 mussels showed respectively a protein content of 14.76 % and 11.46 % with respect to the protein of control group equal to 15.95% ($p < 0.05$). Overall, findings from this preliminary study are consistent with previous studies and highlighted that the differential exposure of *M. galloprovincialis* to the plasticizer DEHT affected its nutritional parameters, especially in terms of total lipids, total protein, and fatty acid profile.

References

- [1] E. Gugliandolo, P. Licata, R. Crupi, A. Albergamo, A. Jebara, V. Lo Turco, A.G. Potortì, H.B. Mansour, S. Cuzzocrea, G. Di Bella, *Frontiers in Marine Science*, **2020**, *7*, 589398.
- [2] A.S. Curpan, F. Impellitteri, G. Plavan, A. Ciobica, C. Faggio, *Comparative Biochemistry and Physiology Part C*, **2022**, 109302.
- [3] A. Jebara, A. Albergamo, R. Rando, A. G. Potortì, V. Lo Turco, H. Ben Mansour, G. Di Bella, *Marine Pollution Bulletin*, **2021**, *163*, 111967.

Migration of mineral oil hydrocarbons from recycled paperboard under accelerated conditions

Laura Barp¹, Chiara Conchione¹, Michele Suman², Francesca Lambertini², Sabrina Moret¹

¹Department of Agri-Food, Environmental and Animal Science, University of Udine,
via delle Scienze 206, Udine 33100, Italy

²Barilla G. R. F.lli SpA – Analytical Food Science, via Mantova 166, 43122 Parma, Italy
laura.barp@uniud.it

Packaging is essential to ensure food protection, preservation, and containment throughout the supply chain. However, the transfer of undesirable substances from packaging to food can have a negative impact on its quality and safety. Specifically, paperboard made from recycled fibers contains a wide mixture of mineral oil hydrocarbons (MOH) from a variety of sources, such as offset printing inks applied to newspapers, solvents used as binders and additive substrates, waxes added to improve the paperboard's water resistance, and adhesive components such as hot melts. In the present work, the migration of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) from recycled paperboard packaging materials was studied under accelerated conditions using both dry foods (semolina and egg pasta), and a food simulant (Tenax). The results were compared with the migration determined during shelf life with appositely produced pasta packages stored under different conditions [1]. In addition, the influence of different aspects affecting migration, such as the amount of food or simulant, particle size, different headspace volumes, and the type of contact (direct or indirect) were investigated.

References

[1] L. Barp, M. Suman, F. Lambertini, S. Moret, *Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment. Foreword*, **2015**, 32, 271.

Green and innovative agrochemical formulations based on liposomal technology

Francesco Corrias¹, Ines Castangia², Salvatore Marceddu³, Arturo Cocco⁴, Roberto Mannu⁴,
Maria Manconi², Ignazio Floris⁴, Alberto Angioni¹

¹Department of Life and Environmental Science, Food Toxicology Unit, University of Cagliari,
University Campus of Monserrato, SS 554, 09042 Cagliari

²Department of Life and Environmental Sciences, University of Cagliari, 09124 Cagliari

³Institute of Sciences of Food Production (ISPA-CNR), Bauladu (Sassari)

⁴Department of Agricultural Sciences, University of Sassari, Viale Italia 39, Sassari, 07100

francesco.corrias@unica.it

Pesticides are widely used to prevent diseases and pests, promoting the development of fruits, vegetables, cereals, and animal foodstuff [1]. Due to a growing awareness by the consumer for the quality of the food and the environmental pollution, eco-sustainable approaches are strongly recommended [2]. However, green formulations showing both suitable physical properties and effectiveness remains a challenging task. In this work etofenprox liposome-like nano-formulations (traditional liposomes, ethosomes and geraniol enriched ethosomes) were prepared and compared with a commercial etofenprox product. The formulations were fully characterized in terms of nano-vesicle size, polydispersion index, zeta potential, and encapsulation efficiency. Stability was evaluated under different thermal condition and after a long-term storage stability test of 90 days. The morphological structure of the vesicles was confirmed by scanning electron microscopy (SEM). The ability of the formulations to release the pesticide was evaluated by release studies, whereas to assess the wettability and the adhesion capacity of the vesicles, surface tension was evaluated and an in vitro leaf retention test on lemon leaves was performed. Open field trials were performed on lemon plants in real conditions, whereas the effectiveness of the formulations was evaluated by acute mortality test on Mediterranean fruit fly (*C. Capitata*). Finally, in vitro cytotoxicity studies on human keratinocytes cells were carried out. The liposomal formulation enriched with geraniol was the best nano-formulation in terms of physicochemical and stability properties and showing results overlapping those obtained with the commercial etofenprox product. In addition, it resulted easy to make. Data obtained evidenced that liposomal technology could represent an effectiveness and very promising strategy for agrochemical application, minimizing environmental pollution and possible toxicity to humans.

References

[1] L. Pareja, V. Cesio, H. Heinzen, R. A. Fernández-Alba, *Talanta*, **2011**, 83, 1613.

[2] F. Corrias, A. Melis, A. Atzei, S. Marceddu, F. Dedola, A. Sirigu, R. Pireddu, F. Lai, A. Angioni, *Pest Management Sciences*, **2021**, 77, 3508.

Optimization of green extraction conditions to recover polyphenols from *Salicornia europea* L.

Francesco Limongelli¹, Marilena Muraglia², Roberta Tardugno², Pasquale Crupi³,
Sabrina Fiorentino⁴, Maria Lisa Clodoveo⁴, Filomena Corbo²

¹Department of Food and Soul Science, University of Bari “Aldo Moro”, 70126 Bari, Italy

²Department of Pharmacy – Drug Science, University of Bari “Aldo Moro”, 70126 Bari, Italy

³Interdisciplinary Department of Medicine, University of Bari “Aldo Moro”, 70126 Bari, Italy

⁴Sestre srl, Nutraceutical supplements, 76015 Trinitapoli BT

francesco.limongelli@uniba.it

Nowadays, the use of natural substances as a health resource has become increasingly popular, especially the recovery of antioxidants, minerals, pigments, and oils from fresh vegetable matrices and agro-industrial by-products. In this regard, we focus our attention on *Salicornia europea*, a halophyte plant that grows on the coasts of the Mediterranean area. *Salicornia* is used not only as a seasoned vegetable but also in traditional medicine for its beneficial effects in protecting against chronic diseases. In numerous studies emerged that *S. europea* exhibits a high level of bioactive molecules, among which, polyphenols are prevalent. Phytochemicals found in *S. europea*, such as tungtungmadic acid, quercetin, chlorogenic acid, and their glycosides, contain many hydroxyl groups that make them highly electrophilic. This characteristic is crucial because it induces stimulation of antioxidant enzymes, thus protecting cells from damage caused by ROS [1].

In this work, a preliminary study was performed on *S. europea* using two different extraction solvents and an HPLC screening was executed. Ultrasound-assisted extraction (UAE) has been carried out on *Salicornia* freeze-dried powder by using hydroalcoholic mixture and Natural Deep Eutectic Solvents (NADESs) [2] at different times and molar ratio in order to select the optimal extraction conditions to improve the polyphenolic yield compounds. The total phenolic content and the antioxidant profiles of *S. europea* extracts have been evaluated by Folin-Ciocalteu, DPPH, ABTS, and FRAP assays [3]. The obtained results show that the most interesting antioxidant profile was detected using the combination of 50% ethanol solution, 10 min, and a molar ratio matrix:solvent of 1:10 (w/v). Furthermore, a preliminary HPLC analysis revealed the presence, in the best extract, of chlorogenic and tungtungmadic acid and quercetin-3O-glycoside. Therefore, this study provides a preliminary investigation to identify a sustainable process to improve the extraction of polyphenols from *S. europea*.

References

- [1] F. Limongelli, P. Crupi, M. L. Clodoveo, F. Corbo, M. Muraglia, *Molecules*, **2022**, *27*, 7954.
- [2] I. Rukavina, M.J. Rodrigues, C. G. Pereira, I. Mansinhos, A. Romano, S. Ślusarczyk, L. Custódio, *Molecules*, **2021**, *26*, 6136.
- [3] M.M. Cavalluzzi, A. Lamonaca, N.P. Rotondo, M. Muraglia, P. Gabriele, F. Corbo, A. De Palma, R. Budriesi, G. Lentini, *Molecules*, **2022**, *27*, 7471.

Towards the standardization of a method for the determination of selected volatile compounds in virgin olive oils

Tullia Gallina Toschi¹, Diego Luis García-González², Enrico Casadei¹, Ramón Aparicio-Ruiz², Maurizio Servili³, Florence Lacoste⁴, Stefania Vichi⁵, Enrico Valli¹, Clemente Ortiz Romero², Roberto Selvaggini³, Julien Escobessa⁴, Beatriz Quintanilla-Casas⁵, Alba Tres⁵, Pierre-Alain Golay⁶, Paolo Lucci⁷, Erica Moret⁸, Anastasios Koidis⁹, Paul Brereton⁹, Lanfranco Conte¹⁰, Alessandra Bendini¹

¹DISTAL, Alma Mater Studiorum - Università di Bologna, Cesena, Italy; ²Instituto de la Grasa (CSIC), Sevilla, Spain; ³DSA3, Università degli Studi di Perugia, Perugia, Italy; ⁴ITERG, Canejan, France; ⁵University of Barcelona, Santa Coloma de Gramenet, Spain; ⁶Nestlé Research Center, Lausanne, Switzerland; ⁷D3A, Università Politecnica delle Marche, Ancona, Italy; ⁸DI4A, Università degli Studi di Udine, Udine, Italy; ⁹Queen's University of Belfast, Northern Ireland, United Kingdom; ¹⁰Società Italiana delle Sostanze Grasse, Milan, Italy
tullia.gallinatoschi@unibo.it

In 2014, the EU, with the support of the IOC, called “*for the development, validation and pre- as well as co-normative activities followed by the standardization of a method for the assessment of the organoleptic characteristics*” of virgin olive oils (VOOs), given the cases of disputes/disagreement between sensory panels. Thus, the H2020 OLEUM project set-up an instrumental method, not alternative but complementary to the Panel test, based on the pre-concentration of volatile organic compounds (VOCs) by solid-phase micro-extraction, separation by gas chromatography, identification, and quantification by mass spectrometry (MS), or flame ionization detector (FID), to cover a wider range of labs' technical facilities [1]. The strategy to design a procedure to be used effectively in the routine quality control, included: i) selection of a widespread instrumentation; ii) targeted approach and a validation process, prerequisite for any standard method; iii) quantification of the minimum number of highly diagnostic compounds; iv) split of selected VOCs into two specifically formulated mixtures (SMA and SMB) to simplify and speed-up the construction of the calibration curves (eliminating the need of a single curve for each VOC). The results of an OLEUM inter-laboratory validation study, followed by a training workshop to publicly revise the method, formed the basis for an international trial [2], resulting in the full validation of the method. At the end of 2022, IOC organized a ring test, to verify the proficiency/easiness of the method, even by novice users to its application, to which 22 labs (8 for FID and 14 for MS) participated. The results are promising and, although at this stage confidential, will inform the progress of the method towards becoming a standard. The objective is the global harmonization of VOOs volatiles analysis to pool experience and collect enough data to propose limits and ranges of the selected VOCs in VOOs. A dataset obtained with a standard method could also be used as a reference targeted database for a virtuous connection with calibrations achieved by non-targeted techniques and/or rapid screenings methods [3].

References

- [1] R. Aparicio-Ruiz, E. Casadei, C. Ortiz-Romero, D.L. García-González, M. Servili, R. Selvaggini, F. Lacoste, J. Escobessa, S. Vichi, B. Quintanilla-Casas, A. Tres, G. Pierre-Alain, P. Lucci, E. Moret, E. Valli, A. Bendini, T. Gallina Toschi, *Methods X*, **2022**, *10*, 101972.
- [2] R. Aparicio-Ruiz, C. Ortiz-Romero, E. Casadei, D.L. García-González, M. Servili, R. Selvaggini, F. Lacoste, J. Escobessa, S. Vichi, B. Quintanilla-Casas, G. Pierre-Alain, P. Lucci, E. Moret, E. Valli, A. Bendini, T. Gallina Toschi, *Food Control*, **2022**, *135*, 108756.
- [3] E. Casadei, E. Valli, R. Aparicio-Ruiz, C. Ortiz-Romero, D.L. García-González, S. Vichi, B. Quintanilla-Casas, A. Tres, A. Bendini, T. Gallina Toschi, *Food Control*, **2021**, *123*, 107823.

Development of a Pressurized Liquid Extraction method for Glucosinolates recovery from by-products of *Camelina sativa* (L.) Crantz seed

Stefania Pagliari¹, Ciro Cannavacciuolo¹, Chiara Maria Giustra¹, Matilde Forcella¹, Paola Fusi¹, Massimo Labra^{1,2}, Luca Campone^{1,2}

¹Department of Biotechnology and Biosciences, University of Milano-Bicocca, 20126 Milan, Italy

²3NBFC, National Biodiversity Future Center, 90133 Palermo, Italy

stefania.pagliari@unimib.it

Increasing population and urbanization have caused a significant growth in the amount of waste produced by the food supply chain. These “wastes” can be considered by-products as they are often rich in active molecules with beneficial effects on human health [1].

Camelina sativa (L.) Crantz is a plant belonging to the Brassicaceae family, cultivated mainly for the oil production that are rich in omega-3 e omega-6 [2]. Following the pressing of the seeds, a seed-press cake by-product remains that could be a source of fibers, mineral proteins, and secondary metabolites such as glucosinolates (GLSs). GLSs are sulfur-containing glucosidic compounds with potential health benefits, which could be used in the pharmaceutical, cosmetics, and food industries. There is an international standard method (ISO9167-1 (Norm, 1992)) [3] for the recovery of GLSs, but it requires large volumes of organic solvent, a long time with low extraction yields. Modern, unconventional extraction methods such as pressurized liquid extraction (PLE) and ultrasound assisted extraction (USAE), which is widely used for the valorization of food by-products could be a more environmentally friendly alternative offering many advantages such as the use of a low amount of organic solvent, higher selectivity and shorter extraction times leading to an efficient process [4].

The present work aims to develop a green extraction method for the recovery of glucosinolates from *Camelina sativa* seed pressed cake by-products using PLE. First, the chemical composition of the *Camelina sativa* by-product extract was evaluated and compared with ISO extraction procedure. Chemical profiling of the PLE extract and quantitative analysis were evaluated by UPLC-HRMS analysis and led to tentative identification of new GLSs in addition to glucoarabinin, glucocamelinin, and homoglucoamelinin already known in this species. The PLE extraction process was optimized using a chemometric approach, with the design of the experiments applied to maximize GLSs recovery. The results showed that the optimized PLE extract improved extraction efficiency by using less organic solvent than the previously developed and optimized ISO and USAE procedures. Finally, under the optimized conditions the glucosinolates were purified using weak anion-exchange solid-phase extraction to test their effects on colon cancer cells, which showed an interesting antiproliferative activity. The enriched GLSs extract increases antioxidative metabolism in cancer cells without toxic effects on healthy lines.

References

- [1] S.A. Varghese, H. Pulikkalparambil, K. Promhuad, A. Srisa, Y. Laorenza, L. Jarupan, T. Nampitch, V. Chonhenchob, N. Harnkarnsujarit, *Polymers*, **2023**, *15*, 648.
- [2] J. Zubr, *Industrial Crops Production*, **1997**, *6*, 113.
- [3] ISO, **1992**, *9167*, 1.
- [4] Q. Deng, K. G. Zinoviadou, C. M. Galanakis, V. Orlien, N. Grimi, E. Vorobiev, N. Lebovka, F. J. Barba, *Food Engineering Reviews*, **2015**, *7*, 357.

Quantitative determination of the lipidic hydroperoxides in virgin olive oils by using a green, easy-to-use, and sensitive spectrophotometric method

Francesco Longobardi¹, Vito Michele Paradiso²

¹Department of Chemistry, University of Bari, Via Orabona 4, I-70126, Bari, Italy

²Department of Biological and Environmental Sciences and Technologies, Food Science Laboratory, University of Salento, I-73100, Lecce, Italy
francesco.longobardi@uniba.it

The official method used for the determination of the peroxide content in olive oil (PV) is based on iodometric titration, i.e., on the redox reaction between the hydroperoxides of the oil with excess potassium iodide; the latter, in an acidic environment, releases molecular iodine in a stoichiometric quantity. The iodine released is complexed by starch which acts as an indicator. By quantifying the iodine liberated with sodium thiosulphate, the concentration of hydroperoxide can be calculated. Nevertheless, this method presents several limitations: time-consuming and labour-intensive procedure, low sensitivity, need of large amounts of sample and to weight the sample according to the presumed number of peroxides, difficulty to determine the endpoint, large amounts of wastes, use of harmful/polluting solvents [1,2,3]. Besides these drawbacks, the main limitation is the co-presence of the interfering reaction between oxygen present in solution and potassium iodide which again produce iodine causing an overestimation of the PV. In addition, absorption of iodine by unsaturated fatty acids (leading to PV underestimation), the oxidation of acetic acid and lipid oxidation (source of peroxides), and the sodium thiosulfate decomposition are possible sources of error. Considering these disadvantages, a novel approach based on the spectrophotometric detection of triiodide was developed. Briefly, virgin olive oils were added with 0.5% HCl-ethanol, with a saturated KI solution and, after incubation, the resulting solution was filtered. Hydroperoxides were determined indirectly by reading the absorbance of the generated triiodide (formed by the reaction between iodine, generated by the redox, and iodide in excess) at 350 nm; the total time of analysis was about 7 min. A good linearity (with correlation coefficient (r) of 0.9997) of the calibration curve was obtained with purified olive oil spiked with tert-butylhydroperoxide at levels ranging from 1.0 to 10.0 meqO₂/kg, with variation coefficients less than 5% ($n = 3$) and limit of detection and quantification of 0.3 and 0.9 meqO₂/kg, respectively. Results obtained with the spectrophotometric method showed good correlation with those obtained with the official method with a r of 0.9819 confirming the reliability of the developed method. In conclusion, the method proposed shows different advantages in comparison with the official method in terms of analytical performances (time, accuracy, reproducibility, LOQ, and LOQ), sustainability of the solvent (substitution of chloroform/acetic acid with 0.5% HCl-ethanol), ease of use (no titration), reduction of sample amounts and solvent volumes, and reduction of interferences from side reactions.

References

- [1] M.C. Dobarganes, J. Velasco, *European Journal of Lipid Science and Technology*, **2002**, 104, 420.
- [2] K.M. Schaich, *Lipid oxidation. Challenges in food systems*, **2013**, 53.
- [3] F. Shahidi, Y. Zhong, *Lipid oxidation: Measurement methods. Bailey's Industrial Oil and Fat Products*, **2005**, 3.

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

Silvia Carlin¹, Fulvio Mattivi^{1,2}, Victoria Durantini^{1,3}, Stefano Dalledonne¹, Panagiotis Arapitsas^{1,4}

¹Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach, 38098 San Michele all'Adige (TN), Italy

²Department of Cellular, Computational and Integrative Biology, University of Trento, 38123 Povo Trento (TN), Italy

³Department of Food and Drug, University of Parma, Area Parco delle Scienze 27/A, 43124 Parma, Italy

⁴Department of Wine, Vine and Beverage Sciences, School of Food Science, University of West Attica, Egaleo, 12243 Athens, Greece

silvia.carlin@fmach.it

Transparent packaging is often used for food products, including wine, milk, beer, and fruit juices. This choice is based on the marketing recommendation that consumers want to see the product before they buy it, although scientists point out that light can harm food quality and nutritional value.

Although the practice of bottling white wine in transparent glass is known to cause a wine defect, the influence of light on the fruity and floral flavor profile of the wine is unknown. The aim of this work was to study the influence of light exposure on the white wine volatilome under the typical supermarket shelf conditions and to monitor the primary aroma compounds that characterize the sensorial identity and flavor of each cultivar using 1,052 bottles of 24 white wines [1]. The volatile profile was studied using a fingerprinting method able to maximize the number of volatiles detected, via comprehensive gas chromatography combined with time-of-flight mass spectrometry (GC×GC-ToF-MS) instrument.

After only 7 days of shelf life in flint glass bottles, a dramatic loss of terpenes (10 to 30%) and norisoprenoids (30 to 70%) was recorded, while colored glass bottles did not show such behavior even after 50 days and the darkness has preserved the fruity and floral aromatic integrity of the wine. Flint glass bottles bring no benefit to the wines, while the multiples changes in the aroma composition can jeopardize the quality, depriving the wine of the identity of the variety and terroir. In other words, the wine is naked. In light of this understanding of the negative impact of flint glass on the aromatic identity and sensory character of white wine, this packaging should be strongly discouraged. The same results should apply to a wide range of different foods consumed daily in which clear packaging is used.

References

[1] S. Carlin, F. Mattivi, V. Durantini, S. Dalledonne, P. Arapitsas, *Proceedings of the National Academy of Sciences of the United*, **2022**, *119*, 29.

Sample preparation strategies followed by GC×GC based techniques for fatty acids and minor lipid components investigation in food-related samples

Marco Beccaria^{1,2}, Angelica Fina³, Chiara Cordero³, Marco Piparo⁴, Pierre Giusti⁴, Pierre-Hugues Stefanuto², Luisa Pasti¹, Alberto Cavazzini¹, Jean-François Focant², Giorgia Purcaro⁵

¹University of Ferrara, Italy.

²University of Liège, OBIAChem Group, Belgium

³University of Torino, Italy

⁴TotalEnergies, France

⁵University of Liège, Gembloux Agro-Bio Tech, Belgium

marco.beccaria@unife.it

Lipids, organic compounds containing hydrocarbon chains, are molecules essential for the structure and function of living cells. They comprise a wide range of structures, characterized by predominantly non-polar and hydrophobic molecular skeletons. However, some exhibit a slight polar or hydrophilic character, giving them amphiphilic properties. Lipids can provide useful information in different fields of chemistry such as the origin of and overall quality of a food product (food chemistry), the physiological state of an individual (clinical chemistry), and the quality of biodiesel (petrol chemistry).

Two-dimensional gas chromatography (GC×GC) methodology, combined with dedicated sample preparation techniques, has been applied to investigate different lipid fractions, namely total fatty acid profile and minor lipid components.

Total fatty acid methyl esters (FAMES) were prepared using a single-step microwave-assisted extraction and derivatization coupled to a flow modulation GC×GC– flame ionization detector. The entire procedure was evaluated according to the PrepAGREE metric for greenness and comparable with reference methods.

Minor lipid components, which represent between 1-5% of total lipid components, have been fractionated and focalized from the lipid matrix by preparative liquid chromatography before GC×GC-high resolution mass spectrometry investigation, leading to the identification of different chemical classes (e.g., intact fatty acids, fatty alcohol, sterols) that together can be considered as the fingerprint of a lipid sample.

A multimethodological approach for the chemical characterization of edible insects: the case study of *Acheta domesticus*

Mattia Spano^{1,2}, Giacomo Di Matteo^{1,2}, Alba Lasalvia¹, Carlotta Fila Totaro³, Stefania Garzoli¹, Maria Elisa Crestoni¹, Luisa Mannina^{1,2}

¹Department of Chemistry and Technology of Drugs, Sapienza University of Rome,
Piazzale Aldo Moro 5, 00185 Rome, Italy

²NMR-based Metabolomics Laboratory (NMLab), Sapienza University of Rome,
Piazzale Aldo Moro 5, 00185 Rome, Italy

³Alia Insect Farm, Via Olmetto, 20123 Milan, Italy

mattia.spano@uniroma1.it

The research of new and innovative food sources represents an ever-growing need, considering the expected large population increase and the necessity of reducing the ecological problems related with intensive food production [1]. In this context, in the last years, the European Union has started to consider edible insects as a potential food source capable to satisfy both sustainability and nutritional demands. The recent introduction of *Acheta domesticus* (house cricket) in the official European list of Novel Food [2], has requiring an improvement of its chemical profile knowledge. In the present work, for the first time, a spray-dried *A. domesticus* powder was investigated by means of a multimethodological approach based on NMR, FT-ICR MS, and GC-MS methodologies. Several classes of compounds namely amino acids, organic acids, fatty acids, terpenes, and other metabolites were identified and quantified. Moreover, the proposed analytical protocol allowed to identify and quantify compounds not previously reported in cricket. In particular, methyl-branched hydrocarbons, previously identified in other insects, together with other compounds such as citrulline, formate, γ -terpinene, *o*-cymene, α -thujene, β -thujene, and 4-carene were detected. The improved knowledge of the chemical profile of this Novel Food opens new horizons both for the use of cricket as food ingredient and for the use of its extracts for the production of new formulations.

References

- [1] L. R. B. Mariutti, K. S. Rebelo, A. Bisconsin-Junior, J. S. de Morais, M. Magnani, I. R. Maldonado, N. R. Madeira, A. Tiengo, M. R. Maróstica, C. B. B. Cazarin, *Food Research International*, **2021**, 149, 110709.
[2] European Union, N. **2022/188**.

Comprehensive metabolomic investigation of Faustrime fruit by LC-MS and GC-MS data fusion coupled with Multivariate Data Analysis

Ciro Cannavacciuolo¹, Stefania Pagliari¹, Chiara Maria Giustra¹, Sonia Carabetta²,
Mariateresa Russo², Paola Branduardi^{1,3}, Massimo Labra^{1,3}, Luca Campone^{1,3}

¹Department of Biotechnology and Biosciences, University of Milano-Bicocca,
Piazza Della Scienza 2, 20126 Milan, Italy

²Department of Agriculture Science, Food Chemistry, Safety and Sensoromic Laboratory (FoCuSS Lab), University of
Reggio Calabria, Via dell'Università, 25, 89124 Reggio Calabria, Italy

³NBFC, National Biodiversity Future Center, 90133 Palermo, Italy
ciro.cannavacciuolo@unimib.it

Several species of Citrus (Rutaceae family) are largely consumed as a traditional food in tropical regions of the Globe. A large literature is available about the content of phytochemicals, such as polyphenolics, coumarins, limonoids, and volatile organic compounds (VOCs), which confer bitterness, bioactivities and fresh aroma to the fruits. *Microcitrus australasica*, better known as “finger limes”, is a specie originated in Australia where are registered by the cultivar registration authority [1]. The native fruits’ phenotype is different in the color of pulp and peels, but a unique composition in limonene/isomenthone/citronellal was assessed as chemical volatile markers for the genus [1]. The large request for novel foods allowed an extended production of finger limes in other temperate climates and sunny weather regions of the world as the Mediterranean area. The Faustrime cultivar is a hybrid of *Fortunella* spp., *Microcitrus australasica*, and *Citrus aurantifolia* [2] well acclimatized in Mediterranean regions. The distinctive aroma of the fruit could be determined by the unusual composition of volatile organic constituents (VOCs) mainly limonene, β -phellandrene, and γ -terpinene, as reported in the Faustrime cultivar from Sicily [3]. Moreover, polyphenols as are usually investigated in Citrus for their antioxidant activity, cancer prevention, and metabolic syndrome alleviation [4]. To the best of our knowledge, Faustrime differed in the occurrence of eriocitrin, neoeriocitrin, diosmin, and neodiosmin content [5]. Another class of bioactive compounds is made by Limonoids, oxygenated terpenoid compounds with several pharmacological properties, as limonexic acid previously reported in the Faustrime cultivar [5]. Despite the large diffusion in cultivation crop and consumption of the fruit, specific studies reporting the metabolomic description of VOCs and secondary metabolites (polyphenols, coumarins, terpenes) are still lacking. A Foodomics approach succeeds to investigate functional foods by integrating complex data from chemical identification and biological response [6]. In the first step, a comprehensive quali-quantitative investigation of VOCs and non-volatile compounds in Faustrime fruit was provided by means of GC-MS and UHPLC-QTOF-MS/HRMS analyses. Then, the characterized chloroform and hydroalcoholic extracts were tested for antioxidant activity by measuring radical scavenging capacity. Finally, the fruit’s metabolomics by multivariate data analysis was performed, by principal component analysis (PCA) and partial least square regression (PLS), to observe the general distribution of compounds among the botanical parts (peel pulp, albedo and seeds) of the fruit and their correlation with antioxidant activity.

References

- [1] E. Delort, A. Jaquier, E. Decorzant, C. Chapuis, A. Casilli, E. Frérot, *Phytochemistry*, **2015**, *109*, 111.
- [2] P. Dugo, L. Mondello, G. Zappia, I. Bonaccorsi, A. Cotroneo, M.T. Russo, *Journal of Essential Oil Research*, **2004**, *16*, 328.
- [3] R. Cozzolino, J.S. Câmara, L. Malorni, G. Amato, C. Cannavacciuolo, M. Masullo, S. Piacente, *Molecules*, **2022**, *27*, 7846.
- [4] M.A. Alam, N. Subhan, M.M. Rahman, S.J. Uddin, H.M. Reza, S.D. Sarker, *Advances in Nutrition*, **2014**, *5*, 404.
- [5] E. Cioni, C. Migone, R. Ascrizzi, B. Muscatello, M. de Leo, A.M. Piras, Y. Zambito, G. Flamini, L. Pistelli, *Antioxidants*, **2022**, *11*, 2047.
- [6] P. Balkir, K. Kemahlioglu, U. Yucel, *Trends in Food Science & Technology*, **2021**, *108*, 49.

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Analysis of pesticides in corn-based samples by comprehensive two-dimensional liquid chromatography

Francesco Cacciola¹, Katia Arena², Paola Dugo^{2,3}, Luigi Mondello^{2,3}

¹Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy

²Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

³Chromaleont S.R.L., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy
cacciolaf@unime.it

Food safety is a stringent everyday challenge for the globalization of production and the lack of traceability of products. In order to address such a topic, there is a continuous need of rapid, ultrasensitive, selective, sustainable and possibly “green” analytical methods to determine contaminants in food. Their analysis is currently based on gas and liquid chromatography coupled to tandem mass spectrometry which offers the feasibility of developing new approaches due to the increasing sensitivity through MRM mode. Advanced liquid chromatography methods e.g. comprehensive two-dimensional LC could represent a valuable tool, thanks to its extreme separation power, and possibility to reduce matrix effects and the amount of solvents employed.

The present contribution illustrates the advantages of the LC×LC technique for the determination of pesticides in complex food matrices; in addition, the capability of dedicated software for data processing will be pointed out.

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Application of Direct Analysis in Real-Time Mass Spectrometry (DART-MS) and multivariate approach for the rapid and automatic evaluation of edible oils

Domenica Mangraviti¹, Francesca Rigano¹, Cinzia Cafarella¹, Paola Dugo^{1,2}, Luigi Mondello^{1,2}

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

²Chromaleont S.R.L., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy
domenica.mangraviti@unime.it

The increasing consciousness of nutrition and health connection has changed greatly consumers' dietary choices towards food products with a strong healthy connotation. At the same time, there has a growing interest for typical products, whose qualitative characteristics are mainly correlated to the area of origin, or reporting labelling brands and/or certification as guarantee product quality (e.g. PDO, PGI). Edible vegetable oils are located in this scenario, including Extra-Virgin Olive Oil (EVOO), flavored and monocultivar EVOOs, with a high nutritional and commercial value, which are recognized for the health protective properties given the high content of bioactive compounds (MUFA, PUFA, phenolic compounds, phytosterols), and satisfying the current market trend.

Many efforts have carried out for development of traceability and characterization protocols, aimed to the analysis of principal or minor chemical components responsible of sensory attributes quality and authenticity assurance in edible oils. In this regard, Ambient Mass spectrometry (AMS) techniques represent attractive tools in foodomics, able to generate unique metabolic fingerprints usable for the assessment of authenticity and quality. Direct Analysis in Real Time MS (DART-MS) was employed in the present work, obtained by hyphenation of an ambient ionization source with a compact single quadrupole (qMS), a user-friendly and low impact environment instrumentation set-up.

Two different sampling devices were used for the database building of Italian high quality EVOOs, quickstrips for the untargeted differenziation of the samples based on main compounds (lipids), and SPE-it kit employing Solid Phase microExtraction for isolation of minority analytes, (polyphenolic profile). Aim of the work was the reliable development of statistical models by applying Principal Component and Linear Discriminant Analyses (PCA/LDA) for clusterization and classification of EVOOs according to the geographical origin and cultivar, and identification of unknown samples by matching with the database built.

DART-MS technique led to satisfactory results in terms of rapid identification reliability of unknown EVOOs, and for a fast qualitative and semi-quantitative evaluation when applied to EVOOs and other edible oils as screening method, confirming the versatile use of AMS for routine control analysis, and safeguard of products against fraudulent activities when employed in combination to multivariate statistical analysis.

Development of authentication models based on $^1\text{H-NMR}$ spectroscopy coupled with chemometrics for chocolate products

Eleonora Truzzi, Davide Bertelli

Department of Life Sciences, University of Modena and Reggio Emilia, Via G. Campi 103, 41125 Modena (Italy)
eleonora.truzzi@unimore.it

Chocolate products are among the most demanded luxury foods in the European market which is currently estimated at around 53 billion euros. The manufacturing of chocolate must comply with European legislation No 2000/36/CE, which allows the addition of specific vegetable fats other than cocoa butter (BC) up to 5% of the total weight of the finished product. These vegetable fats, defined as cocoa butter equivalents (CBEs), display a similar composition in triglycerides (TAG) to BC. The increasing consumption and the high economic value of chocolate, along with the growing request for BC by pharmaceutical, cosmetic, and food industries, have prompted the practice of the addition of illegal amounts of CBEs in this valuable food. The detection of prohibited CBE in products might be a difficult task due to their similar physicochemical features. To date, there are not official methods for authenticating chocolate, and most of the studies present in the literature are based on chromatographic techniques which are time-consuming and require extensive sample preparation. In our previous work, supervised multivariate statistical models built on $^1\text{H-NMR}$ spectral data were demonstrated to efficiently recognize the type and the illegal amount of CBE in dark chocolate bars [1]. In the present work, we aimed the development of a parallel analytical tool for milk chocolate built on a 400 MHz spectrometer instead of a 600 MHz spectrometer (cryo-probe), in order to test if less sensitive spectrometers could provide accurate results. Additionally, we aimed the generation of universal analytical tools for chocolate products with and without the employment of chemometrics. Illipé (*Shorea* spp.), sal (*Shorea robusta*), shea (*Butyrospermum parkii*), kokum gurgi (*Garcinia indica*), palm (*Elaeis guineensis* and *olifera*) and mango kernel (*Mangifera indica*) fats were selected as CBEs. Chocolate fats were extracted from milk chocolate bars and mixed with vegetable fats to obtain all binary mixtures at different concentrations of CBE (5-50%). $^1\text{H-NMR}$ spectra of pure fats, chocolate-extracted fats, and binary mixtures were acquired with a Bruker NMR Av-Neo 400 spectrometer by using the Bruker sequence “zg30”. Two datasets were generated and statistically analyzed: fingerprinting and peak area datasets. Partial least squares (PLS) discriminant analysis (PLS-DA) and regression (PLS-R) models were built on both datasets after evaluating the results of the unsupervised principal component analysis. Results showed that most of the differences were related to the resonances of glycerol moiety of monoglycerides (MAG), diglycerides (DAG), and TAG and the unsaponifiable fraction of fats in the range between 2.6 and 8 ppm. Thus, signals in this region were considered for the creation of classification and quantification models. Fingerprinting PLS-DA model tested on an external dataset demonstrated excellent prediction performances in recognizing the type of CBE or authentic chocolate fats. Specifically, sensitivity and specificity were higher than 95% and 76% respectively, with RMSE lower than 0.338. Concerning the PLS-R quantification model, the regression coefficient and the RMSE in prediction were equal to 0.97 and 3.467 respectively. Similar results were achieved also by models built on peak area dataset. Finally, the universal analytical tools for all types of chocolate products with and without the employment of chemometrics showed promising results, demonstrating the suitability of such strategies based on the NMR spectroscopy for the quality control of these valuable products.

References

[1] E. Truzzi, L. Marchetti, A. Fratagnoli, M.C. Rossi, D. Bertelli, *Food Chemistry*, **2023**, *404*, 134522.

Label-free quantification of the Kunitz inhibitor of trypsin KTI3 in soy products by liquid chromatography – tandem mass spectrometry

Barbara Prandi, Chiara Vacca, Stefano Sforza, Tullia Tedeschi

Department of Food and Drug, University of Parma
barbara.prandi@unipr.it

The sustainability of food chains is receiving increasing attention from consumers, and a trend is being observed to shift part of the consumption of food of animal origin towards similar plant-based products. One of the most important protein sources of vegetable origin is certainly soy, the use of which is widely spread both for animal and human nutrition. In fact, soy has a remarkable high quality protein content when compared to other products of vegetable origin. However, this is accompanied by the presence of various antinutritional factors, including trypsin Kunitz inhibitors. At present, there are few analytical methods available for the direct determination of trypsin Kunitz inhibitors, based on LC-UV techniques or on isotopically labeled peptides. The indirect determination of these inhibitors is much more widespread, based on the principle of measuring the inhibitory activity of the extract on a standard trypsin solution acting on a chromophore substrate. However, this indirect measurement can be affected by the presence of many other compounds that can inhibit trypsin activity, and thus interfere with the Kunitz inhibitor determination.

A direct label-free LC-MS/MS method for the identification and quantification of the trypsin Kunitz inhibitor KTI3 in soybean and derived products will be presented in this contribution. The KTI3 gene codes for the predominant trypsin inhibitor in soybeans [1]. The method is based on the generation of a proteotypic peptide, obtained by extracting the protein fraction with a solution containing TrisHCl, urea and dithiothreitol and performing a subsequent digestion with a combination of trypsin and chymotrypsin. The criteria for the selection of the peptide marker were: length about 8-10 amino acids (MW 800 - 1500 Da), specific cleavages of the enzymes used for digestion, absence of missed cleavages, absence of labile amino acids (e.g., cysteine), correspondence of 100% with the sequence of the protein of interest (Kunitz trypsin inhibitor KTI3) [2]. Quantification was obtained with an external calibration curve in matrix (chickpea flour) with a LOD and a LOQ of 0.75 µg/g and 2.51 µg/g respectively. The developed method was applied both to different soybean varieties and to real food products, such as soy drinks, tofu, yofu and soy burgers. In the present communication, the results of the LC-MS method will also be compared with trypsin inhibition measured spectrophotometrically, highlighting the complementarity of these two different information.

References

- [1] H. B. Krishnan, *Plant Science*, **2001**, 160, 979.
- [2] B. Prandi, M. Varani, *Food Control*, **2019**, 97, 15.

Quali-quantitative screening of bioactive compounds from health food through comprehensive two-dimensional liquid chromatography

Katia Arena¹, Francesco Cacciola², Paola Dugo^{1,3}, Luigi Mondello^{1,3,4}

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

²Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy

³Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

⁴Unit of Food Science and Nutrition, Department of Medicine,
University Campus Bio-Medico of Rome, Rome, Italy

katia.arena@unime.it

Diet evolves over time and is influenced by many social and economic factors that interact in complex ways to shape individual dietary patterns. These factors include income, food prices (which affect the availability and accessibility of healthy foods), individual preferences and beliefs, cultural traditions, and geographic and environmental aspects (including climate change). Therefore, promoting a healthy food environment-including food systems that promote a diverse, balanced, and healthy diet-requires the involvement of multiple sectors and stakeholders, including government and the public and private sectors.

"Health foods" is a general term that may be applied to natural or organic foods, or to regular foods that have undergone less processing than usual, such as stone-ground whole-grain flours.

Fruits and berries are among the world's most popular health foods.

In general, liquid chromatography (LC) coupled with tandem mass spectrometry is the most important techniques employed to investigate the phytochemical components of complex samples. In some cases, one-dimensional (1D) approaches do not often provide the resolving power and selectivity required for the analysis of this samples. One tool to overcome these limitations is the use of multidimensional chromatography (MD-LC).

In the present contribution, the HILIC×RP-LC method allowed the characterization of seven commercial berry juices and six different samples of *Rhus coriaria* L.

Specifically, LC×LC method was explored to improved separation of bioactive compounds with enhanced practical peak capacity and orthogonality with respect to the current conventional available methodologies. In addition, a quantification approach was carried out through external calibration curves and the method was validated yielding satisfactory LODs, LOQs, intraday and interday precision and recovery values.

The developed approach is estimated as a valuable tool for the quali-quantitative study of health food, considering their great complexity and high dimensionality.

Furthermore, their polyphenolic characterization will be of valid aid to confirm their potential use for the human health.

Investigating hazelnut roasting with a multi-analytical technique approach

Maria Mazzucotelli^{1,3}, Iuliia Khomenko¹, Brian Farneti¹, Emanuela Betta¹, Elena Gabetti², Luca Falchero², Andrea Cavallero², Eugenio Aprea³, Franco Biasioli¹

¹Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

²Soremartec Italia srl, Alba, CN, Italy

³Center for Agriculture Food Environment C3A, University of Trento, S. Michele all'Adige (TN), Italy
maria.mazzucotelli@fmach.it

Hazelnut (*Corylus avellana* L.) is a tree nut with relevant industrial importance. 90% of hazelnut consumption is based on processed products obtained from roasted kernels, such as grain and paste, widely used as raw material for confectionery industry [1]. Therefore, roasting is a fundamental step in hazelnut industrial processing. The thermal treatment triggers microstructural modifications and chemical reactions, leading to changes in morpho-textural properties [2] and volatilome profile [3].

In this study, the roasting process was carried out in a pilot scale infrared roaster. Small aliquots (150-200 g) of kernels were collected regularly throughout the thermal treatment, obtaining samples at increasing roasting intensity. Each aliquot was processed to obtain the paste samples, which were characterized for the morpho-textural properties (color and texture) and the profile of volatile organic compounds (VOCs). Hazelnuts from three different geographical and botanical origins were employed in this study: Tonda Gentile Romana monocultivar hazelnuts from Lazio region (Italy), Tonda Gentile delle Langhe monocultivar hazelnuts from Piemonte region (Italy), and Akçakoca hazelnuts from Turkey.

The hazelnut paste VOC profile was determined by applying three analytical approaches, namely gas chromatography - ion mobility spectrometry (GC-IMS), proton transfer reaction – time of flight – mass spectrometry (PTR-ToF-MS) and gas chromatography – mass spectrometry (GC-MS). Outcomes of this study demonstrated the complementarity of these analytic techniques and the prospect to apply them to assess the evolution of hazelnut volatilome during roasting.

References

- [1] A. Romero-Aroca, M. Rovira, V. Cristofori, C. Silvestri, *Agriculture*, **2021**, *11*, 1115.
- [2] S. Saklar, S. Ungan, S. Katnas, *Food Research International*, **2003**, *36*, 19.
- [3] A. Burdack-Freitag, P. Schieberle, *Journal of Agricultural and Food Chemistry*, **2010**, *58*, 6351.

Food metabolomics by GCXGC-TOF MS and tandem ionization: understanding the impact of climate events on edible crops quality

Angelica Fina¹, Nemanja Koljančić², Simone Squara¹, Donatella Ferrara¹, Carlo Bicchi¹,
Ivan Špánik², Chiara Cordero¹

¹Università degli studi di Torino, Dipartimento di Scienza e Tecnologia del Farmaco, Turin, Italy

²Institute of Analytical Chemistry, Faculty of Chemical and Food Technology,
Slovak University of Technology in Bratislava, Bratislava, Slovakia

angelica.fina@unito.it

The increased frequency and severity of extreme climate events around the world, as well as crop diseases and pests caused by climate change, have an impact on global food security and quality. A better and comprehensive understanding of the relationship between climate conditions and nutrition is necessary to provide the human population with safe and secure access to food.

The goal of the current study is to understand the effects of climate change on the detectable metabolome of a selection of edible crops. Hazelnuts and peanuts were selected to understand post-harvest practices impact on aroma precursors; soy was investigated for its metabolic profile in relation to insect bites due to migrations caused by global warming [1].

To validate the hypothesis of a climate change impact on aroma quality, hazelnuts and peanuts were roasted and the key-odorants patterns correlated to aroma precursors distribution [2].

The analytical strategy, aligned to food-metabolomics principles, exploits the information potential of multi-dimensional analysis that combine physicochemical discrimination/separation of analytes with spectrometric detection by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) featuring Tandem ionisation™. The process known as untargeted and targeted (UT) fingerprinting is used in combination with chemometric algorithms to highlight metabolomic variations between composite-class images generated by re-alignment and fusion of raw data collected from samples belonging to distinct classes, thus highlighting metabolites pattern differences [3].

Results indicate that post-harvest connoted by higher relative humidity has an impact on hazelnut metabolome resulting in an up-regulation of about 22 targeted features belonging to different classes (organic acids, amino acids, and mono- and disaccharides), with the latter showing 2-4 fold increment, evidence of a metabolic activation. Metabolism activation in peanuts, resulting in “split” seeds during industrial processing, showed higher concentration in sugars and specifically in ribose, while aroma precursors (L-Val, L-Thr, and saccharose) were present in lower amounts. Soy samples attacked by *Halyomorpha halys* (Stål), the brown marmorated stink bug, showed clear activation of primary metabolism with a general up-regulation of many chemical classes.

References

- [1] T. Zamljen, A. Medič, R. Veberič, M. Hudina, F. Štampar, A. Slatnar, *Horticulturae*, **2021**, 7, 212.
- [2] C. Alasalvar, E. Pelvan, R. Amarowicz, *Journal of Agricultural and Food Chemistry*, **2010**, 58, 8674.
- [3] M.C. Rosso, F. Stilo, C. Bicchi, M. Charron, G. Rosso, R. Menta, S.E. Reichenbach, C.H. Weinert, C.I. Mack, S.E. Kulling, *Applied Sciences*, **2021**, 11, 525.

Computer Vision to analyze chemical signatures: a novel workflow for rationalizing raw data exploration in GC×GC

Andrea Caratti¹, Simone Squara¹, Angelica Fina¹, Stephen E. Reichenbach^{2,3}, Qingping Tao³,
Carlo Bicchi¹, Giorgio Borreani⁴, Francesco Ferrero⁴, Chiara Cordero¹

¹Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Turin, Italy

²Computer Science and Engineering Department, University of Nebraska, Lincoln, NE, USA

³GC Image LLC, Lincoln, NE, USA

⁴Dipartimento di Scienze Agrarie Forestali e Alimentari,
Università degli Studi di Torino, Grugliasco-TO, Italy

andrea.caratti@unito.it

This work delves into Computer Vision in the context of Artificial Intelligence and its ability to extract meaningful information from digital images. This concept is particularly relevant to the field of AI, where the processing of large amounts of data and the ability to derive insights from it is of utmost importance [1]. One of the techniques where AI can be applied is in combination with comprehensive two-dimensional gas chromatography (GC×GC), which provides highly detailed information on the chemical composition of a sample generating chromatogram images with a multidimensional array of data. However, the high amount of these data can make it difficult to interpret and analyze information encrypted. This is where AI techniques such as Computer Vision come into play, as they can help rationalize raw data exploration, leading to an understanding of the biological phenomena related to specific chemical signatures and molecular patterns.

In this work, a new workflow for Computer Vision based on pattern recognition algorithms, such as combined untargeted and targeted (UT) fingerprinting is presented. This workflow involves several steps, starting with the generation of composite class images for representative samples' classes. These images are then processed to create a feature template with reliable peaks and peak-regions. The feature template is then pruned to include targeted components while eliminating bleeding peaks and artifacts. Once the feature template is finalized, it is applied to all samples' images and composite class images. Finally, pair-wise comparisons are made to highlight quantitative pattern differences and link them to the chemistry of targeted compounds and tracked features of untargeted compounds. To demonstrate the effectiveness of this workflow a sample set from a research project on artisanal butter was explicated. The sample set examines the changes in volatile patterns during the production process, from raw sweet cream to ripened butter. The application of the workflow to this sample set shows how the evolution of the volatile fraction along the production chain can be captured, providing valuable insights into the chemical composition and quality changes.

Overall, the importance of Computer Vision in the field of GC×GC was highlighted, particularly when processing large amounts of data to extract meaningful information from it. The workflow described in the contribution provides a structured approach to rationalizing raw data exploration, leading to a better understanding of complex phenomena such as the chemical composition of samples and their changes over time.

References

[1] <https://www.ibm.com/topics/computer-vision>

Green extraction of Hydrolyzed Collagen Peptides (HCPs) obtained from Tuna Yellowfin side-streams after industrial dehydration process

Valentina Orlandi¹, Federica Grasso¹, Lorenzo Dondero², Elena Grasselli²,
Federica Turrini¹, Raffaella Boggia¹

¹Department of Pharmacy, University of Genoa, Genoa, Italy

²Department of Department of Earth, Environmental and Life Sciences,
University of Genoa, Genoa, Italy
valentina.orlandi@edu.unige.it

This study is part of "EcoFISHent"[1], a European Union's Horizon 2020 project (Innovation Action, Grant agreement ID: 101036428) concerning the valorization of fish side-streams for the development of nutraceutical and cosmetic products, starting from previously dehydrated biomasses. The crude biomasses, provided by Generale Conserve/As Do Mar, consisting in canning tuna processing co-products, contain fishbones, fins and heads which have been mixed, avoiding the high-cost, time- and energy-consuming sorting process [2]. Subsequently, they have been dehydrated by an industrial patented process. The dehydration step led to obtainment of powders whose low residual moisture (less than 7%) allows to stabilize this high perishable biomasses for their further extraction and valorization. The oxidation status of the so-dehydrated biomasses has been monitored by thiobarbituric reactive substances (TBARS) assay [3]. Many batches of the above-mentioned side-streams have been considered during a sampling period of 12 months.

After a preliminary proximate analysis for the evaluation of residual moisture, protein, lipid and ash contents, the so stabilized powdered material has undergone an innovative extraction flowchart to obtain Hydrolyzed Collagen Peptides (HCPs), coupling ultrasounds (Ultrasound-Assisted Extraction, UAE) with commercial enzymes (i.e., porcine pepsin). HCPs under 3 kDa have been purified by membranes and freeze-dried as well as PSC. Biological activity tests have been performed (i.e., cell viability and wound healing stimulation).

The HCPs extraction yields, despite the innovative matrix is not the traditional starting material of election, provided competitive yields.

References

[1] <https://ecofishent.eu/>

[2] V.G. Alfio, C. Manzo, R. Micillo, *Molecules*, **2021**, *26*, 1002.

[3] E.D.N.S. Abeyrathne, K. Nam, D.U. Ahn, *Antioxidants*, **2021**, *10*, 1587.

Integrated strategies against food waste: byproducts exploitation for shelf-life extension and packaging development

Antonella Cavazza¹, Maria Grimaldi², Edmondo Messinese¹, Daniel Milanese², Olimpia Pitirollo¹, Corrado Sciancalepore², Claudio Corradini¹

¹Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma

²Dipartimento di Ingegneria e Architettura, Università di Parma

antonella.cavazza@unipr.it

The deterioration of food products is one of the causes of food waste due to enzymatic or oxidative transformations altering organoleptic and nutritional properties. It is therefore advisable to design systems for control and to develop new methods of conservation that allow food shelf-life extension. Besides, food waste also involves a large part of vegetable production that is not considered suitable for commercial distribution as it does not meet requirements related to shape, size, degree of ripeness, etc. These products, together with the accumulation of considerable by-products obtained during technological processes of food transformation, are rich in bioactive substances. Many of them have antioxidant and antimicrobial properties and could represent a potential to be reinserted into the production cycle, in accordance with Circular Economy plan [1].

In this research we have focused on the possible use of extracts derived from by-products of the agri-food industry, such as artichokes and tomatoes, to enrich food products, evaluating in particular the effects on the shelf-life. For this purpose, a chemical characterization of the active molecules was carried out using chromatographic analytical techniques and spectrophotometric assays, highlighting the presence of substances such as polyphenols with marked antioxidant properties, and prebiotics, which could find wide application in cosmetic, nutraceutical, and packaging fields [2]. In particular, by adding these extracts to model foods or products, an overall effect of increased oxidative stability was evaluated using Oxitest instrumentation, allowing to take into account possible synergistic effects, and useful in predicting food preservation time [3].

Besides, new studies on innovative materials for the development of active and sustainable packaging are being addressed. The use of byproducts to obtain active films, or as fillers of biodegradable polymers is being explored with the aim of modulating mechanical and barrier properties, thus improving performances of new materials proposed as plastic substitutes.

Finally, it is remarkable to underline the need for analytical controls for safety assessment of byproducts extracts and new proposed materials, in order to evaluate the presence of possible contaminants endangering consumer's health.

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References

[1] European Commission COM/2015/0614.

[2] M. Grimaldi, O. Pitirollo, P. Ornaghi, C. Corradini, A. Cavazza, *Food Packaging and Shelf Life*, **2022**, 33, 100900.

[3] A. Cavazza, S. Corti, C. Mancinelli, C. Bignardi, C. Corradini, *Journal of the American Oil Chemists Society*, **2015**, 92, 1593.

Composition of discarded Sicilian fruits of *Opuntia ficus indica* L.: phenolic content, mineral profile and antioxidant activity in peel, seeds and whole fruit

Maria Bellumori¹, Marzia Innocenti¹, Luisa Andrenelli², Fabrizio Melani¹, Lorenzo Cecchi², Gaetano Pandino³, Giovanni Mauromicale³, Stefano La Malfa³, Nadia Mulinacci¹

¹Department of NEUROFARBA, University of Florence, Italy

²Department of Agricultural, Food and Forestry Systems Management (DAGRI), University of Florence, Italy

³Department of Agriculture, Food and Environment, University of Catania, Italy

maria.bellumori@unifi.it

Sicily (Italy) is the second producer of *Opuntia ficus-indica* (OFI) fruits after Mexico. FAO considers this plant an important resource for the future mainly because of the low production costs, the low environmental impact and the ability to grow in arid soils without the need for particular agronomic treatments. OFI fruits represent a good source of dietary fiber, vitamins and bioactive compounds which showed interesting biological activities [1-3].

To date, huge quantities of fruit are discarded during the selection for the fresh market, generating a large amount of by-products that requires to be valorized. The aim of the study was to provide data on the composition of OFI discarded fruits from the main Sicilian productive areas, San Cono and Biancavilla districts, over two harvesting periods (end of August and late-October). Peel, seed and whole fruit samples were characterized in terms of minerals and phenolic compounds through ICP-OES and HPLC-DAD-MS, respectively. Commercial OFI fruits destined for the fresh product market were included in the study to compare the quantitative results with those derived from the analyses of discarded fruits. As for the mineral content, potassium, calcium and magnesium were the most abundant elements and particularly, peel samples showed a potassium amount of 15.9-40.9 mg/g dry weight, while it was more than twenty times lower in the seeds. Seventeen phenolic compounds were detected in peel and whole fruit, including flavonoids (mainly glycosylated derivatives of isorhamnetin), phenylpyruvic and hydroxycinnamic acids, while only phenolic acids were found in the seeds. The ability of the hydroalcoholic extracts from peels, seeds and whole fruits to scavenge free radicals *in vitro* was assessed using DPPH assay. One-way ANOVA results showed significant differences between the antioxidant activity of peel and whole fruits samples from that of the seed ($p < 0.05$). A multivariate chemometric approach by principal component analysis (PCA) and linear discriminant analysis (LDA) highlighted a correlation between the mineral and phenolic content and the different parts of the fruit. As already reported in a previous study [4], a significant influence of productive area was also observed for the element and phenolic contents.

References

- [1] E.Y. Abbas, M.I. Ezzat, H.M. El Hefnawy, E. Abdel-Satta, *Journal of Food Biochemistry*, **2022**, *46*, 14310.
- [2] F.J. Barba, C. Garcia, A. Fessard, P.E.S. Munekata, J.M. Lorenzo, A. Aboudia, A. Ouadia, F. Remize, *Food Reviews International*, **2020**, *38*, 930.
- [3] D.M. Amaya-Cruz, I.F. Perez-Ramirez, J. Delgado-Garcia, C. Mondragon-Jacobo, A. Dector-Espinoza, R. Reynoso-Camacho, *Food Chemistry*, **2019**, *278*, 568.
- [4] A.F. Mottese, C. Naccari, R. Vadalà, G.D. Bua, G. Bartolomeo, R. Rando, N. Cicero, G. Dugo, *Journal of Food Science*, **2018**, *83(12)*, 2933.

Integrated and sustainable strategy for the investigation and valorization of Extra Virgin Olive Oil extracts and by-products

Martina Bartolomei, Carlotta Bollati, Jianqiang Li, Carmen Lammi

Department of Pharmaceutical Sciences, University of Milan, 20133 Milan, Italy
martina.bartolomei@unimi.it

Polyphenols are natural compounds extensively used among nutraceutical products for the prevention and/or treatment of chronic disease, immunological and inflammatory disorders [1,2]. Many foods have a high content of polyphenols and extra virgin olive oil (EVOO) stands out in the Mediterranean diet. EVOO is the main product obtained by olives, fruits collect from the *Olea europaea L.* trees which contains about 500 mg/L of polyphenols [3]. Consumption of high-polyphenol EVOO has been shown to have a cardioprotective effect by protecting low-density lipoprotein (LDL) from oxidative stress, reducing triglycerides, and maintaining normal blood cholesterol concentrations of high-density lipoprotein (HDL) [4,5]. Therefore, we evaluate the cholesterol-lowering properties of two EVOO phenolic extracts, obtained from different *cultivars*, demonstrating that it is not only the content of monounsaturated fatty acids that show cholesterol-lowering properties, but also the presence of phenolic compounds. Indeed, the extracts modulate the intracellular cholesterol pathway, leading to an increase of the LDL-R and HMGCoAR protein levels through the modulation of SREBP-2 protein level but were not able to modulate the mature PCSK9 protein levels in human hepatic HepG2 cells [6]. Furthermore, the bioactivity of a *Frantoio* EVOO phenolic extract was assessed through the comprehensive characterization of its antioxidant power both *in vitro* and in two different cellular models, i.e., Caco-2 and HepG2 cells. An aspect of great importance is the absorption and bioavailability of polyphenols. Using differentiated human intestinal Caco-2 cells it was possible to determine the synergistic effect of a mixture of polyphenols and their transepithelial transport [7]. Food waste, by-product and side-stream from agricultural and food industries represent an attractive and cheaper source of proteins and bioactive compounds. In the Mediterranean area, olives and olive oil by-products have a significant environmental impact [8]. Considering the principles of sustainability and circular economy, the antioxidant and hypoglycemic activities of the olive stone hydrolysates were evaluated. In further detail, the effect of olive seed hydrolysates on the secretion of the incretin hormone glucagon-like peptide 1 (GLP-1) and the inhibition of the serine protease dipeptidyl peptidase-IV (DPP-IV) were evaluated using an innovative co-culture system using intestinal Caco-2 and STC-1 cells [9].

References

- [1] C.G. Fraga, K.D. Croft, D.O. Kennedy, F.A. Tomás-Barberán, *Food Funct.* **2019**, *10*, 514.
- [2] M. Abbas, F.Saeed, F.M. Anjum, M. Afzaal, T. Tufail, M. Shakeel Bashir, A. Ishtiaq, S. Hussain, H. Suleria, *International Journal of Food Properties*, **2017**, *20*, 1689.
- [3] M. Gorzynik-Debicka, P. Przychodzen, F. Cappello, A. Kuban-Jankowska, A.M. Gammazza, N. Knap, M. Wozniak, M. Gorska-Ponikowska, *International Journal of Molecular Sciences*, **2018**, *19*, 547.
- [4] M.I. Covas, K. Nyyssönen, H.E. Poulsen, J. Kaikkonen, H.J.F. Zunft, H. Kiesewetter, A. Gaddi, R.De La Torre, J. Mursu, H. Bäuml, S. Nascetti, J.T. Salonen, M. Fitó, J. Virtanen, J. Marrugat; EUROLIVE Study Group, *Annals of Internal Medicine*, **2006**, *145*, 333.
- [5] M.A. Carluccio, M. Massaro, E. Scoditti, R. De Caterina, *Molecular Nutrition & Food Research*, **2007**, *51*, 1225.
- [6] C. Lammi, M. Bellumori, L. Cecchi, M. Bartolomei, C. Bollati, M.L. Clodoveo, F. Corbo, A. Arnoldi, N. Mulinacci, *Nutrients*, **2020**, *12*, 1.
- [7] M. Bartolomei, C. Bollati, M. Bellumori, L. Cecchi, I. Cruz-Chamorro, G. Santos-Sánchez, G. Ranaldi, S. Ferruzza, Y. Sambuy, A. Arnoldi, N. Mulinacci, C. Lammi, *Antioxidants*, **2021**, *10*, 1.
- [8] I.E. Kapellakis, K.P. Tsagarakis, J.C. Crowther, *Reviews in Environmental Science and Bio/Technology*, **2008**, *7*, 1.
- [9] M. Bartolomei, A.L. Capriotti, Y. Li, C. Bollati, J. Li, A. Cerrato, L. Cecchi, R. Pugliese, M. Bellumori, N. Mulinacci, A. Laganà, A. Arnoldi, C. Lammi, *Antioxidants*, **2022**, *11*, 1730.

Valorisation of residual orange peels from PDO cultivars of the Ribera area, Sicily (Italy): extraction, characterization and bioactivity assessment of essential oils and secondary metabolites

Gregorio Peron¹, Sara Marcheluzzo², Giulia Bernabé³, Gokhan Zengin⁴, Kouadio Ibrahime Sinan⁴, Michela Paccagnella², Ignazio Castagliuolo³, Mirella Zancato², Stefano Dall'Acqua²

¹Department of Molecular and Translational Medicine, University of Brescia, Italy

²Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Italy

³Department of Molecular Medicine, University of Padova, Italy

⁴Department of Biology, Science Faculty, Selcuk University, Konya, Turkey

gregorio.peron@unibs.it

Sicily is the main producer of sweet orange (*Citrus sinensis*) in Italy [1]. In 2008, the “Arancia di Ribera” Protected Designation of Origin (PDO) was established [2], and characteristic Navel cultivars such as Washington Navel (WAS) and Navelina (NAV) were included in this label. The Vaniglia (VAN) cultivar, whose fruit is characterized by blonde pulp and particular organoleptic properties, is another typical product of the Ribera area that has been labelled as PDO.

A considerable part of cultivated oranges undergoes industrial processing for orange juice production, and this chain generates tons of pulp and peels as waste *per* year. Peels are mostly discarded and destined to landfilling or composting, causing environmental issues [3]. In this work, peels of WAS, NAV, and VAN oranges were extracted by using an optimized microwave-assisted method, which allowed to obtain both bioactive secondary metabolites and essential oil (EO) in a single step, using water as solvent. Bioactive components of EOs and extracts were characterized, and their antioxidant, antibacterial, and enzyme inhibitory properties were assessed *in vitro*. The EO from the three cultivars showed a similar composition, characterized by an abundance of D-limonene (85%). α -Thujene was the marker of VAN, 3-carene and α -copaene of WAS, and germacrene D and valencene were characteristic of NAV. EOs showed a significant toxicity against gram- and gram+ bacteria of clinical interest, but also on Caco2 cells. This indicates their low suitability as food ingredients but suggests a possible use as anti-proliferative agents. Several flavonoids and coumarins were identified in the aqueous extracts, and narirutin, obacunonic acid, and roseoside were the markers of NAV, VAN, and WAS, respectively. The same extracts were effective in inhibiting the human enzymes tyrosinase, amylase, and glucosidase *in vitro*. Through a multivariate analysis of LC-MS and bioactivity data, correlations between specific chemical constituents and bioactivities of the extracts were highlighted.

Our results are intended to increase the profitability and the sustainability of the orange fruit supply chain following the principles of the Circular Economy. Extracts from peels of PDO orange cultivars from the Ribera area represent valuable sources of bioactive compounds and EOs to be used in novel nutraceuticals and can be obtained using eco-sustainable and low-cost approaches.

References

[1] ISTAT.

[2] S. Tudisca, A. M. Di Trapani, F. Sgroi, R. Testa, *Quality - Access to Success*, **2014**, 14, 99.

[3] V. Negro, B. Ruggeri, D. Fino, D. Tonini, *Resources, Conservation and Recycling*, **2017**, 127, 148.

**Development of sustainable food ingredients from avocado waste and by-products.
Drying technologies and sensory characteristics**

Maria Merlino, Fabrizio Cincotta, Anthea Miller, Martina Buda,
Antonella Verzera, Concetta Condurso

Department of Veterinary Sciences, University of Messina, Viale G. Palatucci, 98168, Messina, Italy
maria.merlino@unime.it

Avocado, scientifically known as *Persea americana* Mill., is a tropical and subtropical fruit that is native to Mexico and Central America [1]. It has had a sharp rise in cultivation over the past few years in south Italy, particularly in Sicily. The main causes are to be found in climate change, and in the consumer's increasing interest in healthy food; avocado fruits are in fact rich in bioactive substances, such as unsaturated fatty acids, ascorbic acid, vitamin E, polyphenols, and carotenoids [2]. However, the avocado processing industry generates a significant quantity of waste and by-products [3]. In the context of a circular economy could be of great interest to valorize waste and by-products of the Avocado productions making flours rich of bioactive compounds to be used in the food sector. Thus, the research aims to develop sustainable food ingredients, with appreciated sensory features, from leaves and seed of the Hass Avocado variety cultivated in Sicily; to reach the goal, the effect of different drying techniques on the aroma compounds and sensory characteristics of the obtained flours have been evaluated. Instrumental and sensory analyses have been carried out. A large number of aroma compounds have been identified by SPME-GC-MS and the sensory descriptors of the flour have been defined by a Qualitative Descriptive Sensory analysis (QDA). The results demonstrated the importance of suitable drying technologies to obtain nutritional and sensory valuable flours from the avocado waste and by-products.

References

- [1] P. Duarte, M. Chaves, C. Borges, C. Mendonça, *Ciência Rural*, **2016**, *46*, 747.
- [2] R. Araújo, R. Rodriguez-Jasso, H. Ruiz, M. Pintado, C. Aguilar, *Trends in Food Science & Technology*, **2018**, *80*, 51.
- [3] T. Tesfaye, M. A. Mebrate, M. Gibril, E. Ferede, D. Y. Limeneh, F. Kong, *Current Research in Green and Sustainable Chemistry*, **2022**, *5*, 100253.

Survey on the presence of acrylamide in street food marketed and produced in Italy and application of possible mitigation measures

Francesco Giuseppe Galluzzo^{1,2}

¹Istituto Zooprofilattico Sperimentale della Sicilia, Via Gino Marinuzzi 3, 90129 Palermo, Italy

²Dipartimento di Scienze della Vita; Università degli studi di Modena e Reggio Emilia,

Via Università 4, 41121 Modena, Italy

francescogiuseppe92@gmail.com

The acrylamide concentration was investigated in different street food products bought in supermarkets (in Southern Italy) or made in a laboratory. High-pressure liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS-MS) was used as a validated method for the analysis. The concentration of twenty-three different food matrices was divided into three categories depending on the cooking method and was analyzed. Products were classified as either: fried food products (arancine, pannelle, cannoli, panissa, crema frita, panzerotti, crocchette, pizza frita, falafel, scagliozzi, courgette flowers, seadas, olive ascolane, sgabei), oven products (bretzel, cecina, erbazzone, fugassa) and pan products (crepes, dosa, piadina romagnola, tacos, tortillas). The highest acrylamide concentration was found in courgette flowers, with a concentration of 1539.8 µg/kg, while the lowest concentration was found in panissa, with a concentration of 8.3 µg/kg. The incidence of positive samples with a detectable amount of acrylamide was highest in baked products (96.3%), followed by products cooked in a pan (73%) and fried foods (68.2%). As for the average values, samples cooked in the oven revealed the highest levels of acrylamide concentration (77.67 ± 83.01 µg/kg), followed by fried products (67.65 ± 129.12 µg/kg) and those cooked in a pan (38.20 ± 49.24 µg/kg). No statistically significant differences were found between products made with the same cereals (wheat flour, manitoba flour), while the Kruskal Wallis test showed differences between products made with different types of flour (wheat flour, chickpea flour, potatoes, rice, and corn). In the fried group, extra virgin olive oil, sunflower oil, and peanut oil were used for frying, and the Kruskal-Wallis test revealed no statistically significant differences between the oils used. One sample of bretzel exceeded the limit imposed by (EC) Regulation No. 2017/2158 at 350 µg/kg. Courgette flowers showed the highest acrylamide levels with a mean of 286.93 ± 345.19 µg/kg, and six samples (30%) exceeded the benchmark levels established by (EC) Regulation No. 2017/2158. A four-factor study design was conducted for mitigation measures, including freezing the samples after cooking, the time and temperature of frying, and the type of oil used. Ten samples of courgette flowers were produced for each mitigation condition, for a total of 160 samples. The courgette flowers obtained were subjected to organoleptic evaluation by seven expert panelists, considering five parameters: color, smell, appearance, consistency, and taste. Each panelist assigned a score from 1 to 7, where one corresponds to poor and 7 to excellent. Three conditions reduced acrylamide content significantly (9, 16, 14). To the best of our knowledge, this was the first study conducted on these food matrices.

References

[1] EC (European Commission) Commission Regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. *OJEU*. **2017**; L304:24–44.

***Calendula arvensis* florets: effect of freeze-drying on the encapsulation of its specialized metabolites and oral bioaccessibility**

Marika Fiorentino, Simona Piccolella, Assunta Esposito, Severina Pacifico

Department of Environmental, Biological and Pharmaceutical Sciences and Technologies,
University of Campania “Luigi Vanvitelli”, Via Vivaldi 43, 81100 Caserta, Italy
marika.fiorentino@unicampania.it

The term “alimurgia”, coined by Giovanni Tozzetti in 1767, refers to the need to consume wild food (mainly from plants) to solve the starvation problem [1]. Phytoalimurgy, the dietary use of wild plants for famine in ancient times, is again gaining popularity due to the increased awareness that these plants are an invaluable source of micro- and macro-nutrients as well as bioactive compounds [2].

Calendula arvensis (Vaill.) L. is a worldwide known medicinal plant, widely exploited in the folk tradition in the Mediterranean countries for making pleasant dishes. In particular, the inflorescences are served raw in salads, employed for preparing candies, whereas a flower vinegar can be made soaking dried *Calendula* inflorescence in the wine for two weeks [3].

Investigating the chemical composition of *Calendula arvensis*, previously harvested in the territory of Cilento, Vallo di Diano and Alburni National Park, the diversity in specialized metabolites of its organs, mainly polyphenols and triterpene saponins, has been unravelled opening new scenario for food and/or nutraceutical usage [4]. Indeed, safeguarding the chemical features of these bioactive compounds is mandatory, since, beyond abiotic and biotic factors, digestion processes in the gastrointestinal tract could affect their native structure, thus decreasing their bioactivity [5].

Herein, the freeze-drying of *Calendula arvensis* floret extract (FE) with maltodextrin (MD) as a carrying agent for encapsulation of bioactive compounds has been exploited. In particular, the alcoholic extract by ultrasound-assisted maceration (UAM) from field marigold florets was encapsulated in maltodextrin (MD) reaching a 1:2 (FE:coating) ratio. The easily-handling powder was preliminarily analysed by Fourier Transform Infra-Red (FTIR) spectroscopy, while UHPLC-HRMS/MS-based profiling was employed to chemically profile FE extract also during *in vitro* simulated digestion. Furthermore, the ability of the encapsulate to preserve FE bioactivity was ascertained by means of DPPH and ABTS tests, and Caco-2 cells viability assessment. The H-interaction established between FE and MD was in accordance with the high biocompatibility of the encapsulate in Caco-2 cells, whereas the *in vitro* digestion significantly modified the structural integrity and/or the diversity of FE compounds and FE antioxidant efficacy.

References

- [1] O. Mattiolo, 2017, V. Bona, Torino, Italy, **1918**.
- [2] M. Marrelli, G. Statti, F. Conforti, *Molecules* **2020**, 25, 649.
- [3] A. Ranfa, M. Bodesmo, *Journal of Applied Botany and Food Quality*, **2017**, 90, 246.
- [4] M. Fiorentino, C. Gravina, S. Piccolella, M.T. Pecoraro, M. Formato, A. Stinca, S. Pacifico, A. Esposito, *Foods*, **2022**, 11, 247.
- [5] K. Ravichandran, R. Palaniraj, N.M. Saw, *Journal of Food Science and Technology*, **2014**, 51, 2216.

NMR and HPLC characterization of *Gentiana lutea* L. aerial parts: flowers as ingredient for functional foods with anti-mycotoxins activity

Giacomo Di Matteo¹, Mattia Spano¹, Massimo Frangiamone²,
Alessandra Cimbalo², Lara Manyes², Luisa Mannina¹

¹Department of Chemistry and Technology of Drugs, Laboratory of Food Chemistry,
Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy

²Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy,
University of Valencia, Burjassot, 46100 Valencia, Spain

giacomo.dimatteo@uniroma1.it

An NMR and HPLC metabolomic characterization of roots, leaves and flowers of *Gentiana lutea* L. harvested in the “Majella National Park” of Abruzzo Region was carried out to find a rational utilization of the aerial parts. The *Gentiana* roots are largely used in the world as source of many bioactive compounds with interesting pharmacological effects, reported also in the Chinese, Japanese and European Pharmacopeias and also to prepare some bitter beverages. Unfortunately, due to the *Gentiana* roots massive collection along century there is a depletion of the natural population. In this scenario, it is of interest the study of the chemical profile of the Italian wild *Gentiana lutea* L. in order to valorize its aerial parts. For this purpose, the untargeted high-field NMR spectroscopy to obtain a whole chemical profile (sugars, amino acids, organic acids, etc.) and the targeted HPLC analysis to quantify the amounts of iridoids, secoiridoids and xanthenes, the most studied *Gentiana* bioactive compounds, were performed on the different plant parts (roots, leaves and flowers). The *Gentiana lutea* leaves had turned out to be a rich source of iridoids, xanthenes and secoiridoids with compound quantity variations during the growing. On the other hand, the *Gentiana lutea* flower presented the highest level of the other secondary metabolites (sugars, organic acids, amino acids). Thus, the flower extract was further studied realizing *in vitro* digestion, bioavailability assay using Caco-2 cell cultures, qPCR analysis to investigate the modulation of selected genes, and proteomics analysis to study the modulation of proteins expression. The *Gentiana* flower duodenal extracts modulate the expression of genes marker of the apoptosis (CASP3) and of the intestinal barrier integrity (ZO-1) in Caco-2 cells contaminated with mycotoxins (AFB1, OTA, BEA). Hence, the flowers could be used as ingredients for functional foods with anti-mycotoxins properties and the leaves for the extraction of bioactive compounds.

Combination of Pomegranate Extract, B Vitamins and Vitamin C against Prolonged Fatigue: a monocentric, randomized, double-blind, placebo-controlled clinical trial

Lorenza Francesca De Lellis¹, Hammad Ullah¹, Daniele Giuseppe Buccato¹, Alessandra Baldi¹, Gaetano Piccinocchi², Roberto Piccinocchi³, Alessandro Di Minno^{1,4}, Maria Daglia^{1,5}

¹Department of Pharmacy, University of Naples Federico II, Naples, Italy

²ComegenS.c.S., Società Cooperativa Sociale di Medici di Medicina Generale, Viale Maria Bakunin 41, 80125 Naples, Italy

³Level 1 Medical Director Anaesthesia and Resuscitation A. U. O. Luigi Vanvitelli, Via Santa Maria di Costantinopoli, 80138 Naples, Italy

⁴CEINGE-Biotecnologie Avanzate, Via Gaetano Salvatore 486, 80145 Naples, Italy

⁵International Research Center for Food Nutrition and Safety, Jiangsu University, Zhenjiang, 212013, China
lo.delellis2@libero.it

Fatigue, classified as prolonged fatigue (PF), which can be considered a physiological condition, and chronic fatigue (CF), a pathological condition also called as Myalgic encephalomyelitis, is characterized by periods of exhaustion, interfering with normal activities that negatively affect quality of life [1–2]. Vitamin deficiency and chronic inflammation seem to be possible causes of fatigue. Due to the unavailability of effective remedies, attenuating fatigue and providing consumer satisfaction, the current investigation is aimed to evaluate the anti-fatigue potential of a commercial food supplement based on pomegranate extract, with known anti-inflammatory activity, and vitamins B and C, in healthy subjects with PF. A monocentric, randomized, double-blind, placebo-controlled clinical trial was conducted on a food supplement containing a chemically characterized pomegranate extract, which consists of ellagitannins, gallotannins, and organic and phenolic acids. For the clinical trial, 58 subjects were randomized into two groups, and received either the food supplement or placebo. The measurement of fatigue (Fatigue Severity Scale - FSS) and quality of life (Short Form Health Survey-12 - SF-12) was evaluated by two validated questionnaires. As secondary outcomes, measurement of biomarkers generally related to fatigue and stress conditions were evaluated (C-reactive protein, cortisol, IL-6, Magnesium, Potassium, Calcium, Creatine phosphokinase, Vitamins of group B and Vitamin D). For the FSS, the treatment induced highly significant effects ($p=0.034$) in comparison with placebo. No statistically significant effects were identified for the other parameters and SF-12 questionnaire. In conclusion, this food supplement can be considered useful for PF. Other studies will be performed to unravel the mechanisms of action underlying the recorded effects on fatigue.

References

- [1] C. Esposito, C. Santarcangelo, A. Di Minno, R. Sacchi, E. Sommella, L.F. De Lellis, M.A De Pasquale, F. Montarolo, P. Campiglia, A. Baldi, C Riccioni, M. Daglia, *Processes*, **2022**, *10*, 208.
- [2] H. Ullah, A. Khan, C. Riccioni, A. Di Minno, A. Tantipongpiradet, D. G. Buccato, L. F. Del Lellis, H. Khan, J. Xiao, M. Daglia, *Phytochemistry Reviews*, **2022**, *1*.

Investigation of the hypocholesterolemic activity of an innovative plant derived extract

Lorenza d'Adduzio, Davide Marangon, Umberto Musazzi, Carlotta Bollati, Martina Bartolomei, Davide Lecca, Carmen Lammi

Department of Pharmaceutical Sciences, University of Milan, 20133 Milan, Italy
lorenza.dadduzio@unimi.it

Plant products are among the most widely consumed foods due to increased consumers awareness about their high nutritional value and the proven correlation between their ingestion and the prevention of cardiovascular disease (CVD) [1]. Plant products contain a wide range of bioactive compounds whose major effects are achieved due to the antioxidant, anti-inflammatory, antimicrobials, and hypocholesterolemic properties [2]. Interestingly, plant products rich in bioactive compounds as vitamin C, glucosinolates and polyphenols show antioxidant and anticancer properties and are implicated in the reduction of the risk of cardiovascular and cognitive diseases [3]. This study was aimed at investigating the hypocholesterolemic effect of plant derived extract (under patent). Indeed, hypercholesterolemia is one of the main risk factors responsible for CVD outbreak. In the present study, experiments were carried out on HepG2 cells, since the hepatocyte is the major cell involved in the low-density lipoprotein (LDL) clearance by the LDL receptor (LDLR) activity. The LDLR expression is tuned by changes in intracellular cholesterol levels and a transcription factor, the sterol-responsive element binding protein-2 (SREBP-2), which plays a key role in LDLR mRNA expression [4]. Among SREBP-2 gene targets, the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoAR) is particularly important [5]. Results showed that the plant extract increases LDL receptor protein levels through the activation of SREBP-2 transcription factor, leading to enhanced ability of hepatic cells to uptake extracellular LDL molecules with a final hypocholesterolemic effect. Moreover, the plant extract regulates the intracellular HMGCoAR activity through the increase of its phosphorylation by the activation of AMP-activated protein kinase (AMPK)-pathways; lastly, unlike statins, the plant extract does not produce any unfavorable effect on proprotein convertase subtilisin/kexin 9 (PCSK9) protein level.

References

- [1] D. Aune, *Advances in Nutrition*, **2019**, *10*, 404.
- [2] M. Taroncher, P. Vila-Donat, J. Tolosa, M. J. Ruiz, Y. Rodríguez-Carrasco, *Current Opinion in Food Science*, **2021**, *42*, 118.
- [3] L. Bell, C. Wagstaff, *Journal of Agricultural and Food Chemistry*, **2014**, *62*, 4481.
- [4] J. L. Goldstein, M. S. Brown, *Nature*, **1990**, *34*, 425.
- [5] J. D. Horton, J. L. Goldstein, M. S. Brown, *Journal of Clinical Investigation*, **2002**, *109*, 1125.

Cold pressed seed oils as a new source of nutraceuticals – A Comprehensive Chemical Characterization

Cinzia Cafarella¹, Francesca Rigano¹, Emanuela Trovato¹, Paola Dugo^{1,2}, Luigi Mondello^{1,2}

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

²Chromaleont S.R.L., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy
cicafarella@unime.it

Edible seed oils have been extracted and used as a source of food ingredients since ancient times. As the body of evidence that links health benefits to the consumption of vegetable oils continues to grow, as well as the diffusion of vegetarian/vegan diet, many consumers prefer vegetable oils instead of animal fats. Due for that, the demand for high quality seed oils has greatly increased. Next to the rising consumption of conventional vegetable oils (olive, soybean, peanut, etc.), unconventional seed oils are appearing on the market (chia, hemp, etc.). Within this context, the aim of the present work was to investigate some unconventional cold-pressed seed oils with an attractive profile. In particular, carrot, raspberry, strawberry, blackcurrant, radish, pomegranate, rosehip and plum seed oils were investigated in terms of their chemical composition, including fatty acids, triacylglycerols, tocopherols (vitamin E), phenols and carotenoids, and the interesting volatile profile. Indeed, the peculiar feature of such seed oils is related to their aroma and color, *viz.* their organoleptic characteristics. The fatty acid composition and volatile fraction were elucidated by Gas Chromatography coupled to Flame Ionization Detector and Mass Spectrometry (MS). Intact lipids, in the form of triacylglycerols, were reliably identified through liquid chromatography (LC) coupled to MS, with an automatic dual-filter identification strategy, which exploits the complementarity between Linear Retention Index and a home-made MS spectral library [1]. Vitamin E was fast determined without sample preparation procedures by means of a simple and cost-effective instrumentation, based on a sensitive and selective fluorimetric detection, coupled to LC. The phenolic and the carotenoid extracts were analyzed by LC coupled to Photodiode Array Detector and MS, using Electrospray and Atmospheric Pressure Chemical Ionization interface, respectively. Besides, a statistical tool was applied to identify similarities and differences in the chemical composition of investigated oils. The qualitative and quantitative data would represent the basis of future applications or selection of those seed oils for health purposes as a source of bioactive compounds.

References

[1] L. Mondello, F. Rigano, Identification of unknown compounds by using a novel retention index system in liquid chromatography, *PCT patent*, 2017.

***Sonchus asper* (L.) Hill: revalorization of wild edible plant of traditional use**

Valentina Santoro¹, Valentina Parisi¹, Luigi Milella², Carla Caddeo³, Antonio Nesticó⁴,
Anna Lisa Piccinelli¹, Luca Rastrelli¹, Nunziatina De Tommasi¹

¹Department of Pharmacy, University of Salerno, 84084 Fisciano, Italy

²Department of Science, University of Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy

³Department of Scienze della Vita e dell'Ambiente, Sezione di Scienze del Farmaco,
University of Cagliari, Via Ospedale 72, 09124 Cagliari, Italy

⁴Department of Engineering, University of Salerno, 84084 Fisciano, Italy

vsantoro@unisa.it

Wild edible plants represent a very important food habits around the world [1]. There are many socio-cultural and economic benefits for local communities and farmers involved in the production and harvesting of these plants. In addition, in the last years the increasing attention to avoid environmental pollution, as well as the rationalization of the agro-industrial cycle has stimulated the search for a possible exploitation of residual vegetables, in a perspective of circular bioeconomy [2]. This research is focused on the valorization of *Sonchus asper* (L.) Hill through a chemical-biological study of the raw extract and its nanoformulated products. *S. asper* is a wild edible plant harvest in the inland areas of the Campania region, typically used in food traditional recipes with a low commercial value.

The raw and cooked extracts obtained from edible leaves, by a green extraction technique, were characterized by liquid chromatography-high resolution mass spectrometry. The analysis led to the identification of 38 compounds belongs to different chemical classes, as flavonoids, phenolic acids, and unsaturated fatty acids, besides coumarins and C13-norisoprenoid glycosides in traces. In this study, the antioxidant and hypoglycaemic activities of the *S. asper* and its by-products, obtained by the discarded leaves incorporated into liposomes, were also investigated. The results showed for *S. asper*-based extracts promising antioxidant and hypoglycaemic activities.

Finally, an economic analysis for *S. asper* utilization was performed. From obtained data, the strategy for the valorization and marketing of *S. asper* based on a co-operation mechanism between the consortium members and the owner of uncultivated land is financially sustainable.

References

- [1] M. de Cortes Sanchez-Mata, J. Tardío, *Mediterranean Wild Edible Plants: Ethnobotany and Food Composition Tables*, Springer, 2016.
- [2] L. Bacchetta, F. Visioli, G. Cappelli, E. Caruso, G. Martin, E. Nemeth, G. Bacchetta, G. Bedini, A. Wezel, T. van Asseldonk, L. van Raamsdonk, F. Mariani, *Journal of Ethnopharmacology*, 2016, 191, 180.

From agri-food waste to zero-impact source: the pomegranate as a model system

Francesco Cairone, Chiara Salvitti, Irene Arpante, Caterina Frascchetti,
Antonello Filippi, Stefania Cesa

Department of Drug Chemistry and Technology "Sapienza" University of Rome
francesco.cairone@uniroma1.it

In recent years, research has been focused on obtaining and evaluating high-value compounds from agricultural waste, for possible re-use in various application fields. Many food matrices' metabolites, such as polyphenolics and many other substances, may be present in the waste deriving from primary productions. In order to promote a more environmentally sustainable approach, the circular economy represents one of the most efficient political-economic strategies [1]. In this perspective, our research activity aims to the valorization, purification and characterization of the phytochemical complex of the non-edible part of different matrices, such as the pomegranate peel and seeds. The pomegranate fruit is a rich source of hydrolysable tannins, such as punicalagin and punicalins, as well as of ellagic acid.

In our previous works, the presence of bioactive polyphenols in the peels was evaluated, highlighting high content of punicalagins and ellagic acid [2,3]. The seeds also represent a high-value waste material. Punicic acid, representing the main fatty acid of seeds' pomegranate oil, is involved in different activities such as antioxidant, anti-diabetic and anti-inflammatory ones [4].

Specifically, the separated peels, of several selected pomegranate cultivars, were subjected to different extraction methods, such as conventional or microwave hydroalcoholic extractions, supercritical CO₂ and solid phase extractions, in order to concentrate punicalagins, and ellagic acid (recovery of about 5-10%) and to extract the fibrous polysaccharide fraction (recovery of about 20-30%). The different obtained extracts were characterized and evaluated by colorimetric (CIEL*a*b* analysis), chromatographic (HPLC-DAD) and spectroscopic analysis (NMR and IR). The biological activity of the ellagitannin-rich extracts was estimated with spectrophotometric assays such as DPPH as well as enzyme inhibition assays on α -amylase and α -glucosidase, and PDIA3. The promising obtained data allowed to evaluate the possibility of using these extracts in supplement and nutraceutical formulations. For the extraction of the polymeric component, in addition to classical methods (enzymatic treatments, acid-base treatments), a less impactful CO₂ extraction method was developed. Nano-celluloses could then be used as supports with applications in pharmaceutical technology, in the preparation of cell scaffolds and in tissue engineering. Finally, an environmentally sustainable CO₂ extraction method of pomegranate seed's oil was optimized, with excellent results in terms of yield and composition, if compared to conventional Soxhlet extraction. The qualitative composition was studied using different analytical techniques, such as MALDI-TOF, ¹H-NMR and GC-MS. The biological activity of the obtained oils was finally evaluated by means of antioxidant and enzyme inactivation assays.

References

- [1] A. Muscio, R. Sisto, *Sustainability*, **2020**, *12*, 5554.
- [2] D. Balli, L. Cecchi, M. Khatib, M. Bellumori, F. Cairone, S. Carradori, G. Zengin, S. Cesa, M. Innocenti, N. Mulinacci, *Antioxidants*, **2020**, *9*, 238.
- [3] F. Altieri, F. Cairone, F. Giamogante, S. Carradori, M. Locatelli, S. Chichiarelli, S. Cesa, *Nutrients*, **2019**, *11*, 186.
- [4] M. A. Shabbir, M. R. Khan, M. Saeed, I. Pasha, A. A. Khalil, N. Siraj, *Lipids in Health and Disease*, **2019**, *16*, 1.

Evaluation of the pistachio's aroma compounds by high-capacity concentration tools coupled with multi-dimensional gas chromatography

Andrea Schincaglia¹, Giorgia Purcaro², Nicola Marchetti¹, Alberto Cavazzini¹, Marco Beccaria¹

¹University of Ferrara, Italy

²Gembloux Agro-Bio Tech, University of Liège, Belgium

schndr@unife.it

Aroma is a key organoleptic characteristic of pistachio that determines its quality and consumer acceptance. As for all-natural products, the aroma is highly dependent on many variables such as the cultivar, geographical origins, harvesting conditions, storage, etc. Most of the pistachio production comes from countries with a warm, arid climate, indeed, the world's leading producers of pistachio are Iran, the United States (US), Italy, Greece, Tunisia, Turkey, Syria, and Spain. Determining their characteristic aroma fingerprints is essential from a consumer acceptance viewpoint and for standardizing pistachio-based product production in industries. The present study evaluated the volatile organic compounds (VOCs) of pistachio from different geographic origins. A high-capacity concentration (HCC) tool, named HiSorb™, was used to perform this evaluation, showing high sensitivity and repeatability, thanks to an automated and easy-handling interface. Different HiSorb™ probes were evaluated to obtain the best results, as well as temperature and time of extraction. VOCs extracted with HiSorb™ were successively analyzed with two-dimensional comprehensive gas chromatography-mass spectrometry (GC×GC-MS), equipped with a reversed fill/flush flow modulator. The use of GC×GC allows an enhancement of separation power and maximises the level of information extracted, obtaining in this way a chromatographic fingerprint of pistachio's VOCs.

Innovative active layer-by-layer edible coating formulation to preserve the qualitative and biochemical traits of 'Della Recca' sweet cherries

Anna Magri^{1,2}, Rosaria Cozzolino³, Livia Malorni³, Gianluca Picariello³,
Francesco Siano³, Milena Petriccione²

¹Department of Environmental, Biological and Pharmaceutical Sciences and Technologies (DiSTABiF), University of Campania Luigi Vanvitelli, Via Vivaldi 43, 81100 Caserta, Italy. ²Council for Agricultural Research and Economics (CREA), Research Center for Olive, Fruits, and Citrus Crops, Via Torrino 3, 81100 Caserta, Italy. ³Institute of Food Science, National Research Council (CNR), Via Roma 64, 83100 Avellino, Italy.

anna.magri@unicampania.it

Sweet cherry (*Prunus avium* L.) belongs to the *Rosaceae* family, *Prunoideae* subfamily, and *Prunus* genus. These fruits originated around the Caspian and Black seas, nowadays they are geographically spread all around the world. In 2020, the annual world production was around 2.5 million tons, with the largest producers being Turkey, the United States, and Chile. Italy was the sixth-largest producer in the world and the largest in Europe [1]. Sweet cherries are among the most well-liked temperate fruits due to their appealing color, flavor, scent, and antioxidant capabilities. These fruits have a limited postharvest due to physiological decay reactions that cause stem browning and deterioration, softness, pitting, and water loss [2]. Fruits' shelf life is extended during postharvest by using a range of methods, including controlled atmosphere storage, modified atmosphere packing, irradiation, and precooling [3]. Besides the application of traditional postharvest practices, the development of coatings and polymer technologies have drawn a lot of interest in the food industry, as several bio-based polymers, used as edible coatings, have proved to extend the postharvest life of very perishable crops. An edible film coating creates a barrier between the fruit surface and the environment, reducing the major loss in quality after harvest of crops [4]. To contribute to this challenge, in this study a functional layer-by-layer (LbL) coating consisting of carboxymethylcellulose (CMC), sodium alginate (SA), oxalic and citric acid (OA and CA) was applied on 'Della Recca' sweet cherry fruits in cold storage conditions (4 ± 0.5 °C, RH $80 \pm 1\%$) for 16 days. To investigate the impact of the LbL coating, changes in the qualitative parameters, such as total soluble solid content, weight loss, titratable acidity, stem color, pH, water activity and firmness, were observed during the storage. Moreover, biochemical traits, including total phenols, flavonoids, anthocyanins, ascorbic acid, antioxidant activity, antioxidant enzymes, and the profiles of phenolic compounds and volatile metabolites were also detected. Results demonstrated that the edible LbL coating can be extended, until the 16th day, the postharvest life of 'Della Recca' sweet cherries, decreasing the weight loss and stem browning, and maintaining the firmness. Furthermore, the LbL coating influenced the production of secondary metabolites in fruits and total chlorophyll content in the stem. Additionally, the coating reduced the fruit browning causing by polyphenoloxidase and guaiacol peroxidase as well as boosted the actions of antioxidant enzymes that lessen oxidative damage by regulating ROS metabolism. The considerable decrease in lipoxygenase activity and malondialdehyde level suggests that the improved storability and delayed onset of senescence provided by this formulation also helped to maintain the membrane integrity. Overall, the results of this study suggest that increasing the shelf-life of sweet cherries on a larger scale can be feasible thanks to the cost-effectiveness of the used polymers.

References

- [1] FAO 2020.
- [2] A Magri, L. Malorni, R. Cozzolino, G. Adiletta, F. Siano, G. Picariello, D. Cice, G. Capriolo, A. Nunziata, M. Di Matteo, M. Petriccione, *Plants*, **2023**, *12*, 610.
- [3] Y. Fang, M. Wakisaka, *Agriculture*, **2021**, *11*, 992.
- [4] S. Chockchaisawasdee, J.B. Golding, Q.V. Vuong, K. Papoutsis, C. E. Stathopoulos, *Trends in Food Science & Technology*, **2016**, *55*, 72.

POSTER

P01

Fish waste as source of vitamin D₃: optimization of extraction and quantification methods for the valorization of an important resource

Laura Alessandroni, Yue Sun, Giovanni Caprioli, Gianni Sagratini

Chemistry Interdisciplinary Project (ChIP), University of Camerino, 62032, Camerino, Italy
laura.alessandroni@unicam.it

World consumption of fish per capita has increased by about 50% in the last 50 years and is continuously growing [1]. As a result, the amount of fish waste has also dramatically increased worldwide. Currently, post-catch fish losses and waste between landing and consumption is an economic and environmental concern that occurs in most seafood distribution chains [2]. In this framework, fish waste can play an important role in the implementation of the circular economy, based on the reuse and recycling of materials to reduce waste production [3]. The benefits of vitamin D for growth and development of humans have long been recognized. The best-known action of the vitamin D endocrine system is its role in calcium uptake and phosphate homeostasis in bone homeostasis [4]. The aim of this work is to use fish wastes as source of vitamin D₃ as bioactive compound for nutraceutical and pharmaceutical purposes. Firstly, an extraction method was optimized from 5 freeze-dried fish samples being salmon, tuna, cod, anchovies, and sardines. Fish powder, fish oil and ultrasound assisted extraction products underwent through saponification and liquid-liquid extraction before HPLC-DAD quantitative analysis. Ergosterol, dihydrotachysterol and vitamin D₂ were tested as internal standards. An HPLC-DAD method was developed using H₂O 5 % and MeOH 95 % as mobile phases and a Gemini C18 column that allowed a clear separation of the analytes monitored at a wavelength of 265 nm. Results showed that vitamin D₂ was the most suitable internal standard and vitamin D₃ calibration curve was prepared calculating the response factor. Cod and salmon resulted to be the most vitamin D₃ rich samples with 0.094 mg/Kg and 0.086 mg/Kg respectively. During the experiments, also 7-dehydrocholesterol (7-DHC), a vitamin D₃ precursor, was quantified in fish samples resulting to range between 13 mg/Kg and 70 mg/Kg. The optimized extraction method and the high amount of 7-DHC provide the basis for the next step of the project which would involve the UV radiations to convert the precursor compound obtaining vitamin D₃ rich extracts. These products can be used in functional foods formulations and in nutraceutical and pharmaceutical sectors. This research highlights the potential of using food waste products to get crucial ingredients to be applied in several fields.

References

- [1] FAO. The State of World Fisheries and Aquaculture **2018**.
- [2] D. Coppola, C. Lauritano, F. Palma Esposito, G. Riccio, C. Rizzo, D. de Pascale, *Marine drugs*, **2021**, 19(2), 116.
- [3] C. Lopes, L.T. Antelo, A. Franco-Uría, A. A. Alonso, R. Pérez-Martín, *Waste management*, **2015**, 46, 103.
- [4] K. Nakamura, M. Nashimoto, Y. Okuda, T. Ota, M. Yamamoto, *Nutrition*, **2002**, 18(5), 415.

P02

Valorization of agro-food waste into artichoke and cauliflower by-products-based food supplements

Donatella Ambroselli¹, Cinzia Ingallina¹, Fabrizio Masciulli¹, Enrico Romano¹, Giacomo Di Matteo¹, Mattia Spano¹, Andrea Salvo¹, Giuliana Vinci², Antonella Di Sotto³, Chiara Di Meo¹, Pietro Matricardi¹, Luisa Mannina¹

¹Department of Chemistry and Technology of Drugs,
Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy

²Department of Management, Sapienza University of Rome, Via del Castro Laurenziano 9, 00161 Rome, Italy

³Department of Physiology and Pharmacology “V. Erspamer”,
Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
donatella.ambroselli@uniroma1.it

Fruit and vegetable supply chains represent one of the most important markets in the world. This sector produces significant quantities of by-products that are often disposed of as waste rather than reintroduced with new purposes into the supply chain. Conventional processing of agro-food waste has economic, social, and environmental impacts, making it a global issue to be addressed by governments and institutions, which are encouraging and supporting innovative solutions towards a zero-waste future. Plant material is a valuable source of nutrients and secondary metabolites, which can be linked to various health benefits. Therefore, the inedible part of the plant, could represent an important raw material to be used for various purposes, depending on its chemical-biological composition. Among vegetables supply chain, artichoke (*Cynara scolymus* L.) and cauliflower (*Brassica oleracea* L.) present a large quantity of waste and by-products, being the inedible part approximately 80% and up to 60% of the entire production, respectively.

Within this context, in the frame of Ri-cicloHorto project [1] the main bioactive compounds present in stems and leaves of cauliflower and artichoke by-products have been analyzed to identify the correct application for the development of new nutraceuticals or functional foods. Spectrophotometric and chromatographic determination of polyphenols (caffeoyl quinic acids) [2] [3] and glucosinolates (sinigrin, glucobrassicin, glucoraphanin, glucoiberin) [4] in leaves and stems hydroalcoholic extracts of vegetable waste was carried out. Moreover, the antioxidant activity of leaves and stems was assessed by applying ABTS and DPPH assays [5]. Obtained results showed that leaves from both matrices were characterized by higher amount of bioactive compounds and promising antioxidant, antimicrobial and anti-inflammatory profile. These findings prompted to develop effervescent sachet (cauliflower leaves) and oral suspension (artichoke leaves) as food supplements.

Further studies need to be performed to assess the bioavailability, absorption rate, and metabolism of the new formulated products. However, these preliminary results represent a starting point for the exploitation and valorization of inedible plant material, with a view to the circular economy.

References

- [1] This work is a part of a project supported by Regione Lazio in Italy (Progetti di Gruppi di ricerca 2020), entitled “Valorizzazione degli scarti agroalimentari del comparto ortofruitticolo del Lazio: dai biostimolanti per l’agricoltura agli integratori per la salute umana” CUP B85F21001170002.
- [2] N. Jiménez-Moreno, M.J. Cimminelli, F. Volpe, R. Ansó, I. Esparza, I. Mármol, M.J. Rodríguez-Yoldi, C. Ancín-Azpilicueta, *Nutrients*, **2019**, *11*(8), 1723.
- [3] A. Di Sotto, L. Abete, C. Toniolo, L. Mannina, M. Locatelli, A.M. Giusti, M. Nicoletti, M. Vecchiato, S. Di Giacomo, *Journal of Functional Foods*, **2018**, *40*, 679.
- [4] C. López-Berenguer, M.C. Martínez-Ballesta, C. García-Viguera, M. Carvajal, *Plant Science*, **2008**, *174*(3), 321.
- [5] G. Vinci, F. D’Ascenzo, L. Maddaloni, S.A. Prencipe, M. Tiradritti, *Beverages*, **2022**, *8*, 18.

Integration of pomace and grape seeds in feed of broiler chickens: effect on the chemical characteristics of the meat

Carla Buzzanca, Vita Di Stefano, Magda Greco, Mirella Vazzana, Manuela Mauro

Department of Biological, Chemical, and Pharmaceutical Science and Technology (STEBICEF),
University of Palermo, via Archirafi 32, 90123 Palermo, Italy.
carla.buzzanca@unipa.it

Recently, consumer demand for healthier, safer, good quality food products has increased. The use of grape pomace and grape seeds, natural antioxidants rich in polyphenols and known for their ability to prevent lipid oxidation, has attracted significant interest and could become an important alternative as a partial substitute for vitamin E which is the most commonly used antioxidant in animal diets. In this context, the valorization and reuse of wine industry wastes could be a way to reduce costs for companies and damage to the environment [1]. The objective of this study was to evaluate the effect of adding different percentages of pomace and grape seeds to the diet of broiler chickens. Three experimental sets were prepared of broiler chicken feeds containing: 0%, 3% and 6% pomace, 0%, 3% and 6% grape seeds and a mixture of 3% pomace and 3% grape seed. The animals were and butchered fed for 7, 21 and 42 days of age; meats were weighted, cut into small pieces, and stored at -80°C. Then, they were freeze-dried to evaluate total polyphenolic content (TPC), antiradical activity by DPPH e ABTS methods [2], fatty acid content by GC-M. The results of dietary supplementation with different percentages of grape pomace and grape seeds show an increase in anti-radical activity (DPPH) and TPC with respect to control samples. The highest TPC and the highest TEAC (Trolox equivalent antioxidant activity) value, were recorded in broiler meats with the simultaneous presence of pomace and grape seeds in the diet (3%+3%), 4.21 mg GAE/g and 7.82 mmol TEAC/100g respectively. The same set of samples showed an increase in % PUFA (40.71%) compared to the control (21.00%) and this could be due to the fact that the grape by-products, slowed down lipid degradation reactions reducing oxidative rancidity and lengthening the shelf life of chicken meat [3]. The color, texture and oxidative stability of the meat were evaluated. Further research on the use of grape by-products in chicken broiler diets will be essential to evaluate the best supplementation rate that will ensure the meat's beneficial potential without compromising the birds' growth performance.

References

- [1] M. Nardoia, C. Romero, A. Brenes, I. Arija, A. Viveros, C. Ruiz-Capillas, S. Chamorro, *Animal*, **2020**, *14*(7), 1371.
- [2] H. I. Yong, H. J. Kim, S. Jung, D. D. Jayasena, Y. S. Bae, S. K. Lee, C. Jo, *Korean Journal for Food Science of Animal Resources*, **2013**, *33*(1), 83.
- [3] M. Olteanu, T. D. Panaite, R. P. Turcu, M. Ropota, P. A. Vlaicu, M. Mitoi, *Revista Mexicana De Ciencias Pecuarias*, **2022**, *13*(1), 43.

From waste to worth: pomegranate seeds extraction, characterization and potential applications

Francesco Cairone¹, Irene Arpante¹, Laura Di Muzio¹, Antonia Iazzetti², Chiara Salvitti¹, Caterina Frascchetti¹, Stefania Cesa¹

¹Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza, Roma

²Università Cattolica del Sacro Cuore, Campus di Roma

francesco.cairone@uniroma1.it

Pomegranate, *Punica granatum* L., has an outstanding medical history throughout the world. The excellent composition of the fruit in all its parts (edible arils and discarded peels and seeds), in terms of bioactive healthy compounds, make the valorization of these products of great interest for the scientific community. Pomegranate seeds coming from the side streams of pomegranate juice production, rather than representing one of the main problems of agri-food waste processing, could rise to an added value product. Globally, the recycling of food waste streams to obtain more sustainable chemical products through greener technologies is one of the actions more strictly joined to the circular economy. Wastes, through recycling, create benefits for the environment, the human health and the global economy. The aim of the present work was to optimize and evaluate new and more environmentally friendly extraction procedures of pomegranate seeds, to create a zero-impact circuit, with a focus on the oily component and the fibres. Fruit seeds belonging to “Granata” and “Roche” cultivars were studied and compared. A previous work only focused on the “Granata” cv, had shown significant differences in the lipid composition according to the performed extraction procedure [1]. The presence of high content of conjugated isomers of linolenic acid (CLNA isomers and namely punicic acid) in the extracted oil, makes this matrix of high interest. Many studies highlight the significant health effects of these acids as anticancer, anti-diabetic, and anti-inflammatory bioactives [2]. So, the classic Soxhlet extraction (n-hexane) and supercritical CO₂, assisted by alcoholic co-solvent, extraction, were performed on both these cultivars and compared. Different results were obtained, both in terms of extraction yield and of quali-quantitative composition, ¹H and ¹³C-NMR, AP-MALDI-MS and Head Space GC-MS analyses were performed. Differences in the volatile compounds and triacylglycerols composition, with particular regard to punicic acid and other CLNA contents were shown. Results confirmed, in the triacylglycerol mixture, the prevalence of punicic acid joined to a better and more relevant aromatic component, when the oil is extracted by supercritical fluids. In addition, the polyphenolic component of the oily phase was extracted by a solid-phase extraction (C18) and deepened. The HPLC-DAD and DPPH analysis, to evaluate the antiradical potential, showed that the oil extracted with supercritical fluids was richer in polyphenolic components and showed greater antioxidant activity. Finally, in view of a complete circular approach, the solid residues, obtained after the first extraction step, were furtherly submitted to extraction and/or purification, in order to obtain and characterize the contained fibres, to be used as food supplements or as bio-packaging.

References

- [1] F. Cairone, C. Salvitti, A. Iazzetti, G. Fabrizi, A. Troiani, F. Pepi, S. Cesa, *Foods*, **2023**, 12(8), 1592.
- [2] M.T. Boroushaki, H. Mollazadeh, A.R. Afshari, *International Journal of Pharmaceutical Sciences Review and Research*, **2016**, 7(2), 430.

Comparison of green extraction methods of carotenoids for the valorisation of carrots by-products

Lidia Favaretto¹, Ciro Cannavacciuolo¹, Luca Campone^{1,2}

¹Department of Biotechnology and Biosciences, University of Milano-Bicocca,
Piazza Della Scienza 2, 20126 Milan, Italy

²NBFC, National Biodiversity Future Center, 90133 Palermo, Italy
ciro.cannavacciuolo@unimib.it

Throughout the industrial processing of vegetables and fruits, serious amounts of by-products are generated in the form of non-edible parts, such as peels and pomace, which can still be refined into biologically active compounds. Amongst these, the production of carrot juice shows a significant loss rate, with up to 50% of the weight of the raw material being discarded in the form of pomace. However, carrot pomace still contains large amounts of carotenoids, vitamins, dietary fibres, and minerals [1]. Carrots (*Daucus carota* subsp. *sativus*) are widespread roots recognized as an important natural source of bioactive components such as dietary fibres, carotenoids, polyphenols, and antioxidants. Phytonutrients such as carotenoids and phenolics may play a significant role, in addition to vitamins, in protecting biological systems from the effects of oxidative stress [2]. Carotenoids in foods are commonly classified into carotenes and xanthophylls, pigments that give attractive red or yellow colour and contribute to food quality. The presence of high concentration of carotenoids, especially β -carotene, may account for the biological and medicinal properties of carrots. Carotenoids are active as precursors of vitamin A, but they also show some biological effects attributed to their antioxidant property. Moreover, they are related to the enhancement of the immune system and reduced incidence of degenerative diseases such as cancer, cardiovascular syndromes, and age-related macular degeneration among others [2]. The valorisation of carrot pomace can be achieved through the development of sustainable techniques for the extraction of its carotenoids. Compounds ranked in carotenoid class are extremely hydrophobic and mostly show little or no solubility in water. They are, however, soluble in organic solvents which are therefore used in the traditional extraction methods. Compared to nonconventional methods, advanced carotenoid extraction technologies are at the same time efficient in the recovery and environmentally friendly. In particular, the optimisation and evaluation of Ultrasound-Assisted Extraction (UAE), Pressurised Liquid Extraction (PLE) and Supercritical Fluid Extraction (SFE) will outline the most suitable technique for the recovery of carotenoids from carrot pomace. Requiring low temperature and low pressure, SFE with CO₂ (SC-CO₂) is an effective method for extraction of thermolabile compounds avoiding carotenoids. SC-CO₂ achieves a rapid penetration into the pores of complex matrices, thus enhancing extraction efficiencies [3]. Additionally, it avoids the use of hazardous solvents, since CO₂ is defined as a “generally recognized as safe” (GRAS) solvent, and it is also easy to remove from the target compounds [4]. In the current work a comparison between advanced methods and their optimisation have been performed, extracting carotenoids in high yield and selectivity from carrots pomace of common variety and red variety, thus providing an environmentally friendly technique to valorise the by-products of carrot juice processing. A quali-quantitative investigation of non-polar carotenoids in carrot pomace was provided by means of HPLC and LC-MS analyses.

References

- [1] E. Kultys, M.A. Kurek, *Molecules*, **2022**, *27*, 518.
- [2] K.D. Sharma S. Karki, N.S. Thakur, S. Attri, *Journal of Food Science and Technology*, **2012**, *49(1)*, 22.
- [3] K.S. Ramesh, K. Young-Soo, *Food Chemistry*, **2018**, *240*, 90.
- [4] A. Viñas-Ospino, D. López-Malo, M.J. Esteve, A. Frígola, J. Blesa, *Foods*, **2023**, *12*, 863.

Evaluation of chemical characteristic of bread enriched with different percentages of hemp flour

Lorenzo Del Vecchio, Sebastiano Ricci, Eleonora Carini, Gianni Galaverna, Martina Cirlini

Department of Food and Drug, University of Parma, Viale Parco Area delle Scienze 27/A, 43124, Parma, Italy
lorenzo.delvecchio@unipr.it

Hemp flour is a by-product obtained from the seed of the hemp plant (*Cannabis sativa L.*), also known as industrial hemp [1]. The crop or part of it is mainly used for the textile industry due to the quality of natural fiber as in the cosmetic industry thanks to a good amount of oil extracted by seeds. Hemp seeds and their by-products are also utilized as animal feed but there is growing interest from the food industry in using them as a source of nutrients for human nutrition [2]. In the last few years, hemp flour is becoming an interesting ingredient for bakery products as it is a rich source of proteins, essential amino acids, fiber, and essential fatty acids [3].

This research investigated the potential use of hemp flour to fortify wheat bread formulation to increase its nutritional profile. A previous study was necessary to characterize hemp flour to highlight its high nutritional value compared to commercially available wheat flour in terms of proteins, lipids and fatty acid fraction, total phenolic content (TPC), and antioxidant activity (DPPH and ABTS). Afterwards, bread with different percentages of substitution of wheat flour with hemp flour (10%-15%-25%) were characterized by chromatographic (GC-MS) and spectrophotometric techniques to identify the effect of hemp flour addition on the nutritional quality and volatile profile of bread. The results obtained allowed us to select the most appropriate percentage for breadmaking, concluding that 15% of hemp enrichment was optimal for a better compromise between technological and nutritional quality. Indeed, a significant increase ($p < 0.05$) in protein and amino acids, lipid and fatty acids, volatile organic compounds, TPC and antioxidant activity was observed compared to control (100% wheat flour bread)

It should be emphasized that hemp flour could be an interesting ingredient also for gluten-free bread formulation, and the optimal addition level investigated by the present work could be the starting point for gluten-free bread development. This work is part of the CATERPILLAR research project financed by Emilia Romagna region, focused on the chemical characterization of by-products derived from the processing of fiber hemp such as flour and related food enriched with hemp flour as bread and gluten-free products.

References

- [1] B. Farinon, R. Molinari, L. Costantini, N. Merendino, *Nutrients*, **2020**, *12*, 1935.
- [2] S. Bonacci, V. Di Stefano, F. Sciacca, C. Buzzanca, N. Virzì, S. Argento, M.G. Melilli, *Foods*, **2023**, *12*, 774.
- [3] I.E. Rusu, R.A. Marc (Vlaic), C.C. Mure şan, A.E. Mure şan, V. Mure şan, C.R. Pop, M.S. Chi ş, S.M. Man, M.R. Filip, B.-M. Onica, *Plants*, **2021**, *10*, 1558.

A green extraction to recover polyphenol and polysaccharides from red bell pepper fruit by-products

Mohamad Khatib¹, Lorenzo Cecchi², Enrica Bargiacchi³, Maria Bellumori¹, Beatrice Zonfrillo¹,
Marzia Innocenti¹, Nadia Mulinacci¹

¹Department of NEUROFARBA, Division of Pharmaceutical and Nutraceutical Sciences, University of Florence

²Consortium INSTM – Florence (Italy)

³Department of Agricultural, Food and Forestry Systems Management (DAGRI), University of Florence

marzia.innocenti@unifi.it

The pepper fruit contains several phenolic compounds, mainly glycosides of apigenin, luteolin and chrysoeriol [1], and polysaccharides which have shown anticancer activity and anti-inflammatory properties in *in vitro* and *in vivo* tests [2]. The processing of fresh fruit and vegetables produces high quantities of waste (about 25%-60%), today known as agricultural by-products and not waste [3]. If the processes applied to treat these materials are ecological and sustainable, these by-products can become interesting as economical sources of valuable bioactive molecules. The by-products of red pepper (RPB) obtained from the processing of the fruit for the vegetable canning industry and consisting mainly of the discarded tops of the fruit and the seeds, have so far been little studied.

The objectives of this study were to investigate the content of polyphenols and polysaccharides in the RPB by-product. Furthermore, in order to define an extraction process suitable for a scale-up, the Timatic® extractor was used, with water as the extraction solvent at a pressure of approx. 7 bars, applying different extraction cycles. The extraction time was 60 and 120 min, the temperature from 50°C to 90°C. All extractions were performed applying an RPB/solvent ratio of 1:20 (w/v). The phenolic content was determined by HPLC-DAD-MS, while size exclusion chromatography (SEC) and dynamic light scattering (DLS) were applied to study the molecular weight distribution of the polysaccharides. ¹H-NMR was useful to determine galacturonic acid, methylation and degree of acylation after acidic hydrolysis according to Khatib et al 2021[4]. The phenolic content in RPB was low and reached max. 11.2 mg/g on dry extract. At the same time, the samples contained approx. 7%-10% of highly methylated polysaccharides determined on the dry aqueous extract. Five fractions were obtained from the pool of polysaccharides by adding increasing aliquots of ethanol at 0°C, of which Fraction 3 covered 40% of the total polysaccharides. The hydrodynamic volume of the main fractions was determined by SEC, which resulted close to 2000 kDa counting 80% of the total components detected. The acidic hydrolysis of polysaccharides by trifluoroacetic acid (TFA) allowed pointing out the presence of galacturonic acid. The ¹H-NMR, used to verify the methylation and acylation grade of the fractions, confirmed the presence of O-methyl groups and O-acetyl groups in several fractions along with a high content of galacturonic acid indicating the presence of pectin. The extraction by hot water is suitable to recover up to 10 % of polysaccharides from RPB. This work describes a sustainable and green extraction process to obtain new functional food ingredients mainly based on polysaccharides from red pepper by-products.

References

- [1] A. Marin, F. Ferreres, F. A. Tomás-Barberán, M.I. Gil, *Journal of Agricultural and Food Chemistry*, **2004**, *16*, 3861.
- [2] E.R. Adami, C.R. Corso, N. Mulinari Turin-Oliveira, C. Martins Galindo, L. Milani, M.C. Stipp, G. Erdmann do Nascimento, A. Chequin, L. Mota da Silva, S. Faloni de Andrade, R. Locatelli Dittrich, J. Ederaldo Queiroz-Telles, G. Klassen, E.A.S. Ramos, L.M.C. Cordeiro, A. Acco, *Carbohydrate Polymers*, **2018**, *201*, 280.
- [3] FAO Statistics Data, **2017**.
- [4] M. Khatib, A. Al-Tamimi, L. Cecchi, A. Adessi, M. Innocenti, D. Balli, N. Mulinacci, *Food Chemistry*, **2022**, *395*, 133591.

Avocado peels and seeds from Hass varieties: from industrial by-products to circular reuse

Vita Di Stefano¹, Carla Buzzanca¹, Ilenia Tinebra², Roberta Passafiume², Vittorio Farina²

¹Department of Biological, Chemical, and Pharmaceutical Science and Technology (STEBICEF),
University of Palermo, via Archirafi 32, 90123 Palermo, Italy;

²Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo,
Viale delle Scienze, 90128 Palermo, Italy
vita.distefano@unipa.it

From a sustainable progress perspective, the circular economy model is widely used today. One of the main purposes of last years is to valorize wastes of agri-food industries, as new sources of bioactive components, from the approach of a circular and biorefinery economy both for environmental and economic reasons. Avocado (*Persea americana* cv Hass) seeds and peels are an example of promising bio-sustainability raw materials with a high nutritional value that can be obtained from industrial by-products. The objective of this work was to study also chemical qualities of avocado peels and seeds. Avocado peel and seeds samples from fruit collected in Sicily were dried in a convective hot-air dryer at a temperature of 60°C for 4 hours, then processed into flour. Analysis of enzymatic browning and color quality, assessed using the CIELab colorimetric system, showed that the flours derived from both peels and seeds had very low levels of browning, with high brightness values ($L^*=51$; $a^*=2$; $b^*=29$ for peels; $L^*=60$; $a^*=40$; $b^*=45$ for peels and seeds respectively).

Furthermore, analyses show, mostly in peels, a very interesting value of total phenolic compound (386.80 mg GAE/100g) and antiradical activity (127.86 mmol TEAC/100g).

The fatty acid profile of the powder of dried peels and seeds flour was also evaluated after oil extraction via Soxhlet. Particularly interesting was the amount of total fatty acid in peels, with a value of 23.77 g of total fatty acid/100 g, with a percentage of 9.5% SFA, 78.3% MUFA, and 12.2% PUFA. Free and bound phenols, were determined by UHPLC-Orbitrap-MS using a method previously optimized [1]. The main bioactive compounds present in avocado by-products include hydroxycinnamic acids, hydroxybenzoic acids, flavonoids, catechins, quercetins, proanthocyanins and tannins [2].

This approach is essential in order to give a second life to by-products, due to their potential health and industrial value.

Avocado by-products supplementation in fact could be a potential option for the production of high-quality, nutritionally rich, low-cost functional products with good organoleptic properties, and higher nutritional value. [3]

References

- [1] V. Di Stefano, C. Buzzanca, M.G. Melilli, S. Indelicato, M. Mauro, M. Vazzana, V. Arizza, M. Lucarini, A. Durazzo, D. Bongiorno, *Sustainability*, **2022**, *14*, 6702.
- [2] V. Di Stefano, G. Avellone, D. Bongiorno, S. Indelicato, R. Massenti, R. Lo Bianco, *International Journal of Food Properties*, **2017**, *20*, 1302.
- [3] F. Shahidi, J. Yeo, *Molecules*, **2016**, *21*, 1216.

Systematic multistep extraction process for the total valorization of fiber fraction from fruit by-products

Pio Viscusi, Andrea Fuso, Clara Pedrazzani, Veronica Lolli, Augusta Caligiani

Dipartimento di Scienze degli Alimenti e del Farmaco, Università di Parma
veronica.lolli@unipr.it

Fruit seeds/kernels constitute a significant part of the food industry waste/by-products. Considering their important dietary fiber content and its potential, various fruit seeds/kernels (mango, cherry, lemon, pumpkin, avocado, litchi, peach and apricot) have been considered in this study to design and set up a new method for a revalorization approach. While fiber quantification methods are widely reported in literature, e.g., enzymatic gravimetric AOAC Official Method 991.43 [1], isolation and fractionation ones are limited [2]. This study aimed to find a systematic multistep extraction process able to fractionate and isolate fiber portions, from the most available for removal (soluble fibers), to the less available fractions (pectin and hemicellulose), adopting increasingly more drastic treatments. A first mild treatment, i.e., a protease assisted extraction, was made to separate a part of the soluble fibers and to aid the disaggregation of the plant cell structure, facilitating the following extraction steps [3]. Then, insoluble residue was treated with a more aggressive approach (hot acid extraction), in order to solubilize and separate the most insoluble pectin fraction eventually present. Then, the remaining solid material was subjected to hydrothermal treatment, aimed at recovering hemicellulose, even more recalcitrant to be extracted. For each fraction, extraction yields were calculated, and molecular characterization was performed in terms of molecular weight, monosaccharides composition and degree of methylation (DM) and acetylation (DA). Cherry, lemon and apricot seeds/kernels turned out to be the matrices with the highest total dietary fiber content on dry matter, i.e., 80 ± 3 ; 81 ± 7 and 92 ± 5 , respectively. Soluble fiber extraction yield, calculated on the soluble fiber content of the fresh sample, was very floating depending on the matrix, ranging from the 27 to the 91%. Pectin extraction yield was very low in almost all the matrices tested, ranging from the 0 to the 17% of the soluble fiber content of the fresh matter. Hemicellulose extraction yield was more promising, reaching values from the 40 to the 60% of the insoluble fiber content of the fresh matter. Pectin and hemicellulose extracts were generally characterized by their typical monosaccharides, i.e., galacturonic acid and xylose, respectively, with some differences related to the raw materials. Arabinose e galactose were detected in most of the soluble fiber fractions, probably indicating the presence of arabino-galactans. For some fruit by-products, high glucose amount was found in the different fiber fractions, suggesting that starch with very different extractability was also present in the fruit seeds/kernels.

References

- [1] AOAC, *Journal of AOAC International*, **2012**.
- [2] Y. Maphosa, V. A. Jideani, *Food Reviews International*, **2016**, 32(1), 98.
- [3] A. Fuso, P. Viscusi, S. Larocca, F. S. Sangari, V. Lolli, A. Caligiani, *Foods*, **2023**, 12(1), 148.

P10

Characterization of the phenolic profile of cocoa (*Theobroma cacao* L.) processing by-products for their possible use in animal feed

Francesca Mercogliano¹, Corinne Bani¹, Marco Tretola^{2,3}, Luciano Pinotti³,
Patrizia Restani^{1,4}, Chiara Di Lorenzo¹

¹Department of Pharmacological and Biomolecular Sciences, University of Milan, 20133 Milan, Italy

²Agroscope, Institute for Livestock Sciences, La Tioleyre 4, 1725 Posieux, Switzerland

³Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Milan, 26900 Lodi, Italy

⁴Coordinating Research Center (CRC) "Innovation for Well-Being and Environment", University of Milan, 20133 Milan, Italy
francesca.mercogliano@unimi.it

The food value chain is responsible for significant resource and environmental pressures and it is estimated that around 20% of the total produced food is lost or wasted in the EU. The European Circular Economy Action Plan provides a future-oriented agenda for achieving a cleaner and more competitive Europe and in this context, it's essential to reduce waste and ensure high-quality secondary raw materials [1]. The valorization of cocoa (*Theobroma cacao* L.) processing waste is arising great interest from both nutritional and functional points of view; through processing, about 80% of the cocoa fruit is discarded [2] and this waste includes cocoa pod shells, mucilages, and bean shells, which contain compounds of interest for various industries, such as the food, cosmetics and chemicals producers [3]. Cocoa beans by-products contain numerous biocompounds, including polyphenols (flavonols, phenolic acids), methylxanthines, dietary fibres and an interesting lipid profile. These compounds make cocoa processing waste an interesting matrix for reuse also in animal feed, in line with the circular economy aim of optimizing available resources and reducing food waste [4]. In this study, we evaluated the possible use of these matrices as ingredients in animal nutrition. The analysis were performed on three samples, S1 (flakes form), SK1 (flakes form) and S2 (pellets), supplied by a Swiss former foodstuff processor and obtained from cocoa bean processing waste, by the application and comparison of some *in vitro* methods: the Folin-Ciocalteu assay for the evaluation of the total polyphenol content, the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay for the evaluation of the overall antioxidant capacity of the samples and the vanillin test for the determination of flavan-3-ols; moreover, the antioxidant capacity was evaluated by HPTLC, following derivatization with the DPPH and Fast Blue B Salt reagents. From the comparison of the data obtained with the spectrophotometric methods, the sample showing the greatest antioxidant activity is sample S2 (pellets). This result can be explained by the high concentration of phenolic compounds, measured by the Folin-Ciocalteu assay. This sample also showed a higher content of flavan-3-ols. The S1 and SK1 samples showed comparable values in terms of antioxidant activity, total polyphenols and flavan-3-ols, but lower than sample S2. The results related to flavan-3-ols obtained with the vanillin test show that sample S2 is the richest source of these compounds compared to the other samples. The characterization of the phenolic profile was also evaluated by HPTLC, showing a higher concentration of antioxidant molecules for sample S2 than for samples S1 and SK1, when derivatized with Fast Blue B Salt. These considerations are in line with the results obtained by spectrophotometric methods. By comparing the results, it was possible to obtain an in-depth characterization of the phenolic profile and the antioxidant activity of the cocoa processing waste and to evaluate the potential of these matrices in animal feed. Nevertheless, further approaches will be applied to integrate the results obtained so far and to obtain a more complete characterization of the samples under examination.

References

- [1] European Commission, Circular economy action plan: for a cleaner and more competitive Europe, **2020**
- [2] Z.S. Vásquez, D.P. de Carvalho Neto, G.V. Pereira, L.P. Vandenberghe, P.Z. de Oliveira, P.B. Tiburcio, H.L. Rogez, A.G. Neto, C.R. Soccol, *Waste Management*, **2019**, *90*, 72.
- [3] C.J. Mendoza-Meneses, A. A. Feregrino-Pérez, C. Gutiérrez-Antonio, *Journal of Chemistry*, **2021**, *2021*, 1.
- [4] M. Tretola, M. Ottoboni, A.R. Di Rosa, C. Giromini, E. Fusi, R. Rebusci, F. Leone, V. Dell'Orto, V. Chiofalo, L. Pinotti, *Journal of Food Quality*, **2017**, *2017*, 1.

Toxic and essential mineral elements in fermented agro-food waste

Clara Naccari¹, Nicola Cicero^{2,3}, Teresa Gervasi², Giacomo Dugo^{2,3}, Ernesto Palma¹

¹Department of Health Sciences, University “Magna Græcia” of Catanzaro (Italy)

²Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy

³Science4life S.r.l., start-up of University of Messina, Messina, Italy

nicola.cicero@unime.it

The agro-food waste is considered a renewable resource of great economic and environmental impact [1] for the production of bio-fertilizers, single cell proteins (SCP) for animal feed [2], biofuels of second generation [3] and biomaterials. For a safe and effective reuse of agro-food wastes, however, it is needed a toxicological monitoring [4] to avoid potential risks to animals, humans and the environment, due to the exposure to several contaminants.

The aim of this study was to evaluate the content of micro-elements (Cu, Co, Ni, Se, Fe, Zn, Mn, Mo, V, Sb), toxic metals and metalloids (Cd, Pb, Cr As) and macro-elements (Na, Ca, K, P, Mg) in several agro-food wastes (tomatoes, bananas, eggplants, pineapples, cauliflowers, pears and peaches), collected from the large and medium distribution markets at the end of shelf-life and after fermentation process. In particular, a comparative analysis of metals levels has been carried out to evaluate a possible bioremediation in fermented samples respect to agro-food wastes just collected.

Each sample was subjected to homogenization with a stainless steel blender, kept in PET containers and frozen at -20°C until to analysis. For the fermentation of agro-food waste was used strain of *S. cerevisie*, cultivated on Malt Extract Agar. All agro-food waste samples, at the end of shelf-life and after fermentation, were submitted to acid digestion and then analyzed by ICP-MS for the determination of mineral content.

The results obtained showed the presence of all minerals analyzed, with a lower content in fermented agro-food waste samples compared to those at end of shelf-life. Relating to toxic metals Cd, Pb, As and Cr significant differences were found in the most of all waste samples ($P < 0,01$ and $P < 0,05$ vs fermented samples), except in pears and peaches. Among micro-elements Se showed significant differences in all samples analyzed, followed by Co and Zn, while among macro-elements only Na in eggplants and pears, K in eggplants and cauliflowers.

The results confirm the importance of toxicological monitoring of agro-food waste rich of essential nutrients before their reuse. The lower content of metals in fermented agro-food waste samples admit to hypothesize a possible bioremediation, although furthermore studies are needed to better understand this process.

References

- [1] J. Parfitt, M. Barthel, S. Macnaughton, *Waste Management*, **2011**, 31(1), 108.
- [2] C.F. Silva, S.L. Arcuri, C.R. Campos, D.M. Vilela, J.G. Alves, R.F. Schwan, *Waste Management*, **2011**, 31(1), 108.
- [3] V.N. Nkemka, D.H. Marchbank, X. Hao, *Waste Management*, **2015**, 43, 123.
- [4] Y.N. Jolly, A. Islam, S. Akbar, *Springer Open Journal*, **2013**, 2, 385.

Quercetin 3-*O*-glucuronide from the leaves of *Vitis vinifera* cv. Aglianico: a selective sustainable recovery

Lara Comune, Simona Piccolella, Severina Pacifico

Department of Environmental, Biological and Pharmaceutical Sciences and Technologies,
University of Campania ‘Luigi Vanvitelli’, Via Vivaldi 43, 81100 Caserta, Italy
lara.comune@studenti.unicampania.it

In recent years, as part of the “sustainable development” policy, the revaluation of “end-of-life” products, in the form of reuse of materials, has become more and more widespread. Indeed, food waste is a global problem involving a loss of 1.6 billion tons per year with related economic, environmental, and social issues. In terms of sustainability, in a scenario of circular economy (“less raw materials”, “less waste”, “fewer emissions”) food sustainability aims, *inter alia*, at making effective utilization of natural resources. Well integrated into this topic, the concept of “Food Waste Recovery” includes the re-evaluation of agri-food wastes and by-products (e.g. leaves, peels, seeds and/or other inedible items), which are usually underutilized and cause serious disposal problems for the environment [1].

In the wine industry, these by-products represent about 30% of the processed grapes [2]. The leaves of *Vitis vinifera*, as a food waste, are a source of flavonoids and, in particular, of quercetin 3-*O*-glucuronide [3]. This is a powerful antioxidant, removes free radicals, and has a neuroprotective effect, resulting in a lower risk of central nervous system disorders [4].

With the aim of promoting the enhancement of the wine sector wastes, the present study took into account the leaves of *Vitis vinifera* cv. Aglianico, harvested at different heights from the grape-vine. The use of alternative extraction methods, such as deep eutectic solvents (DES), joint to spectroscopic and mass spectrometric analyses, allowed us to define the main differences in the quali-quantitative profile in terms of flavonol derivatives and to draw valuable information regarding the influence of abiotic factors, e.g. the exposure to sunlight, in the biosynthesis of such molecules that should be strictly considered for their full recovery.

References

- [1] D. A. Teigiserova, L. Hamelin, M. Thomsen, *Science of The Total Environment*, **2020**, 706, 136033.
- [2] L. Del Rio Osorio, E. Flórez-López, C. D. Grande-Tovar, *Molecules*, **2021**, 26, 515.
- [3] S. Piccolella, G. Crescente, M. G. Volpe, M. Paolucci, S. Pacifico, *Molecules*, **2019**, 24, 3630.
- [4] A. Ishisaka, R. Mukai, J. Terao, N. Shibata, Y. Kawai, *Archives of Biochemistry and Biophysics*, **2014**, 557, 11.

Quantification of polyols in sugar-free foodstuffs by qNMR

Anna Scettri, Elisabetta Schievano

Department of Chemical Sciences, University of Padova, via Marzolo 1, 35131 Padova, Italy
elisabetta.schievano@unipd.it

Polyols are valuable food additive thanks to their properties, as cooling agents or sweet-tasting products [1,2]. In food industry, the control of polyols amounts in sugar-free foods is essential in terms of nutritional information and quality control [3].

Polyols detection and quantification in mixtures, employing analytical methods based on chromatographic separation, pose several challenges due to their structural similarity, the lack of chromophores and their high boiling point and low volatility.

We present a qNMR method for the determination of low calories sweeteners (erythritol, mannitol, maltitol, sorbitol, isomalt and xylitol) in sugar-free foodstuff. The structural similarities of these compounds determine often a severe spectral overlap that hampers their quantification via conventional 1D and 2D NMR spectra. This problem is here overcome by exploiting the resolving capabilities of the CSSF-TOCSY experiment (Fig.1), allowing the quantification of all six polyols, with satisfactory results in terms of LoQ (2.8–7.4 mg/L for xylitol, mannitol, sorbitol, 15 mg/L for erythritol, 38 mg/L for maltitol and 91 mg/L for isomalt), precision (RSD% 0.40–4.03), trueness (bias% 0.15–4.81), and recovery (98–104%).

Polyol's quantification in different sugar-free confectionary products was performed after a simple water extraction without any additional sample treatment [4]. While these results demonstrate the robustness of the proposed method for polyols quantification in low calories foods, its applicability can be further extended to other food matrices or biofluids.

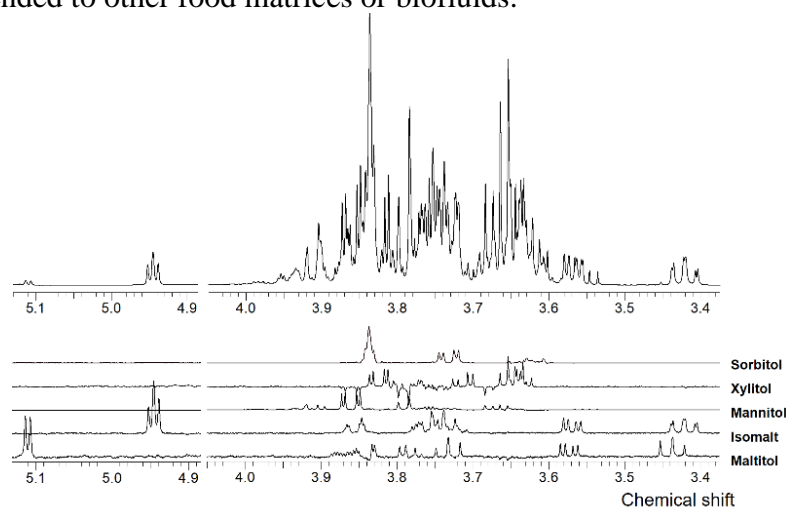


Fig. 1. ¹H NMR spectrum of a sugar-free chewing-gum sample (top)
 Selective spectra of the contained polyols (bottom).

References

- [1] R. Hadjikinova, N. Petkova, D. Hadjikinov, P. Denev, D. Hrusavov, *Journal of Pharmaceutical Sciences*, **2017**, 9(8), 1263.
- [2] M. A. Radeloff, R. H. F. Beck, *Sugar Industry*, **2013**, 138, 226.
- [3] T. Rice, E. Zannini, K. E. Arendt, A. Coffey, *Critical Reviews in Food Science and Nutrition*, **2020**, 60 (12), 2034.
- [4] A. Scettri, E. Schievano, *Food Chemistry*, **2022**, 390, 133125.

Milk Fatty Acids profile in Modicana and Holstein cows under the same feeding management

Annalisa Amato¹, Sonia Bonacci², Marianna Oteri¹, Carmelo Cavallo¹,
Vincenzo Lopreiato¹, Luigi Liotta¹

¹University of Messina, Messina, Italy

²University Magna Grecia of Catanzaro, Catanzaro, Italy

luigi.liotta@unime.it

In Sicily, the achievement of high production levels in dairy farms promoted the expansion of Holstein cows replacing less productive breeds. Modicana is a local breed, mainly used for milk production, that originates from Ragusa area. It is particularly suitable to pasture-based production system and characterized for its adaptability to challenged condition (climate and feed requirements). This study aimed to assess the influence of breed on milk fatty acids profile in mid lactating Modicana (Mo) and Holstein (Ho) cows. 20 Mo and 20 Ho cows, balanced for days in lactation and housed in the same farm, were enrolled and individual milk sample were collected. Fatty acids (FA) were extracted, methylated, and separated with a gas-chromatographer fitting a CP-Sil88 column. Peaks of individual FAs were identified and quantified using a 37 FAME standard. Data were analyzed with the PROC GLIMMIX of SAS. Values are expressed as a percentage of total FAME. Results showed no differences ($P < 0.05$) of short-chain (SCFA, C4:0 and C6:0) and medium-chain FA (MCFA, C8:0 and C12:0) between breeds. Regarding long-chain FA (LCFA, from C13:0), milk C14:1 (Ho:1.96 vs. Mo:1.06 %), C15:0 (Ho:1.94 vs. Mo:1.51 %), C16:1 (Ho:2.65 vs. Mo:1.52 %), C18:1t9 (Ho:4.02 vs. Mo:1.27 %) and C18:2n6 (Ho:2.65 vs Mo:1.77 %) were greater in Ho than in Mo group ($P < 0.05$). Milk C18:0 was greater in Mo compared with Ho breed (8.89 vs. 4.97 %, respectively; $P < 0.05$). Regarding very long chain FA (VLCFA) Mo group had greater amount of C20:0 (Mo:0.22 vs. Ho:0.03 %). Interesting, only in milk of Mo cows were identified and quantified C18:3 n6 (0.05 %), C20:4 (0.12 %), C22 (0.10 %), C22:2 (0.07 %), C24 (0.05 %), and C20:5n3 (0.04 %). Finally, while atherogenic index (AI) was no statistical different between groups (Ho:5.22 vs Mo:5.53; $P = 0.53$), thrombogenic index (TI) was greater in Mo than in Ho group (3.96 vs. 3.41, respectively; $P < 0.05$) and desaturation index (DI) resulted higher in Ho than in Mo group (13.37 vs. 7.97, respectively; $P < 0.05$). Although results showed no differences between the two breeds in the amount of SCFA and MCFA, MUFA was higher in Ho group, maybe for the higher DI. Desaturation is a process led by an enzyme, stearoyl Co-A desaturase (SCD), which add double bond to SFA in the mammary gland and other tissue [1]; as reported by Garnsworthy et al.[1], genetic effects (thus genetic differences between breeds) are responsible for a significant proportion of the phenotypic variation in SCD activity in dairy cows and thus the differences in MUFA amount between the two breeds. The higher presence of VLCFA in Mo than in Ho group, is an interesting result since they have anti-inflammatory activity and are associated to lower the risk of coronary heart disease [2]; their presence in milk is related to their presence in pasture, but the fact that only milk in Mo group showed their content should be thoroughly investigated to evaluate if it is related to the different capacity of this breed to better metabolize and transfer in milk these FAs. Thus, from these results, we could assume that identifying breed has a significant influence of FA profile and the use of Mo milk could be one way of revitalizing this indigenous breed. *Study supported by P.O. FESR SICILIA 2014/2020, Project BIOTRAK Grant number 08SR1091000150 -CUP G69J18001000007*

References

[1] P. C. Garnsworthy, S. Feng, A. L. Lock, M. D. Royal, *Journal of Dairy Science*, **2010**, 93(4), 1743.

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Fatty acid profile, conjugated linoleic acid content and antioxidant activity of probiotic fermented milks

Alessia Fazio, Chiara La Torre

Department of Pharmacy, Health and Nutritional Sciences, University of Calabria,
Edificio Polifunzionale, 87036 Rende, CS, Italy
a.fazio@unical.it

Development of probiotic fermented milks is of great interest as they combine improved nutritional values, particular sensory properties, health benefits and extended shelf life. Among all of them, kefir an ancient milk beverage, has a creamy consistency, mild acid flavor, a natural carbonated effervescence and may contain between 0.08 to 2% alcohol [1]. Its production involves the use of kefir grains as starter. They contain a complex mixed population of lactic acid bacteria (LAB), acetic acid bacteria (AAB) and yeast that are able to modulate the chemical composition of the fermented product. It is subject to variations, depending on several factors including the type of milk. The present study investigates the influence of the type of milk on fatty acid profile, phenolic content, antioxidant activity and also rheological behaviour of kefir. Cow, buffalo, goat, camel, donkey and sheep milks are used for the growing of kefir grains, having the same microbial composition. After 24 hours, the chemical composition and rheological behaviour of milks before and after fermentation are evaluated. The fatty acid profile is subjected to changes according to the type of milk used for the fermentation. kefir from cow, goat and sheep milks show an increase in the saturated fatty acid content, especially in myristic (C14:0) and palmitic acids (C16:0). Among monounsaturated fatty acids (MUFA), oleic acid content is 17.9, 2.0 and 2.9 folds higher in fermented cow, goat and sheep milks, respectively, compared to their corresponding unfermented milk. Among the polyunsaturated fatty acids (PUFA), the initial linoleic acid content in cow milk ($25.3 \pm 1.1 \text{ mg} \cdot \text{g}^{-1}$) increases during fermentation to $453.7 \pm 1.81 \text{ mg} \cdot \text{g}^{-1}$. The main conjugated linoleic acid (CLA) isomer found is *cis*-10 *trans*-12, C18:2, which increases during fermentation, especially in sheep milk where it quadruples. Fermentation leads to a significant increase in phenolic content of fermented milks. The best results are found for fermented buffalo ($260.4 \pm 5.5 \text{ } \mu\text{g GAE/mL}$), camel ($204.7 \pm 2.5 \text{ } \mu\text{g GAE/mL}$), and sheep milks ($218 \pm 1.0 \text{ } \mu\text{g GAE/mL}$), which increase by 46, 53 and 54 %, respectively, compared to the starting milks. The increase in phenolic content was also confirmed by the FRAP (ferric reducing antioxidant power) and ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assays. ABTS assay shows increased activity in all fermented milk being highest in goat milk ($76.2 \pm 0.5\%$) followed by cow milk ($74.1 \pm 0.6\%$). The effect of rheological properties on kefir quality is largely important, so the apparent viscosity of all samples, before and after fermentation is evaluated [2]. According to flow curves, viscosity decreases with increasing shear rate, meaning that the kefir behaves as a pseudoplastic fluid. At low shear rate, the highest viscosity value is observed in the sheep sample, while the lowest viscosity value is found in the buffalo one. The results highlight that fermentation enhances the nutritional and therapeutic values of the products, to a variable extent according to the type of milk.

References

- [1] A. Fazio, C. La Torre, M.C. Caroleo, P. Caputo, R. Cannataro, P. Plastina, E. Cione, *Molecules*, **2020**, 25, 2706.
- [2] D. Saygili, D. Döner, F. İçiercem, C. Karagozlu, *Food Science and Technology*, **2022**, 42, 32520.

P16

Food Design of Cereal Products: chemical, nutritional, technological and hedonistic aspects

Stefania Ruggeri, Valeria Turfani, Valentina Narducci, Mena Ritota, Francesca Antonazzi

CREA- Research Centre for Food and Nutrition. Via Ardeatina, 546 - 00179 Roma
francesca.antonazzi@crea.gov.it

Food Design is a new methodology to project food (food product design, eating design) and for food (products to cook, contain, preserve, cut, chop, mix, store and present food).

In November 202, the Sustainable Food Design Hub was founded by a group of researchers at CREA with the aim to project new healthy and sustainable foods. The hub projects are carried out with a transdisciplinary approach: several experts from different disciplines such as genetic, anthropology, biotechnology, human nutrition, agronomy, food chemistry, design, digital engineering, gastronomy, social sciences, and geography work as a whole in order to find innovative design solutions for a truly sustainable and healthy food.

A Food Design methodology was applied in the frame of the PRO-FORNO project - “*Sviluppo di PROdotti da FORNO ad alta valenza salutistica, ambientale e di sicurezza d’uso per la valorizzazione della filiera cerealicola laziale. (acronimo: PRO-FORNO)*” funded by Lazio Innova. The project aim is to realize new cereal products for subjects with Irritable Bowel Syndrome (IBS), characterized by low FODMAPS, high nutritional value (high folate and polyphenols content) and low acrylamide levels.

To this scope, 19 Italian soft wheat cultivars from three breeder industries were evaluated for their chemical and nutritional characteristics: folate content [1,2,3], mineral profile (by ICP-OES atomic absorption spectrophotometry), dietary fiber [4], alkylresorcinols [5] sugar content (HPAEC-PAD) and asparagine levels [6].

In the meanwhile, a questionnaire for the evaluation of food preferences and nutritional needs of subjects with IBS was developed and administered by dietician and nutritionists to their IBS patients. Three soft wheat cultivars with high folate content, low asparagine and FODMAPS levels were subjected to different pearling and air classification processes to obtain soft wheat by-products very rich in folate.

By using wheat by-products and on the basis of our questionnaire results, three experimental food design plans were carried out to formulate some cereals products (i.e. bread, snack) with high nutritional values, low acrylamide content and hedonistic properties really targeted for IBS patients.

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References

- [1] J.W. DeVries, J.I. Rader, P.M. Keagy, C.A. Hudson, G. Angyal, J. Arcot, M. Castelli, N. Doreanu, C. Hudson, P. Lawrence, J. Martin, R. Peace, L. Rosner L, H.S. Strandler, J. Szpylka, H. van den Berg, C. Wo C Wurz, *Journal of AOAC International*. **2005**, 88 (1), 5.
- [2] M. Edelman, S. Kariluoto, L. Nyström, V. Piironen, *Food Chemistry*, **2012**, 135, 1938.
- [3] S. Ruggeri, A. Aguzzi, E. Carnovale, *Proceedings of the First International Conference of Foliates: analysis, bioavailability and health*, **2004**, Warsaw (Poland), 27.
- [4] AOAC 991.43, **1995**.
- [5] A. Gajda, M. Kulawinek, A.J. Kozubek, *Journal of Food Composition and Analysis*, **2008**, 21, 428.
- [6] T.Y., Curtis, N. Muttucumar, P.R. Shewry, M.A.J. Parry, S.J. Powers, J.S. Elmore, D.S. Mottram, S. Hook, N.G. Halford, *Journal of Agricultural and Food Chemistry*, **2009**, 57(3), 1013.

Chemical characterization and biological activities of *Rhus coriaria* L. extract: antioxidant, antiglycation and dpp4 inhibitory effects

Laura Dugo¹, Elisa Pannucci¹, Ludovica Spagnuolo¹, Luca Santi²

¹Department of Science and Technology for Sustainable Development and One Health, University Campus Bio-Medico of Rome, Via Alvaro del Portillo 21, 00128 Roma, Italy

²Department of Agriculture and Forest Sciences, University of Tuscia, Via San Camillo de Lellis snc, 01100 Viterbo, Italy

L.dugo@unicampus.it

Rhus coriaria L., commonly known as “sumac”, belongs to Anacardiaceae family. It is a wild edible plant growing in tropical and temperate regions worldwide. Several research highlighted the health properties of sumac drupes including antioxidant, antidiabetic, and anti-inflammatory activities; indeed, drupes are rich in phytochemicals which are responsible of the biological properties [1]. In this work a phytochemical characterization of an extract obtained from sumac drupes was carried out through HILICxRP-LC-PDA-ESI/MS. Furthermore, the total phenolic content (TPC) was determined using the Folin-Ciocalteu method (174,24 mgGAE/g) [2]. Antioxidant activity, one of the most prominent biological properties associated with sumac fruit, was explored through ORAC (225.836,14 $\mu\text{mol TE}/100\text{ g}$) [2], DPPH and ABTS (DPPH-IC₅₀: 0,41 mg/ml; ABTS-IC₅₀: 0,21 mg/ml) assays [3] and, also, ascorbate and glutathione levels were measured. Moreover, the inhibitory effects on DPP-IV, an enzyme secreted after a meal that degrades incretins, were evaluated by an *in vitro* enzyme activity inhibition assay. Results indicate a broad inhibition activity on DPP-IV, comparable with the sitagliptin reference compound. Besides, since AGEs and ROS formation has been considered as risk factors in the pathogenesis of diet-related disorders such as diabetes and insulin resistance, antiglycation effects of sumac extract were evaluated using the BSA-MGO model and the measurement of intracellular ROS generation was evaluated in THP-1 cell line through the H₂DCF-DA assay. Results indicate the ability of sumac extract to inhibit the *in vitro* AGEs formation in a dose-dependent behaviour and to inhibit ROS production in oxidative-stressed cells. In conclusion, results suggest that sumac extract has a marked antioxidant activity, can inhibit DPP-IV enzyme activity, can inhibit AGEs formation, and can protect cells from the oxidative stress. The overall results suggest that sumac extract represents a promising natural source for developing nutraceuticals or dietary supplements with antioxidant activity and boost its potential use in the food, nutraceutical, and pharmaceutical industries. Moreover, although it is still early to suggest the use of sumac in diabetes, this work would lay the basis to encourage further studies on antidiabetic activities of sumac.

References

- [1] H. Alsamri, K. Athamneh, G. Pintus, A. H. Eid, R. Iratni, *Antioxidants*, **2021**, *10*(1), 73.
- [2] K. Arena, E. Trovato, F. Cacciola, L. Spagnuolo, E. Pannucci, P. Guarnaccia, L. Santi, P. Dugo, L. Mondello, L. Dugo, *Molecules*, **2022**, *27*(5), 1727.
- [3] C. Isgrò, L. Spagnuolo, E. Pannucci, L. Mondello, L. Santi, L. Dugo, A.M. Sardanelli, *International Journal of Molecular Sciences*, **2022**, *23*(21), 12774.

Optimization of enzymatic treatment to increase prebiotic potential of cocoa beans shells

Vincenzo Disca, Yassine Jahouari, Manuel Martoccia, Jean Daniel Coisson, Marco Arlorio

Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale
vincenzo.disca@uniupo.it

Cocoa bean shells (CBS) (or cocoa “hulls”) are a valuable by-product of the chocolate manufacture process. They are dehulled from the rest of the cocoa bean during the roasting phase and discarded. Almost 10 kg of shells are produced for every kg of cocoa powder obtained [1]. These numbers and the fact that this food by-product is very rich in Dietary Fiber (DF) [2,3] and antioxidant compounds [4] open up to possibilities of valorization to obtain functional ingredients useful for the *food design*.

In this work was assessed the potentiality of enzymatic treatment to valorize the DF fraction of CBS. A commercial mixture of cellulase, xylanase and polygalacturonase was used to process CBS (2, 4, 24 and 48h of digestion). Enzymatic treatment was standardized and performed in duplicate. Moreover, it was assessed the impact of two different drying methods after enzymatic processing, comparing i) freeze drying with ii) oven drying.

Samples were characterized on their Total Phenolic Content, TPC (Folin-Ciocalteu assay), Antioxidant Activity, AA (DPPH assay) and Total Flavonoid content (Aluminum Chloride assay) as described by Papillo et al. [5].

Total Dietary Fiber (TDF) was assessed by quantify the Insoluble Dietary Fiber, IDF and Soluble Dietary Fiber (SDF) as percentage over dry weight, following the AOAC protocol (991.43).

Results showed a marked influence of the enzymatic hydrolysis of IDF portion into SDF specifically after 24h of enzymatic treatment, where a 2x fold increase in soluble fiber was observed.

CBS are strongly rich in antioxidant compounds, and it was evaluated the recovery of TPC and TFC and the AA after the enzymatic treatment and with the impact of two drying method. While freeze drying is an almost completely conservative method it is quite expensive, in term of energy and cost, while the oven drying results in a higher thermic shock, despite a relatively low cost.

These results opens new perspective regarding the use of bio-based processing to valorize CBS, highlighting a new scenario to further valorize and exploit its prebiotic and antioxidant potential.

References

- [1] M. Li, A. Younes, S. Karboune, *Critical Reviews in Food Science and Nutrition*, **2022**, 0, 1.
- [2] O. Rojo-Poveda, G. Zeppa, I. Ferrocino, C. Stévigny, L. Barbosa-Pereira, *Antioxidants*, **2021**, 10, 1533.
- [3] A. S. Younes, Karboune, L. Liu, E.S. Andreani, S. Dahman, *Polymers*, **2023**, 15, 745.
- [4] O. Rojo-Poveda, L. Barbosa-Pereira, G. Zeppa, C. Stévigny, *Nutrients*, **2020**, 12, 1123.
- [5] V.A. Papillo, M. Locatelli, F. Travaglia, M. Bordiga, C. Garino, J.D. Coisson, M. Arlorio, *Food Research International*, **2019**, 115, 511.

Chitin from black soldier fly: extraction, purification and enzymatic hydrolysis

Clara Pedrazzani¹, Lara Righi², Ferdinando Vescovi², Massimiliano Rinaldi¹, [Augusta Caligiani¹](mailto:augusta.caligiani@unipr.it)

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

²Department of Chemistry, Life sciences and Environmental Sustainability, University of Parma,
Parco Area delle Scienze 11/A, 43124 Parma, Italy
augusta.caligiani@unipr.it

In recent times, chitin and its derivatives, including chitosan and chitooligosaccharides (e.g. N,N'-diacetylchitobiose), have been well studied with great evidence of their many applications in agriculture, biomedicine, food, cosmetic and pharmaceutical field¹. Applications in these areas require a chitin with a high degree of purity. Extraction methods for chitin have been extensively studied and optimised from crustacean shells². This has not yet been achieved for insects. In addition, insect chitin has recently been considered as an alternative to chitin from crustaceans due to the increasing demand for food and feed, which has led to the research for new protein sources to be exploited³. Among insects, Black Soldier Fly (BSF) represents a great protein and chitin source⁴. In this study, different chemical and enzymatic methods for chitin extraction from BSF were tested and compared with chitin extracted from shrimp shells. In addition, two pre-treatment methods were also tested: ultrasonication and mechanochemical milling, with the aim of improving the extraction by altering the crystalline structure of insect chitin. The results showed that the different extraction methods lead to a chitin with different degrees of purity and a variable content of protein bound to it. In shrimp shells, on the other hand, the proteins were effectively removed. These results were also supported by the structural characterisation of the extracted chitins by ESEM (Environmental Scanning Electron Microscope) and X-ray analysis. Subsequently, the extracted chitin samples were treated with chitinase from *Streptomyces griseus* to produce the N,N'-diacetylchitobiose derivative. Again, differences were shown between chitin extracted from shrimp shells and insects, where in the case of shrimp there was a higher yield. In the case of the BSF, an improvement was shown due to the mechano-chemical milling pre-treatment. It is therefore evident that insect chitin cannot be treated in the same way as shrimp shells, but it is necessary to obtain an extraction protocol and consequently an enzymatic hydrolysis protocol that is optimised on the insect, which can lead to a product with an acceptable degree of purity, necessary for the different applications.

References

- [1] A. Fuso, S. Barbi, L. I. Macavei, A. V. Luparelli, L. Maistrello, M. Montorsi, S. Sforza, A. Caligiani, *Foods*, **2021**, *10*, 1773.
- [2] M. Pakizeh, A. Moradi, T. Ghassemi, *European Polymer Journal*, **2021**, *159*, 1.
- [3] J. A. Cortes Ortiz, A. T. Ruiz, J. A. Morales-Ramos, M. Thomas, M. G. Rojas, J. K. Tomberlin, L. Yi, R. Han, L. Giroud, R. L. Jullien, *Insects as Sustainable Food Ingredients, Academic Press (USA)*, **2016**, *6*.
- [4] A. Caligiani, A. Marseglia, G. Leni, S. Baldassarre, L. Maistrello, A. Dossena, S. Sforza, *Food Research International*, **2018**, *105*, 812.

Chemical characterization of shrimp: impact of different cooking methods on nutritional parameters

Manuella Lesly Kouamo¹, Giovanna Baffa¹, Maria Elena Telloni²,
Gianni Sagratini¹, Elisabetta Torregiani¹

¹School of Pharmacy, University of Camerino, 62032 Camerino, Italy

²D.I.MAR. S.r.l, Via Enrico Mattei, 180 62014 Corridonia, Italy
manuella.kouamo@unicam.it

Food waste and losses have been globally recognized among the most important manifestations of food system inefficiencies [1]. This become worst when talking about fishery industry were up to 70% of the production can be discarded even if it provides major consumable resources of n-3 PUFAs important to prevent metabolic, liver, and CVDs disorders [2]. Shrimps especially are an extremely good source of protein, yet are very low in fat and calories, making them a very healthy choice of food. However, they are mostly consumed cooked and as several experimental studies have demonstrated that method of food processing and preparation can adversely affect the quality and the valuable biological health benefits leads usually to an over-restrict consumption. Since consumers are responsible for a high percentage (65 %) of the total food waste, reducing it at household level is considered one of the most important actions necessary to improve the quality of the environment as well as food security. Many studies have reported the importance of careful communication of risks and benefits to increase food consumption [3]. The present study aims to give a broad characterization of shrimp highlighting the impact of different cooking methods on nutritional parameters. Three varieties of shrimp mostly consumed in Marche region Italy, *Pandalus Baurealis*, *Solenocera Crassicornis* and *Pleoticus Muelleri* were supplied by D.I.MAR. Sarl and their content in proteins, carotenoids, lipids, minerals as well as the antioxidant activity was assayed in their raw frozen form, as cooked in 3 different ways (boiled, grilled, and fried). Furthermore, the physical parameters that are known to be play a significant role in maintaining a high consumer acceptance were monitored in the different states. The protein content represents more than 80% of the dry matter throughout the cooking process with higher amount found in *Pandalus B.* ranging from 15.40-28.7g/100g. Also, the minerals content as well as the lipids remained constant in the raw, boiled, and grilled form whereas we noticed and oxidation when frying. Moreover, the index of Atherogenicity (IA) was reduce when applying the cooking process ranging respectively from 0.7 to 6.2 and 0.54 in raw, boiled, and grilled *Pandalus B.*, from 1.28 to 0.75 and 0.42 in *Solenocera C.* and from 0.64 to 0.58 and 0.57 in *Pleoticus C.* Regarding the index of thrombogenicity, the values were containing below the recommended 0.54 in all the sample. These results were sustained by the one of antioxidant activity of the powder which remains constant even if the cooking process where applied. The appealing pink colour of shrimp also tends to be levied by the cooking process.

References

- [1] M.Vittuari, L. Garcia, M. Masotti, E. Iori, C. Caldeira, Qian Z, H. Bruns, E. van Herpen, G. Obersteiner, G. Kaptan, G. Liu, B. E. Mikkelsen, R. Swannell, G. Kasza, H. Nohlen, S. Sala, *Sustainable Production and Consumption*, **2023**, 38, 104.
- [2] N.A. AlFaris, G.M. Alshammari, J.Z. AlTamimi, L.A. AlMousa, R.I. Alagal, N.M. AlKehayez, D. H. Aljabryn, M. M. Alsayadi, M. A. Yahya, *Saudi Journal of Biological Sciences*, **2022**, 29(1), 640.
- [3] H. Engelberth, M.F. Teisl, E. Frohmberg, K. Butts, K.P. Bell, S. Stableford, A. E. Smith, *Environmental Research*, **2013**, 126, 232.

Olive oils flavoured with chili peppers by different flavouring techniques: a comparison of the evolution of sensory and chemical characteristics over 1 year of storage

Lorenzo Cecchi¹, Silvia Urciuoli², Diletta Balli³, Matteo Bordiga⁴,
Fabiano Travaglia⁴, Bruno Zanoni¹, Nadia Mulinacci³

¹Department of Agricultural, Food and Forestry Systems Management (DAGRI), University of Florence, Italy

²PHYTOLAB-DiSIA, University of Florence, Via U. Schiff, 6, Sesto Fiorentino, 50019, Italy

³Department of NEUROFARBA, University of Florence, Via Ugo Schiff 6, 50019 Sesto F.no (Florence), Italy

⁴Department of Pharmaceutical Sciences, Università del Piemonte Orientale, Largo Donegani 2, Novara, 28100, Italy
nadia.mulinacci@unifi.it

Depending on the flavouring agent, flavoured olive oils show improved shelf-life and different types of sensory characteristics, which are strongly appreciated by consumers, helping in promoting olive oil in countries with people unfamiliar to it [1,2]. In a recent work of our research group, it was demonstrated that co-milling of fresh chili peppers with sound olives allows improving the quality of olive oils flavoured with chili peppers with respect to the typical infusion technique, and that the use of either green or red fresh chili peppers allows obtaining oils with different sensory characteristics [1]. Capsaicinoids, volatile compounds such as the typical esters of chili peppers and those linked to sensory defects, and sensory characteristics such as the pleasant hotness sensation and the fresh pepper flavour strongly discriminated among the samples prepared by the two different flavouring techniques and using chili peppers at different ripening levels.

The aim of the second part of the study was to evaluate the evolution of the chemical and sensory characteristics of the flavoured and non-flavoured oils over 1 year of storage. Samples were stored in the dark at room temperature into 250-mL dark glass fully filled bottles and were analyzed at time 0, and after 2, 6 and 12 months. Samples were subjected to a wide spectrum of analysis: basic legal parameters such as free acidity, peroxide number and spectrophotometric indices; tocopherols, phenolic compounds and capsaicinoids by HPLC-DAD; volatile compounds by both HS-SPME-GC-MS and HS-GC-IMS; sensory analysis with trained panelists.

The basic legal parameters only slightly increased over time in all samples, remaining well below that the limits established for the extra virgin olive oil category. The total content of phenolic compounds did not change over time, while some single molecules showed strong differences over time. Capsaicinoid content showed a slight increase in sample infused over the whole period. The typical esters of chili peppers characterized the oils flavoured by the co-milling technique over the whole period as the molecules linked to defects did for the oils flavoured by infusion. On the contrary, tocopherols were reducing over time for all samples, with the strongest decrease highlighted for the samples flavoured by the co-milling technique.

Finally, samples flavoured by infusion showed the presence of sensory defects over the whole storage period, with very light positive sensory attributes. The non-flavoured samples become defective (i.e., rancid) after the 12 months of storage. Interestingly, all the samples flavoured by the co-milling techniques showed no defects over the whole period of storage, also maintaining all the fresh and positive sensory characteristics such as pleasant hotness sensation and the fresh pepper flavour.

References

- [1] L. Cecchi, D. Balli, S. Urciuoli, A. Urciuolo, M. Bordiga, F. Travaglia, B. Zanoni, N. Mulinacci, *Food Chemistry*, **2023**, *404*, 134696.
[2] S. Lamas, N. Rodrigues, A.M. Peres, J.A. Pereira, *Trends in Food Science and Technology*, **2022**, *124*, 108.

Fingerprinting cold-pressed oils from apricot (*Prunus armeniaca*) and peach (*Prunus persica*) kernels: a comparative study

Francesca Carrà¹, Lorenzo Cecchi², Vincenzo Disca¹, Matteo Bordiga¹,
Lorella Giovannelli¹, Nadia Mulinacci³, Marco Arlorio¹

¹Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale

²Department of Agricultural, Food and Forestry Systems Management (DAGRI), University of Florence, Italy

³Dipartimento di Neuroscienze, Area del Farmaco e Salute del Bambino, Università degli Studi di Firenze
francesca.carra@uniupo.it

The valorisation of by-products and wastes from agri-food chains is a global trend within the circular economy, supporting the EU Green Deal. The application of resource-efficient and competitive processes in food area will lead to new food ingredients of high added value [1].

Plant edible oils can be prepared using different approaches: the cold-pressing method (hydraulic- or strew-pressing process) is largely used in order to recover oils from seeds and kernels.

Apricot (*P. armeniaca*) and peach (*P. persica*) cold-pressed oils from kernels are interesting principally because of their composition and their flavouring volatile fraction, but also relating to their potential functional properties, despite their high cost and the difficulty to prepare the kernels for the extraction from the seeds [2].

The aim of this work has been the characterization of samples of peach and apricot cold-pressed oils, kindly provided by an Italian Company. This preliminary study reports their fatty acids profile (GC-FID), the peroxide number, their volatile organic compounds (VOCs) fingerprints obtained in parallel by HS-SPME-GC-MS and HS-GC-IMS. All the analyses were performed on raw pressed oils. Moreover, their thermal stability (accelerated thermal stress simulated in oven), focusing on the changes of VOCs profiles, was also investigated, at a comparative level. TGA (Thermo-Gravimetric Analysis) was also considered in order to study the thermal impact on oils.

The overall analysis showed a different chemical fingerprint for these oils; an oleic acid-rich profile (particularly regarding peach oil sample, reaching 70% of fatty acids) was established, confirming interesting perspectives for these products, particularly concerning their use as “gourmet” oils, or oils useful as starting ingredient/material in nutraceutical or cosmetic applications.

References

[1] Green Deal Europeo, COM/2019/640.

[2] S. A. Siddiqui, S. Anwar, B. M. Yunusa, G. A. Nayik, A. M. Khaneghah, *Food Bioscience*, **2022**, *51*, 102336.

Phytochemical characterization of yellow (*Sinapis alba*) and oriental mustard (*Brassica juncea*) seed fractions as new sources of bioactive compounds

Raquel Torrijos¹, Laura Righetti², Martina Cirlini², Luca Calani², Jordi Mañes¹,
Giuseppe Meca¹, Chiara Dall'Asta²

¹Department of Food Science and Toxicology, Faculty of Pharmacy, University of Valencia,
Ave. Vicent Andrés Estellés s/n, 46100, Burjassot, Spain

²Food and Drug Department, University of Parma, Viale delle Scienze 27/A, 43124, Parma, Italy
raquel.torrijos@uv.es

Yellow mustard (*Sinapis alba*) and oriental mustard (*Brassica juncea*) are two plant species of the Brassicaceae family cultivated worldwide for their gastronomic value and are characteristic for their richness in bioactive compounds such as glucosinolates, polyphenols, β -carotene, and dietary fibre [1]. Mainly two fractions, flour and bran, can be obtained during mustard seed processing. Mustard bran, its main by-product, is discarded and could be a promising source of bioactive compounds with potential application due to its reported antifungal properties [2,3]; however, only a few reports are focused on describing their phytochemical profile, especially regarding yellow mustard. Therefore, to find new potential sources of bioactive compounds, the study aimed to comprehensively characterize the phytochemical profile of yellow and oriental mustard seed fractions (flour and bran). The volatile profile was identified by HS-SPME coupled to GC/MS, while free and bound bioactive compounds were determined by UHPLC-MS/MS. Moreover, the total phenolic content (TPC) and antioxidant activity (measured by DPPH assay) were evaluated. A total of 53 volatile compounds, including isothiocyanates, alkanes, alcohols, ketones, and esters, were identified in the mustard powders. In addition, up to 26 phenolic compounds (including phenolic acids, flavonoids, and glucosinolates) were determined. In particular, *Sinapis alba* presented a higher average of sinapic, *p*-hydroxybenzoic, and ferulic acids compared to *Brassica juncea* seed fractions ($p < 0.05$); however, some particularities were described in *Brassica juncea*, such as the higher allyl isothiocyanate content and the presence of glycosylated flavonoids that were not found in the yellow mustard samples. The principal component analysis (PCA) confirmed the singularity of each mustard specie and fraction according to their bioactive profile, achieving a 75.6% of total variance with the sum of the two principal components. Besides, *Sinapis alba* seed fractions evidenced a higher TPC than *Brassica juncea* seed fractions, while the flour fractions of both mustard species presented higher antioxidant activity than the bran fractions ($p < 0.05$). Thus, the results highlighted differences between mustard species and demonstrated that the mustard bran fraction could be revalorized for its richness in bioactive components and could be applied in agro-industrial or pharmaceutical applications.

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References

- [1] Y. Tian, F. Deng, *CyTA-Journal of Food*, **2020**, *18*(1), 704.
- [2] V. Reungoat, L.M.M. Mouterde, M. Chadni, J. Couvreur, E. Isidore, F. Allais, H. Ducatel, I. Ioannou, *Food and Bioproducts Processing*, **2021**, *130*, 68.
- [3] R. Torrijos, T.M. Nazareth, J.M. Quiles, J. Mañes, G. Meca, *Foods*, **2021**, *10*(2), 431.

Characterization of different cultivar of *Fagopyrum esculentum*: a high value nutritional source

Manuel Martoccia¹, Matteo Donna², Monica Locatelli¹, Jean Daniel Coïsson¹,
Massimo Blandino², Fabiano Travaglia¹

¹Dipartimento di Scienze del Farmaco - Food Chemistry, Biotechnology and Nutrition Unit –
Università del Piemonte Orientale "A. Avogadro", Largo Donegani 2, 28100 Novara (NO), Italy

²Dipartimento di Scienze Agrarie, Forestali e Alimentari Università di Torino, Largo Braccini 2,
10095 Grugliasco (TO), Italy
manuel.martoccia@uniupo.it

The common buckwheat (*Fagopyrum esculentum*, Moench) belongs to Polygonaceous family, and originates from Northern Europe and Asia [1]. It has strong adaptability to aversive environmental conditions, for this reason it is mainly cultivated in mountainous area in Russia, China and Ukraine [1,2]. *F. esculentum* can grow in poor soil with limited agronomic treatments, for this reason is considered an emergency crop [3]. Furthermore, due to the short cycle, in fertile plain soil, buckwheat could be cultivated as intercrops, after wheat and other winter cereals, increasing the profitability of cereal farms. This dicotyledon is considered a pseudocereal that have similarity with cereal grains in the physical appearance and in starch content, but they differ in their anatomy [1]. Buckwheat has also an excellent nutritional value and a low allergenic impact. Proteins are not toxic for celiac patients, but their total digestibility is reduced due to the presence of protease inhibitors (tannins and fiber) [1]. The amino acids composition is well-balanced if compared to the cereals one, due to a high content in lysine and arginine [1]. For these reasons buckwheat flour is used for formulation of gluten-free products and as high valued ingredient.

In this context, a variety screening focused on the identification of cultivars with high added value is underway, considering the cultivar actually cultivated in North Italy and in other European Countries. The characterization of different buckwheat cultivars may be useful to identify varieties with suitable agronomic and productive characteristics and responding to specific qualitative requirements of the processing industry in Piedmont. Seven different cultivars, such as “Panda”, “Lileja”, “Harpe”, “Billy”, “MHR Korona”, “MHR Smuga” and “Misto Tudori” (a mixture of “Kora” and “Smuglianka” cultivar) were analysed in their proximate composition and total polyphenol and flavonoid contents. Preliminary results have shown that three cultivars stand out for total dietary fiber (“MHR Korona”), lipid (“Panda”) and total flavonoid content (“Misto Tudori”). Further investigation will be carried out on protein quality and the flavonoid composition will be characterized.

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References

- [1] L. Aubert, C. Decamps, G. Jacquemin, M. Quinet, *Plants*, **2021**, *10*(2), 258.
- [2] S. Bhinder, A. Kaur, B. Singh, M.P. Yadav, N. Singh, *Food Research International*, **2020**, *130*, 108946.
- [3] B.K. Joshi, *Neglected and Underutilized Crops*, Academic Press (USA), **2023**, *7*, 151.

Chemocatalogation of antioxidant components of typical products from the Kroton area

Emanuela Marchese¹, Antonio Curcio¹, Stefano Alcaro¹⁻³

¹Dipartimento di Scienze della Salute, Università degli Studi “Magna Græcia” di Catanzaro, Campus “S. Venuta”, Viale Europa, 88100 Catanzaro, Italy

²Net4Science Academic Spin-Off, Università degli Studi “Magna Græcia” di Catanzaro, Campus “S. Venuta”, Viale Europa, 88100 Catanzaro, Italy

³Associazione CRISEA - Centro di Ricerca e Servizi Avanzati per l’Innovazione Rurale, Località Condoleo, 88055 Belcastro (CZ), Italy
e.marchese@unicz.it

The Mediterranean diet (MedDiet), one of the most studied and well-known dietary patterns worldwide, has been associated with a broad range of benefits for health [1]. The MedDiet is characterized by an abundance of plant food (fruit, vegetable, cereals, potatoes, beans, nuts, and seeds); minimally processed, seasonally fresh and locally grown foods; principal source of fat represented by olive oil; dairy products (mainly cheese) consumed daily in low to moderate amounts; fish and poultry in low to moderate amounts; red meat in low amounts; and wine consumed in low to moderate amounts, normally with meals. This diet also promotes natural versus processed foods, which can maximize the health-promoting micronutrient and antioxidant content of these foods [2].

Natural antioxidants have been proposed to have beneficial effects on different disease states, including neurodegenerative and cardiovascular diseases, diabetes and cancer. A lot of the biological activities of natural antioxidants have been ascribed to their ability to scavenge reactive oxygen species (ROS) that counteract oxidative stress [3]. In detail, the main structural features in antioxidants are the presence of the hydroxyl (-OH) and thiol (-SH) groups, as in polyphenols, carotenoids and vitamins.

In this context, we conduct a scientific investigation providing the state of the art on the natural antioxidant components present in several typical products from the Kroton area (such as *Mosto Cotto*, *Pipareddru*, *Olio Evo Pennulara*).

Chemocatalogation is scheduled to summarise the whole pool of chemical structures among the relevant antioxidant classes. Hence, a detailed description of the structures and antioxidant mechanisms involved for each product studied will be given. Finally, balanced combinations of them will be proposed in order to reach the daily requirement level of antioxidants.

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References

- [1] M. Guasch-Ferré, W.C. Willett, *Journal of internal medicine*, **2021**, 290, 549.
- [2] A.K. Sikalidis, A.H. Kelleher, A.S. Kristo, *Encyclopedia*, **2021**, 1, 371.
- [3] S. Hrelia, C. Angeloni, *Antioxidants (Basel)*, **2020**, 9, 344.

Polyamines for supplements development: microalgae as promising sources

Annalisa Maietti¹, Costanza Baldisserotto², Simonetta Pancaldi², Nicola Marchetti¹

¹Department of Chemical Pharmaceutical and Agricultural Science, University of Ferrara, 44121 Ferrara, Italy

²Department of Environmental and Prevention Sciences, University of Ferrara, 44121 Ferrara, Italy
annalisa.maietti@unife.it

Polyamines are a group of linear or branched aliphatic compounds containing multiple amino groups. Almost all organisms, from bacteria to higher plants and animals, can synthesize polyamines, which are derived from amino acids. Polyamines are involved in growth and differentiation of cells and in the stabilization of membranes and nucleic acids. They have a recognised role as antioxidant compounds against the cellular oxidative stress. Spermidine is the most abundant polyamine in a majority of different human tissues, with intracellular spermidine concentrations declining during the natural course of organism aging [1]. Spermidine homeostasis is influenced by nutritional uptake, intestinal sources (microbiota), endogenous biosynthesis, degradation, and active transporter systems between compartments [2]. Different foods are rich in polyamines such as mushrooms and soy products [3], and in 2020 EFSA authorized wheat germ rich in spermidine as novel food [4].

If the demand for spermidine increases, it will be necessary to find a natural source for the production of this molecule on a large scale and with sustainable methods to limit environmental stress. Microalgae constitute a rich reservoir and an excellent renewable resource of bioactive metabolites for pharmaceutical, food and cosmetic applications. These microorganisms are a relevant source of bioactive components such as proteins, vitamins, polyunsaturated fatty acids, polysaccharides, carotenoids and polyphenols [5]. During their exponential growth phase, microalgae also produce and release polyamines that promote cell division and growth.

In this study, polyamines content of *Neochloris oleoabundans*, already known to produce and release these compounds [6], was compared to that of well-known polyamines-rich foods. HPLC-UV method after derivatization with dansyl chloride was employed.

Polyamines produced by microalga were determined in both biomass and exhausted medium and under both autotrophic and mixotrophic conditions in axenic environment. In addition, microalgae extracts were evaluated in terms of total polyphenols content, antioxidant activity and fatty acid profile by GC-MS.

The results evidenced that the content of polyamines in microalgae is similar to spermidine rich foods and depends from the growing conditions. Polyamines are significantly released into the culture medium. Furthermore, microalgae provide antioxidant compounds (mainly polyphenols) and unsaturated fatty acids, and for this reason *N. oleoabundans* microalga can be considered as a promising starting material for the development of innovative food supplements and many other food and feed applications.

References

- [1] K. Soda, *Cells*, **2022**, *11*, 164.
- [2] S. Kiechl, R. Pechlaner, P. Willeit, M. Notdurfter, B. Paulweber, K. Willeit, P. Werner, ..., J. Willeit, *The American Journal of Clinical Nutrition*, **2018**, *108*, 371.
- [3] N.C. Muñoz-Esparza, J. Costa-Catala, O. Comas-Basté, N. Toro-Funes, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. C. Vidal-Carou, *Foods*, **2021**, *10*, 1752.
- [4] Commission Implementing Regulation (EU), **2020**/443.
- [5] M. V. Vieira, L. M. Pastrana, P. Fuciños, *Marine Drugs*, **2020**, *18*, 644.
- [6] A. Sabia, C. Baldisserotto, S. Biondi, R. Marchesini, P. Tedeschi, A. Maietti, M. Giovanardi, L. Ferroni, S. Pancaldi, *Applied Microbiology and Biotechnology*, **2015**, *99*, 10597.

New functional foods: characterisation of products derived from *Cannabis sativa* L. by advanced chromatographic techniques

Roberto Laganà Vinci¹, Katia Arena¹, Emanuela Trovato¹, Roberta La Tella¹,
Francesca Rigano¹, Paola Dugo^{1,2}, Paolo Guarnaccia³, Luigi Mondello^{1,2,4}

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, 98168 Messina, Italy

²Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

³Department of Agriculture, Food Science and Environment (Di3A), University of Catania, Catania, Italy

⁴Department of Sciences and Technologies for Human and Environment, University Campus Bio-Medico of
Rome, Rome, Italy

roberto.laganavinci@studenti.unime.it

The increasing demand for high nutritional and nutraceutical foods has generated, in recent years, a growing interest in *Cannabis sativa* L., a versatile plant with an eco-sustainable culture. In particular, the low ratio of ω -6/ ω -3, together with the high content of polyunsaturated fatty acids, phytosterols, tocopherols, polyphenols, terpenes and phytocannabinoids, make hemp seed foods sources rich in beneficial properties for health. In the present work, a comprehensive characterization of the minor components occurring in hemp seed-based food products *i.e.* oil, flour and flour by-product, is reported. For this goal, volatile (*i.e.* terpenes, hydrocarbons, furans and ketones) and non-volatile (*i.e.* tocopherols, cannabinoids and phenolic compound) metabolites were investigated by means of different chromatographic techniques. HPLC in combination with PDA, fluorescence, and MS detection was employed to analyse non-volatile fraction. Furthermore, GC coupled with FID and MS detectors were used for the analysis of volatiles and unsaponifiable compounds, the latter after conversion into more volatile trimethylsilyl derivatives. Terpenes represented the most abundant compounds among volatiles. A total of 58 compounds belonging to the unsaponifiable matter was identified only in hempseed oils. Among tocopherols, γ -tocopherol was quantified at the highest level. Phenols and cannabinoids were also investigated, and a total of 52 compounds were identified and quantified. The results obtained confirm the importance of *Cannabis sativa* L. as a high nutraceutical value source, emphasizing the qualitative-quantitative differences of its by-products.

Polyphenols in cold-pressed hemp and flax seed oils: effect of 11 months of storage under LED and neon light exposure

Yassine Jaouhari, Monica Locatelli, Jean Daniel Coïsson, Marco Arlorio,
Matteo Bordiga, Fabiano Travaglia

Dipartimento di Scienze del Farmaco - Food Chemistry, Biotechnology and Nutrition Unit - Università del Piemonte Orientale "A. Avogadro", Largo Donegani 2, 28100 Novara (NO), Italy
yassine.jaouhari@uniupo.it

During the last decade, cold-pressed hemp seed (*Cannabis sativa* L.) and flax seed (*Linum usitatissimum* L.) oils have been gaining a popularity as niche products. With their nutritional value and minor components content, such as antioxidants, the market price and demand for these oils used as value-added products in our diet and skincare are gradually increasing [1]. Generally, cold-pressed oils refer to those oils extracted from seeds under pressure at room temperature without applying heat. Several studies have shown that natural antioxidants, especially polyphenols present in the lipid's unsaponifiable fraction, are positively correlated with obesity and cardiovascular diseases [2]. More importantly, these compounds have the ability to reduce the oxidation of the fatty acids, especially in those oils rich in unsaturated fatty acids like flax and hemp seed oils [3].

One of the biggest issues of hemp and flax seed oils is their high susceptibility to oxidation and photo-oxidation reactions, which induce degradation of the polyphenolic compounds and subsequent fatty acid oxidation, generating rancid off-flavors and unpleasant odors.

Polyphenolic oxidation depends on several factors, including the extraction method, oxygen availability, and light exposure. Considering the market storage condition, where edible oils are exposed to intense artificial light (LED or neon) for several hours, the qualitative characteristics may eventually differ from those indicated on the label. An investigation of the polyphenolic compounds alteration in those oils that undergo into a rapid rancidification, like hemp and flax seed oils, during their storage under different light exposure conditions, will certainly provide useful information.

With this in mind, the aim of this work was to evaluate the influence that different lighting conditions have on the phenolic patterns in bottled cold-pressed flax and hemp seed oils during storage. The oils were bottled in dark glass bottles and kept under LED and neon light at room temperature. The sampling times were the following: 3, 5, 7, 9, and 11 months after bottling (T0).

The phenolic profile was obtained with HPLC-DAD and resulted belonging mainly to the classes of phenolic acids (as caffeic acid, vanillic acid, and ferulic acid) and flavonoids (luteolin and epicatechin). The starting value of the main phenolic compounds identified in fresh hemp seed oil was $612 \pm 24 \mu\text{g}/\text{kg}$, calculated as the sum of the individual phenolic compound concentrations. In comparison, flax seed oil contained a lower phenolic content, which resulted in $506 \pm 5 \mu\text{g}/\text{kg}$. After 11 months of storage, total polyphenols quantified in hemp seed oil stored under neon light showed a significant decrease ($542 \pm 25 \mu\text{g}/\text{kg}$) compared to the control, while under LED light condition they weren't significantly affected ($559 \pm 47 \mu\text{g}/\text{kg}$). On the other hand, the total polyphenolic content of flax seed oil significantly dropped in both lighting conditions as compared to the control oil, decreasing to 24% and 12% under neon ($383 \pm 15 \mu\text{g}/\text{kg}$) and LED ($443 \pm 22 \mu\text{g}/\text{kg}$) light, respectively.

References

- [1] N.T. Dunford, Specialty Oils and Fats in Food and Nutrition, Woodhead Publishing (UK), 2015, 2, 39.
- [2] R.V. Giglio, A.M. Patti, A.F.G. Cicero, G. Lippi, M. Rizzo, P.P. Toth, M. Banach, *Current Pharmaceutical Design*, 2018, 24, 239.
- [3] R. Abuzaytoun, F. Shahidi, *Journal of the American Oil Chemists' Society*, 2006, 83, 855.

Combination direct immersion - headspace solid-phase microextraction to extract and analyse volatile substances of DOCG wines from Marche Region

Lucia Lenti¹, Marco Cespi², Paolo Lucci³, Deborah Pacetti³, Dennis Fiorini¹

¹School of Science and Technology, Chemistry Division, ChIP research center, University of Camerino, via Madonna delle Carceri, 62032-Camerino (MC), Italy

²School of Pharmacy, ChIP research center, University of Camerino, Via Madonna delle Carceri, 62032-Camerino (MC), Italy

³Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche, 60131, Ancona, Italy
dennis.fiorini@unicam.it

Volatile substances profile of a wine is a key feature to define wine's quality and peculiarities. To assess volatile substances profile of wine, we have recently developed a method [1] aiming to extract a representative pool of substances, avoiding the possible loss of the least volatile, or of the most volatile substances, that can occur if using only headspace or only direct immersion SPME, respectively. The method makes use of the commercially available polydimethylsiloxane/divinylbenzene (PDMS/DVB) overcoated fiber and performs a two steps extraction, where the first step is a direct immersion extraction (DI) and the second is the headspace (HS) extraction. This approach has been demonstrated to represent a good compromise when the analytes have a broad range of volatility. After this two-step DI-HS extraction, analytes are desorbed and analysed by gas chromatography coupled to mass spectrometry. We have applied this method to determine the profile of volatile substances of DOCG wines from Marche region, that have never been characterized from this point of view and that represent important food excellences produced in Marche region, that need to be better known in order to authenticate and better promote them. DOCG wines produced in Marche region are: the white wine "Castelli di Jesi Verdicchio Riserva", the white wine "Verdicchio di Matelica Riserva", "Offida" (white "Pecorino" and "Passerina" wines and red "Offida"), the red wine "Conero", and the red sparkling wine "Vernaccia di Serrapetrona" (sweet and dry types). In the present study, sampling has been performed selecting wine samples provided by the producers recording higher sales, in order to investigate the Marche DOCG wines being more commonly consumed. The obtained results were elaborated by one way analysis of variance and principal component analysis allowing to highlight several peculiarities. "Verdicchio di Matelica Riserva" resulted to be characterized by high abundances of ethyl butyrate, ethyl octanoate and ethyl decanoate, compounds associated with yellow pulp fruits scents. "Castelli di Jesi Verdicchio Riserva" and white "Offida" showed more similar composition, even if "Castelli di Jesi Verdicchio Riserva" was characterized by a higher amount of decanoic acid, while white "Offida" of 2-phenyl ethyl acetate, isoamyl acetate and hexyl acetate, which are compounds associated with fruit and floral olfactory attributes. Concerning red DOCGs, "Offida" was found to be enriched in nerolidol. This DOCG showed also relatively high abundances of isopentyl hexanoate, while "Conero" was characterized by ethyl hydrogen succinate and diethyl succinate. Sweet "Vernaccia di Serrapetrona" contained relatively high percentage abundances of ethyl nonanoate as compared to the other DOCGs. The contribution will present more in detail the results that need to be further elaborated and correlated with other chemical and sensory parameters investigated to comprehensively define these high-quality food products.

References

[1] L. Lenti, S. Scortichini, D. Pacetti, M. Cespi, D. Fiorini, *Food Research International*, **2021**, *148*, 110632.

Extraction, characterization and antioxidant evaluation of unripe Apulian carob: a possible source of functional health foods

Marilena Muraglia¹, Mauro Niso¹, Filomena Corbo¹, Pasquale Crupi², Francesca Curci¹,
Francesco Limongelli¹, Nicola Garofalo¹, Maria Lisa Clodoveo²

¹Department of Pharmacy-Pharmaceutical Sciences, University of Bari “A. Moro”, 70125, Bari, Italy

²Interdisciplinary Department of Medicine, University of Bari “A. Moro”, 70124, Bari, Italy

filomena.corbo@uniba.it

Ceratonia siliqua L., commonly known as carob, is a Mediterranean tree, which has recently been the focus of attention due to the presence of a significant percentage of potentially beneficial polyphenols for human health [1,2,3]. To the best of our knowledge, this is the first study of two unripe (U-CAR) varieties of Apulian carob (*Amele* and *Selvatica*) to investigate their bioactive properties as a possible source of functional health foods.

For this purpose, the extraction, characterization and profiling of the antioxidant and antiradical potential of the U-CAR polyphenolic compounds were planned. The extraction methodologies adopted aimed to improve polyphenol extraction yields and the quality preservation of bioactive compounds. The extraction approaches such as ultrasound-assisted extraction (UAE) and the traditional maceration process (MAE) were selected, using an aqueous mixture with ethanol in different proportions. The total phenolic content and antioxidant profile of U-CAR extracts were determined using Folin Ciocalteu, flavonoid, DPPH, and ABTS tests. HPLC chromatography was performed to detect polyphenols in the extracts.

As part of this study, the cytotoxic activity and effects of U-CAR extracts on H₂O₂-induced cell viability against SH-SY5Y and MCF7 cells were investigated [4]. The obtained results provide a valuable starting point to plan a research program aimed at in vitro and in vivo screening of the polyphenolic antioxidant profile of the Apulian U-CAR fruit to find a molecular target that simultaneously regulates inflammatory-related diseases.

References

[1] V. Goulas, E. Georgiou, *Foods*, **2020**, *9*, 20.

[2] M.L. Clodoveo, P. Crupi, M. Muraglia, F. Corbo, *Processes*, **2022**, *10*, 983.

[3] M.L. Clodoveo, P. Crupi, M. Muraglia, F. Corbo, *Foods*, **2022**, *11*, 284.

[4] M. Mastromarino, M. Niso, C. Abate, E. Proschak, M. Dubiel, H. Stark, M. Castro, E. Lacivita, M. Leopoldo, *Molecules* **2022**, *27*, 1297.

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Effect of fortifying the diet with flaxseed, vitamin E and selenium on the broiler breast meat: nutritional and functional traits

Rossella Vadalà¹, Ambrogina Albergamo¹, Vincenzo Nava¹, Giovanni Bartolomeo³,
Rossana Rando¹, Nadia Colombo⁴, Roberto Gualtieri⁴, Massimiliano Petracci⁵,
Giuseppa Di Bella¹, Rosaria Costa¹, Giacomo Dugo^{1,3}, Nicola Cicero^{1,3}

¹Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali,
Università di Messina, Viale G. Palatucci, 98168 Messina, Italy

²Dipartimento di Scienze Veterinarie, Università di Messina, Messina, Italy

³Science4life S.r.l., start-up dell'Università di Messina, Messina, Italy

⁴Avimecc Spa, C.da Fargione, Agglomerato Industriale ASI, 97015 Modica, Italy

⁵Dipartimento di Scienze e Tecnologie Agroalimentari (DISTAL), Alma Mater Studiorum,
Università di Bologna, Bologna, Italy

ncicero@unime.it

The effect of fortifying the diet with flaxseed, selenium, and vitamin E, and market class on the nutritional and functional value of breast meat was investigated. The experimental unit (n = 6000 birds) received conventional or enriched diets and was placed on the 37th (light class), 47th (medium class), or 57th (light class) days of life. Therefore, functional and standard *Pectoralis major* muscles from each market class were analyzed for FA composition, inorganic elements, and vitamin E. A multiple linear model revealed that in breast, the dietary treatment significantly influenced (p < 0.05) the FA profile, lipid metabolism and health lipid indices while the slaughtering weight was related (p < 0.05) to most of elements (e.g., Na, Mg, K, Mn, and Se) and vitamin E. The interdependence of the two factors had strong relations (p < 0.05) with total PUFAs, including linolenic acid, desaturase activities, health lipid indices, trace essential elements and vitamin E [1, 2]. The consumption of functional breast meat can effectively contribute to the coverage of the daily requirement of EPA + DHA, inorganic elements and vitamin E, as established by the Italian Society for Human Nutrition (SINU) with the nutrient and energy reference values for the Italian population (LARN). The effect of feeding manipulation on breast meat was profitable, since functional products were better able than conventional products to meet the daily requirements of all nutrients studied. In addition, consumption of enriched meat from heavy broilers would be preferable, as they can provide the highest nutrient intake, considering the nutrient requirements of an adult male (30-59 years old). These products can also meet the daily intake of Mg up to 20.00%, Fe and Se up to 35.61% and 62.85%, and vitamin E up to 24%. Overall, the study showed that both market class and dietary influence are two relevant factors to be considered in the production of breast meat with higher nutritional value and functionality.

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References

- [1] M. Petracci, S. Mudalal, F. Soglia, C. Cavani, *World's Poultry Science Journal*, **2015**, *71*, 363.
- [2] E. A. Decker, Y. Park, *Meat Science*, **2010**, *86*, 49.

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Determination of regulated pyrrolizidine alkaloids in aromatic herbs using liquid chromatography–tandem mass spectrometry combined with rapid-easy extraction

Rita Celano¹, Serena Rizzo¹, Anna Lisa Piccinelli¹, Simona Serio¹,
Luca Campone², Luca Rastrelli¹

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

²Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy
rcelano@unisa.it

PAs are natural toxins produced by the secondary metabolism of plants as a defense mechanism against herbivores and insects. Currently, more than 600 different types of PAs and their PANOs have been identified in a wide variety of plant species (>6000), but the great majority of them (about 95%) belong to the families of Asteraceae, Fabaceae, Boraginaceae, Orchidaceae and Apocynaceae [1]. 1,2-Unsaturated PAs are defined as hepatotoxic, genotoxic, and carcinogenic agents and they are one of the most significant hepatic phytotoxins class. The major sources of PAs consumption in humans seem to be products contaminated with these PAs-producing plants. In the last two years, these alerts have noticeably increased for other products such as spices and aromatic herbs, highlighting the striking number of alerts raised for the relatively high amounts of PAs found in oregano [1].

SALLE was evaluated as extraction and clean-up technique for the analysis of PAs/PANOs in aromatic herbs. UHPC-MS/MS was selected as highly sensitive and selective multiresidue method. The analytical method was properly validated, with extraction efficiency from 75 to 104% and satisfactory intra-day (< 11) and inter-day (< 13) precisions. The proposed method showed good method detection and quantification limits 1.5-4.9 µg kg⁻¹ and 4.8-16.3 µg kg⁻¹ respectively.

The method proved to be a sustainable analytical strategy which meets green analytical chemistry principles as it reduced use of organic solvents and used green solvents. Its feasibility was verified through the analysis of 105 aromatic herbs samples. Of the samples analyzed, 75% were contaminated. Lasiocarpine, lasiocarpine N-oxide, europine, europine N-oxide, senecivernine, senecionine, lycopsamine N-oxide and intermedine N-oxide were the alkaloids which significantly contributed to the contamination of the samples.

The excellent performance, simplicity, speed and sustainability make it suitable for PAs and PANOs monitoring and occurrence studies in aromatic herbs, in order to guide future regulations for these products.

References

[1] N. Casado, S. Morante-Zarcelero, I. Sierra, *Trends in Food Science & Technology*, **2022**, *120*, 123.

Evaluation of bioactive compounds in bergamot peels and juice

Giovanna Cafeo¹, Marina Russo¹, Paola Dugo^{1,2}, Luigi Mondello^{1,2}

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

²Chromaleont S.R.L., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy
giovanna.cafeo@studenti.unime.it

Bergamot (*Citrus bergamia*) is a rich source of bioactive molecules such as flavonoids and limonoids. These molecules showed many biological properties: anticancer, antioxidant, antiviral, antimicrobial and anti-inflammatory activities. For this reason, each part of bergamot fruit (peel, pulp, juice, seeds) was a long investigated.

The main product of bergamot industry is represented by cold-pressed essential oil while juice is considered a by-product of the bergamot essential oil production. Unlike other *Citrus* juices, the taste of bergamot juice is not appreciable, however it can be used to isolate bioactive molecules useful as ingredients in food, pharmaceutical and cosmetic industry.

In this context, fruits belonging of three different bergamot cultivars (*Fantastico*, *Femminello* and *Castagnaro*) were analyzed in order to elucidate a quali-quantitative profile of bioactive molecules. Each fruit was peeled and subjected to solvent extraction before HPLC analysis. Bioactive molecules (flavonoids and limonoids) were determined in all the samples analyzed using a previously validated RP-HPLC/PDA/MS method [1]. In accordance with literature data, results showed that bergamot peels are the richest in bioactive molecules. All the samples showed the same qualitative profile, with a total of 26 bioactive molecules, but the three bergamots cultivar differed from a quantitative point of view: *Fantastico* sample was the richest in bioactive molecules. On the other hand, bergamot juices are the qualitative and quantitative poorest in molecules of our interest but 12 bioactive compounds were however found.

Taking into account the results obtained and the numerous studies that prove the beneficial effects of the molecules present in the sample analyzed, we can assert that isolation of bioactive molecules from bergamot juice represent a valid way to reevaluate this by-product in order to obtain nutraceuticals for human dietary intake.

References

[1] M. Russo, A. Arigò, M.L. Calabrò, P. Dugo, L. Mondello, *Journal of Functional Foods*, **2016**, *20*, 10.

Tannins from different parts of the chestnut (*Castanea sativa* Mill.) trunk: a green and efficient extraction method and their profile by HPLC-DAD-MS

Mohamad Khatib¹, Margherita Campo², [Maria Bellumori](mailto:maria.bellumori@unifi.it)¹, Lorenzo Cecchi³, Pamela Vignolini², Marzia Innocenti¹, Nadia Mulinacci¹

¹Department of NEUROFARBA, Division of Pharmaceutical and Nutraceutical Sciences, University of Florence

²Department of Statistics, Computer Science, Applications "Giuseppe Parenti", University of Florence

³Department of Agricultural, Food and Forestry Systems Management (DAGRI), University of Florence

maria.bellumori@unifi.it

Sweet chestnut (*Castanea sativa* Mill) is one of the most common chestnut species in European countries, with the cultivation for fruit production mainly concentrated in southern Europe, particularly in the Mediterranean area [1]. In Europe in 2016, the sweet chestnut covered more than 2.5 million hectares, with approximately 90% of the cultivated areas concentrated in few countries as France, Italy, Spain and Portugal. Chestnut wood is a rich source of hydrolysable tannins, which include gallotannins and ellagitannins, such as castalin, vescaline, castalagin and vescalagin, interesting for designing new, sustainable and innovative ways of use this source of hydrolysable tannins [2-4]. Since 2018, in Italy the regulation of the use of vegetable substances and preparations for food supplements include the cortex of *Castanea sativa* Mill.

The aim of this study was to investigate hot water extraction procedures to recover tannins from different parts of the chestnut tree and from the whole trunk using wood samples with short and long seasoning times. Different extraction conditions (e.g. wood-chips/water ratio and temperature) were evaluated and the tannin profile was determined by HPLC-DAD-MS. More than fifty ellagitannins and gallotannins were tentatively identified in the extracts. Higher temperatures (100 °C) allowed an efficient recovery of tannins and the highest yields (about 26%) were obtained from samples characterized by a longer seasoning time. A different content of tannins was highlighted in the internal and basal parts of the trunk as well as in the bark. Of note for producers, the tannin extracts obtained from different wood samples had similar percentages of total tannins, indicating a low variability in the composition of the final marketable extract. Folin-Ciocalteu method was also applied to evaluate the tannin content and to compare the results with those obtained by HPLC-DAD, proposing a correction factor of 3-3.5 suitable to correlate the results obtained applying the two methods.

Overall, the results of the study contribute to a deeper understanding of the active secondary metabolites of chestnut wood extract, which is a natural, sustainable and versatile product with proven antioxidant and antimicrobial efficacy and potentially useful for applications in different commodity sectors, including food.

References

- [1] T.R. Freitas, J.A. Santos, A.P. Silva, H. Fraga, *Plants*, **2021**, *10*(7), 1463.
- [2] P. Arapitsas, *Food Chemistry*, **2012**, *3*, 1708.
- [3] L.F.M de Melo, V.G.Q.A Martins, A.P. da Silva, H.A. Oliveira Rocha, K.C. Scortecci, *Food Chemistry*, **2023**, *414*, 135645.
- [4] A. Romani, M. Campo, P. Pinelli, *Food Chemistry*, **2012**, *130*(1), 214.

Aroma evaluation of omega-3 fortified biscuits: quality and stability in biobased packaging

Eloisa Bagnulo¹, Monica Locatelli², Chiara Cordero¹, Erica Liberto¹

¹Dipartimento di Scienza e Tecnologia del Farmaco,
Università degli Studi di Torino, Via Pietro Giuria 9, Turin, Italy
²Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale,
Largo Donegani 2/3, Novara, 28100
erica.liberto@unito.it

Functional foods, nutraceuticals and dietary supplements as part of a controlled lifestyle can provide opportunities to reduce health risk factors and address the need for prevention and control of diet-related chronic diseases to save healthcare resources è [1].

The development of functional foods that address the needs of specific consumer groups (especially infants and the elderly) is motivated by specific market demands and needs [2].

Environmental sustainability is also an important aspect, driven by the design and use of innovative, biodegradable and recycled packaging, but at the same time able to maintain the aromatic and nutritional quality of the product unchanged [3].

The aim of this work is to evaluate the quality and evolution over time of the aromatic fraction of biscuits functionalised with oils enriched with omega-3 fatty acids and packaged in innovative and more sustainable packaging (BB961, NK, NKME, NKPLA). For the characterisation of the compounds contained in the volatile fraction of the biscuits, the technique of online headspace analysis was used in conjunction with gas chromatography with mass spectrometry as a detector (HS-SPME-GC-MS).

The analysis of aromatic properties and their stability over time, when packed in different packages, showed that the biscuit recipes formulated with the blend of N6 oils (10% hemp, 90% EVO) are less accustomed to the development of oxidation compounds than those formulated with the N29 blend (20% hemp, 40% EVO, 40% OO) [4-5].

Empty packaging has its own profile of volatile organic compounds, with components such as propylene glycol, acetone and toluene being relatively abundant. Depending on the type of formulation, some packaging have slightly different interactions (NK and NKME). The material BB961 is the least inert of all. It can even be said that this packaging tends to absorb compounds from the biscuit that give it characteristic aromatic notes and instead release compounds that characterise the packaging material. In contrast, the situation is more favourable for the other two packaging. In particular, with the NKPLA packaging, there seems to be a tendency to adsorb those components that can be associated with lipid oxidation of the biscuit.

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References

- [1] S.R.B.M. Eussen, H. Verhagen, O.H. Klungel, J. Garssen, H. van Loveren, H. J. van Kranen, C.J.M. Rompelberg, *European Journal of Pharmacology*, **2011**, 668, S2.
- [2] I. Sioen, L. van Lieshout, A. Eilander, M. Fleith, S. Lohner, A. Szommer, C. Petisca, S. Eussen, S. Forsyth, P.C. Calder, C. Campoy, R.P. Mensink, *Annals of Nutrition and Metabolism*, **2017**, 70(1), 39.
- [3] P. Salgado, L. Di Giorgio, Y.S Musso, A.N. Mauri, *Frontiers in Sustainable Food Systems*, **2021**, 5, 1.
- [4] S. Krist, G. Stuebiger, S. Bail, H. Unterweger, *European Journal of Lipid Science and Technology*, **2006**, 108(1), 48.
- [5] Y. Xu, J. Li, J. Zhao, W. Wang, J. Griffin, Y. Li, S. Bean, M. Tilley, D. Wang, *International Journal of Food Science & Technology*, **2021**, 56(2), 530.

Chemical characterization of the aroma of new functional ingredients with high nutritional value: sensomic approach

Marta Viglietti¹, Eloisa Bagnulo¹, Monica Locatelli², Chiara Cordero¹, Erica Liberto¹

¹Dipartimento di Scienza e Tecnologia del Farmaco,
Università degli Studi di Torino, Via Pietro Giuria 9, Turin, Italy

²Dipartimento di Scienze del Farmaco,
Università del Piemonte Orientale, Largo Donegani 2/3, Novara, 28100
erica.liberto@unito.it

Most of today's chronic diseases, especially non-communicable diseases, are multifactorial and for some, the onset is associated with poor diet. It is believed that functional foods and dietary supplements, as part of healthy eating habits, can have significant benefits for human health by providing both primary and secondary prevention. As part of the regional NUTRAcore project, extra virgin olive oil, hemp oil, virgin olive oil and flaxseed oil were selected for the preparation of different blends that could become new functional and healthier ingredients used as raw materials for seasoning food or making sweet and savoury baked goods [1]. While the consumption of extra virgin olive oil and virgin olive oil has been widespread and promoted by the knowledge of their health benefits, the use of hemp oil and flaxseed oil has been promoted more recently as their nutritional and health values have been recognised [2]. Hemp oil and flaxseed oil have an optimal $\omega 6/\omega 3$ ratio, which is essential for maintaining the balance of the human organism and preventing cardiovascular diseases [2-4]. However, due to their high content of polyunsaturated fatty acids, hemp and flaxseed oil are much more susceptible to oxidative degradation, which can affect the stability and pleasant aroma of the finished oil ingredient and baked goods. During the heating process, some volatile compounds develop which are responsible for rancid off-flavours leading to a less appealing sensory profile of the oil. The aim of this study was to characterise the volatile content and thus the flavour profile of these vegetable oils as individual components and as part of blends in the innovative ingredient. In addition, their stability to oxidative degradation was evaluated by observing the changes in the volatile fraction, especially during heating processes. The first screening was carried out with HS-SPME-GC-MS. Two-dimensional comprehensive gas chromatography (GCxGC- MS) was then used to extend the characterisation of the oils. Several oxidation marker compounds were identified in each sample and their changes during the heating process were assessed [5]. Furthermore, these markers were quantified using multiple headspace extraction (MHE) to calculate their OAVs (Odour Activity Values), which describe the sensory profile of the studied sample in terms of odour impact. The optimal blend from a nutritional and sensory point of view has been used as a base lipid fraction in the production of biscuits further fortified with fibre, fibre and edulcorant, and Vitamin D.

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References

- [1] NUTRACORE - PIATTAFORMA TECNOLOGICA “BIOECONOMIA” Project FESR-FSE 2014-2020.
- [2] R Yang, L. Zhang, P. Li, L. Yu, J. Mao, X. Wang, Q. Zhang, *Trends in Food Science & Technology*, **2018**, 74, 26.
- [3] I. Sioen, L. van Lieshout, A. Eilander, M. Fleith, S. Lohner, A. Szommer, C. Petisca, S. Eussen, S. Forsyth, P.C. Calder, C. Campoy, R.P. Mensink, *Annals of Nutrition and Metabolism*, **2017**, 70(1), 39.
- [4] Y. Xu, J. Li, J. Zhao, W. Wang, J. Griffin, Y. Li, S. Bean, M. Tilley, D. Wang, *International Journal of Food Science & Technology*, **2021**, 56(2), 530.
- [5] F. Stilo M. del Pilar Segura Borrego, C. Bicchi, S. Battaglini, R.M. Callejón Fernadez, M. Lourdes Morales, S.E. Reichenbach, J. Mccurry, D. Peroni, C. Cordero, *Journal of Chromatography A*, **2021**, 1650, 462232.

Medium roasting specialty coffee fingerprint: discrimination among different brewing methods

Agnese Santanatoglia^{1,2}, Giovanni Caprioli¹, Laura Alessandroni¹,
Lauro Fioretti², Gianni Sagratini¹, Sauro Vittori^{1,2}

¹School of Pharmacy, University of Camerino, 62032, Camerino, Italy

²Research and Innovation Coffee Hub, Via Emilio Betti 1, 62020, Belforte del Chienti, Italy

agnese.santanatoglia@unicam.it

Coffee beverages, made with specialty coffees using filter brewing methods, have become increasingly popular. Specialty coffee segment has emerged in the last years as a response to consumer's demand for equity between the producer and the processing side [1]. From a chemical-physical perspective, coffee brewing is a solid-liquid extraction that takes place between hot water and ground coffee beans, when water passes through a bed of coffee grounds [2]. The extraction process plays a key role in the volatile composition of the filter coffee beverages [3]. Nowadays, Pure Brew system represents a real innovation as it allows to obtain a fast filter coffee with an espresso machine without the need for the barista to purchase additional equipment [4].

This study aimed to investigate the volatile compounds differences of a medium roasting specialty coffee between Pure Brew (PB) (automatic system), French Press (FP) (full immersion system), V60 (pour-over/drip system) and AeroPress (AP) (pressure system) brewing methods. A semi-quantitative analysis of volatile organic compounds (VOCs) of 6 resulting beverages from each method was performed.

A total of 49 VOCs were screened and quantified by headspace-solid phase microextraction gas chromatography-mass spectrometry and obtained data were elaborated through multivariate statistical approaches. The partial least squared discriminant analysis (PLS-DA) allowed a partial discrimination of the groups; according to the variable importance in projection (VIP) plot values, furans resulted to be the main influencing terms. In particular, 2-furanmethanol acetate, furfural and 2-acetylfuran showed the highest scores. Heatmap hierarchical cluster analysis of the VOCs showed a first clusterization of PB and FP samples and AP and V60 samples. This could be explicated as FP and PB extractions share the use of a metal filter, while V60 and AP methods involve a paper filter, as already reported by Santanatoglia et al. 2023 [4]. Moreover, a clusterization of coffee samples according to the extraction method was possible using the volatile profile.

In conclusion, our findings represent a preliminary point, to establish the basis for a more in-depth study in understanding the fingerprint of different coffee brewing methods.

References

[1] S. Ponte, *World development*, **2002**, 30(7), 1099.

[2] N. Córdoba, F.L. Moreno, C. Osorio, S. Velásquez, Y. Ruiz, *Food Research International*, **2021**, 141, 110141.

[3] F. Vezzulli, G. Rocchetti, M. Lambri, L. Lucini, *Foods*, **2022**, 11(6), 807.

[4] A. Santanatoglia, G. Caprioli, M. Cespi, D. Ciarlantini, L. Cognigni, L. Fioretti, F. Maggi, A. Mustafa, F. Nzekoue S. Vittori, *LWT*, **2023**, 114471.

Characterization of chemical properties of spent coffee ground and coffee silverskin as possible use for nutraceuticals and fertilizer products

Giovanni Caprioli, Samanta Corsetti, Simone Angeloni,
Massimo Ricciutelli, Gianni Sagratini, Sauro Vittori

School of Pharmacy, Chemistry Interdisciplinary Project (ChIP),
University of Camerino, 62032, Camerino, Italy
samanta.corsetti@unicam.it

According to the latest statistics, the global consumption of coffee is more than 150 million of 60 kg bags per year, this makes coffee one of the most consumed beverages worldwide. Consequently, a large amount of coffee residues is produced and needs to be disposed of. The main generated by-products are coffee silverskin (CS) and spent coffee grounds (SCGs). Coffee silverskin is the thin tegument that covers coffee beans, and it is obtained during the roasting process of green coffee, while SCGs are mainly formed during the extraction and preparation of instant coffee. The disposal of high amount of CS and SCG each year represent an environmental problem but, at the same time, they are potential sources of valuable by-products. Indeed, they are a good source of nutrients and bioactive compounds such as soluble dietary fibre, protein, minerals, fat, caffeine, and polyphenols [1, 2]. In our research group, the two matrices have been fully chemically characterized in terms of polyphenols, fat and fatty acid profile, volatile organic compounds, cholesterol-lowering phytosterols, and vitamins [1-4].

The content of polyphenols and phytosterols have been monitored also in all the coffee production chain, starting from green coffee, to CS, roasted coffee, espresso coffee and SCG allowing a complete view of theirs evolution. From a biological point of view, this research highlights that CS and SCG extracts protect cells against H₂O₂-induced oxidative stress by upregulating endogenous antioxidant enzymes such as thioredoxin reductase, heme-oxygenase 1, NADPH quinone oxidoreductase, and glutathione reductase. Moreover, the hydroalcoholic and methanolic SCG and CS extracts were shown to be the most active against selected enzymes such as tyrosinase, α -glucosidase, α -amylase and α -cholinesterases. Furthermore, the effect of CS/SCG-containing fertilizers on plant growth and modulation of soil microbiome was evaluated. SCG and CS application as organic amendment are known to modify soil chemical-physical properties, provide fundamental macro and micronutrients for plant growth, reducing the need for inorganic fertilizer, and stimulate soil microflora exerting a beneficial effect on soil-plant systems. Finally, a novel granulated formulation of SCG and CS was developed and assayed for its potential toxicity on seed germination and root elongation of four crop plants.

References

- [1] F.K. Nzekoue, G. Khamitova, S. Angeloni, A. Nacher Sempere, J. Tao, F. Maggi, J. Xiao, G. Sagratini, S. Vittori, G. Caprioli, *Food Chemistry*, **2020**, 325, 126836.
- [2] F.K. Nzekoue, G. Borsetta, L. Navarini, D. Abouelenein, J. Xiao, G. Sagratini, S. Vittori, G. Caprioli, *Food Chemistry*, **2022**, 372, 131188.
- [3] G. Zengin, K.I. Sinan, M.F. Mahomoodally, S. Angeloni, A.M. Mustafa, S. Vittori, F. Maggi, G. Caprioli, *Foods*, **2020**, 9, 713.
- [4] S. Angeloni, M. Freschi, P. Marrazzo, S. Hrelia, D. Beghelli, A. Juan-García, C. Juan, G. Caprioli, G. Sagratini, C. Angeloni, *Oxidative Medicine and Cellular Longevity*, **2021**, 45, 6620913.

Flavonoids and limonoids from Bergamot (*Citrus bergamia*) differentially modulate the expression of PCSK9 and LDLR in human hepatocarcinoma cell line Huh7

Irene Ferrarese¹, Maria Giovanna Lupo², Ilaria Rossi¹, Stefania Sut¹, Francesca Loschi¹, Pietro Allegrini³, Antonella Riva³, Nicola Ferri^{2,4}, Stefano Dall'Acqua¹

¹Department of Pharmaceutical and Pharmacological Sciences, University of Padova, 35131, Padua, Italy

²Department of Medicine-DIMED, University of Padua, 35122 Padua, Italy

³Indena Spa, 20139, Milan, Italy

⁴Veneto Institute of Molecular Medicine (VIMM), 35129 Padua, Italy

irene.ferrarese@phd.unipd.it

Citrus bergamia extracts have been studied for the management of hypercholesterolemia disorders [1], [2]. Up to now limited information is available concerning the activity of its main phytoconstituents towards the main targets of the cholesterol homeostasis. In this study, the effects of bergamot peel extract and isolated constituents, namely glycosidic and non-glycosidic flavonoids, one coumarin and one limonoid were concurrently evaluated on the low-density lipoprotein receptor (LDLR) and proprotein convertase subtilisin/kexin type 9 (PCSK9), by using an *in vitro* HuH7 cell line, under the same experimental conditions. Furthermore, for the first time the effects of bergamot peel extract have been studied to describe a potential hypolipidemic action. Results are expressed as LDLR/PCSK9 ratio to emphasize, unlike statins, the ability of bergamot extract and its derivatives to exert a potential cholesterol-lowering effect through increase in LDLR expression and the inhibition of PCSK9 expression. Significant differences were observed due to glycosylation and different substitution on flavanone moiety (*O*-methylation). Considering the thirteen isolated compounds, naringenin-7-*O*-rutinoside and apigenin-6,8-*C*-glucoside showed a potential adjuvant action with statins, such as those that do not alter the PCSK9 expression. However, hesperetin derivatives (hesperetin, hesperetin-7-*O*-glucoside and hesperetin-7-*O*-neohesperidoside) and eriodictyol showed a statin-like effect since a significant increase of both LDLR and PCSK9 expression were detected. Furthermore, bergamot peel extract firstly demonstrated a significant reduction of PCSK9 expression, indicating a potential adjuvant action to statins. Based on the present data, bergamot peel constituents can play a role in the management of hypercholesterolemia, on one hand they may produce an adjuvant action in combination with statins, while on the other hand they may have a statin-like effect. Additionally, bergamot peel extract may be a good candidate as an adjuvant to statins action.

References

[1] Y. Huang, R. Tocmo, M.C. Nauman, M.A. Haughan, J.J. Johnson, *Nutrients*, **2021**, *13*, 3156.

[2] L. Di Donna, D. Iacopetta, A.R. Cappello, G. Gallucci, E. Martello, M. Fiorillo, V. Dolce, G. Sindona, *Journal of functional foods*, **2014**, *7*, 558.

Identification and quantification of *Vaccinium corymbosum* L. in yogurt using molecular approaches

Valeria Fochi, Tamara Attard, Jean Daniel Coïsson, Marco Arlorio

Dipartimento di Scienze del Farmaco - Food Chemistry, Biotechnology and Nutrition Unit,
Università del Piemonte Orientale "A. Avogadro", Largo Donegani 2, 28100 Novara, Italia
valeria.fochi@uniupo.it

Blueberries (*Vaccinium corymbosum* L.) are considered one of the most high-valued fruits and are becoming popular “superfoods” due to their high content of polyphenolic compounds responsible for the protective effects against cell damage related to oxidative stress [1]. Thanks to their numerous health benefits but also to commonly-recognized taste properties, blueberry has been transformed into fruit juice, jam, jelly and also incorporated in several food products such as yogurt and baked goods.

With the rapid growth of the blueberry market, adulteration became a serious challenge. In addition to economically motivated fraud, there is also a concrete risk linked to the presence of hidden ingredients derived from a cross contamination during the production line. The correspondence of what is declared on the label and what is used in these products sometimes remains an unsolved problem. Therefore, a reliable authentication system can constitute an essential instrument to strengthen the control system and consequently to protect the consumers. The use of DNA-based approaches in food authenticity and traceability purposes is continuously increasing, thanks also to the developing of new techniques with improved performances in terms of specificity, sensitivity, speed, and multiplexing [2]. Digital PCR (dPCR) is a powerful technique that provide an absolute measurement of nucleic acid concentration, without the use of standard curves. Compared with the real-time PCR, it can eliminate the effects of matrixes, improving the sensitivity and precision [3]. This work is part of the INNO.PI.FRUT project (leader partner ‘Fondazione per la Ricerca, Innovazione e lo Sviluppo Tecnologico dell’Agricoltura Piemontese – Agrion) and its aim was the development of a robust and sensitive PCR-based method for the detection and quantification of blueberry DNA in commercial yogurt. Different yogurt mixtures were developed in the laboratory, simulating commercial products with different blueberry content. Real-time and digital PCR were performed both to estimate the percentage concentration of blueberry fruit in several commercial yogurt using a specific marker developed on the gene coding for an Anthocyanidin synthase (ANS), key enzyme of anthocyanidins production. The results of this work demonstrated that innovative techniques, like digital PCR are very sensible and can be used to improve the traceability system of blueberry-based products.

References

- [1] J.S. Câmara, M. Locatelli, J.A. Pereira, H. Oliveira, M. Arlorio, I. Fernandes, R. Perestrelo, V. Freitas, M. Bordiga, *Nutrients*, **2022**, *14*, 5133.
- [2] K. Böhme, P. Calo-Mata, J. Barros-Velázquez, I. Ortea. *Journal of Agricultural and Food Chemistry*, **2019**, *67*(14), 3854.
- [3] C.M. Hindson, J.R. Chevillet, H.A. Briggs, E.N. Gallichotte, I. K. Ruf, B. J. Hindson, R.L. Vessella, M. Tewari, *Nature Methods*, **2013**, *10*, 1003.

Characterization by LC/MS and GC/MS of Sicilian extra virgin olive oils

Claudia Lino¹, Rosa Pitonzo¹, David Bongiorno², Giuseppe Avellone^{1,2}

¹AteN Center, Università di Palermo, viale delle Scienze, 90128 Palermo (Italy)

²Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche,
Università di Palermo, via Archirafi 32, 90123 Palermo (Italy)

claudia.lino@unipa.it

The aim of this study was to evaluate the nutraceutical qualities of extra virgin olive oils samples produced from olive trees belonging to three Sicilian cultivars *Nocellara*, *Biancolilla* and *Cerasuola*.

Several studies have shown how a diet rich in extra virgin olive oil leads to healthy nutritional effects mainly due to its bio-phenol compounds content, such as antioxidant, anti-inflammatory, anti-cancer, antimicrobial, antiviral, hypoglycaemic, hepatic, cardiac and neuroprotective properties [2].

The bio-phenols involved in the nutraceutical properties of olive oils have been determined and quantified using a selective, sensitive and versatile analytical technique such as liquid chromatography coupled to high resolution mass spectrometry (UPLC-Q Exactive Orbitrap-HRMS system) [3-4]. Moreover, extra virgin olive oils samples were characterized by determining the fatty acid profile using GC/MS (Trace 1310/ISQ LT) [5].

The European Food Safety Authority (EFSA) has allowed health recognition based on bio-phenols in olive oil, with reference to food claims, present on the label and summarized in EC Regulation 432/2012, which qualify the oil as a functional food. This Regulation affirms that "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress" and the "health claim may be used only for olive oil which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) for 20 g of olive oil" [6-7].

The different claim values obtained for the analyzed samples have been affected by the different olive cultivars, by the different milling systems used and by the different olive harvesting periods. Were selected three samples, one for each cultivar, which showed the highest claim values according to EC Regulation 432/2012.

The three selected samples will be used to evaluate the effects of EVOO on lipid metabolism and on the improvement of global health status and prevention of cardiovascular risk in the metabolic syndrome.

This research was supported by the Production Activities Department of the Sicilian Region funded as part of the FESR 2014-2020, within the project "Trial: food, nutraceuticals, and health". Scientific partner was the "PROMISE" department supported by "ATeN Center", both belonging to the University of Palermo. Project leader was "Manfredi Barbera & Figli S.p.A", a Sicilian company that deals with the production and marketing of EVOO, and "Nuova Farmaceutica" was a company that has developed nutraceutical compounds for contrasting several clinical pathologies.

References

- [1] D. Bongiorno, V. Di Stefano, S. Indelicato, G. Avellone, L. Ceraulo, *Mass Spectrometry Reviews*, **2022**, e21744.
- [2] M. Servili, B. Sordini, S. Esposto, S. Urbani G. Veneziani, I. Di Maio, R. Selvaggini, A. Taticchi, *Antioxidants* **2014**, *3*, 1.
- [3] F. Grilo, M.E. Novara, M.C. D'Oca, S. Rubino, R. Lo Bianco, V. Di Stefano, *International Journal of Food Sciences and Nutrition*, **2020**, *71(4)*, 397.
- [4] M. Ricciutelli, S. Marconi, M.C. Boarelli, G. Caprioli G. Sagratini, R. Ballini, D. Fiorini, *Journal of Chromatography A*, **2017** 1481, 53.
- [5] S. Rizwan, C. Benincasa, K. Mehmood, S. Anjum, Z. Mehmood, G.H. Alizai, M. Azam, E. Perri, A. Sajjad, *Journal of Oleo Science*, **2019**, *68(1)*, 33.
- [6] Commission Regulation (EU) 432/2012.
- [7] M.Z. Tsimidou, N. Nenadis, M. Servili, D.L. García-González, T. G. Toschi, *European Journal of Lipid Science & Technology*, **2018**, *120*, 1800098.

Study of the oxidative stability of thermally stressed vegetable oils

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

Department of Agricultural and Food Sciences,
University of Bologna, Piazza Goidanich 60, 47521, Cesena (Italy)
silvia.marzocchi4@unibo.it

Most vegetable oils used as cooking oils or as ingredients in many foods undergo heat treatments that partially undermine their quality stability. All chemical changes in oils subject to high temperatures are promoters of phenomena such as oxidation, hydrolysis, polymerization or isomerization that can change the sensory, nutritional and safety properties of oils. The aim of the study was to evaluate the thermo-oxidative stability of vegetable oils subjected to mild heat treatments to simulate cooking conditions or home preparations. Peroxide number [1], hexanal [1], *p*-anisidine [2] and oxidized fatty acid (OFA) [1] content were determined in samples of extra virgin olive oil (EVOO), grape seed oil (GS), low oleic sunflower oil (LOSO) and high oleic sunflower oil (HOSO), treated thermally at 100 °C for different times (0; 0,5; 1; 1,5; 2; 3; 5 hours). Although remaining below the maximum permitted limits, the EVOO showed the highest values of peroxides and hexanal, this because EVOO was not subjected to the refining process, so it contains pro-oxidant compounds, and to the deodorization process, so the hexanal is a main representative of its aromatic pattern. In contrast, the OFA content was lower in EVOO than in other oils and remained constant during heat treatment. After EVOO, GS sample was the one with the highest peroxide and hexanal contents at 0h, probably due to the use of high temperatures in the refining process. These parameters, however, remained constant over time, that means that, under the used conditions, the rate of formation of secondary compounds of degradation of hydroperoxides did not exceed the rate of formation of the same but remained almost constant. *p*-anisidine was also constant over time and showed values similar to EVOO and LOSO. OFA showed a significant increase after 1h of treatment due to the increased susceptibility to oxidation of polyunsaturated fatty acids, the main constituents of GS sample. The LOSO sample showed the lowest peroxide and hexanal content at all times, while the values of *p*-anisidine and OFA were among the highest. These seemingly contradictory data could be affected by the refining process suffered that has displaced much of volatile compounds and hydroperoxides. Finally HOSO, had peroxide and hexanal values that increased significantly as the thermal stress time increased. The value of *p*-anisidine remained low and constant; while the content in OFA was among the highest, along with GS and LOSO, and showed an increase in time.

The results show that the heat treatment conditions were not sufficient to trigger the oxidation of many oils exponentially; consequently refined oils, such as LOSO or GS, showed oxidative values still acceptable, while the EVOO had higher oxidative values due to the presence of prooxidants. Moreover, while *p*-anisidine varied according to the type of oil but not so much according to the heat treatment, OFA represent a parameter that is affected by the effect of heat treatment and therefore of extreme importance for the quality of a heat treated oil.

References

- [1] S. Marzocchi, M.F. Caboni, *Journal of Agricultural and Food Chemistry*, **2018**, 66, 12555.
- [2] AOAC, Official Methods Cd, 18-90/2017.

Effect of light on the initiation of oxidation in selected vegetable oils

Silvia Marzocchi¹, Cesare Ravagli¹, Maria Fiorenza Caboni^{1,2}, Federica Pasini^{1,2}

¹Department of Agricultural and Food Sciences, University of Bologna, Cesena, Italy

²Inter-Departmental Center for Agri-Food Industrial Research (CIRI Agroalimentare),
University of Bologna, Cesena, Italy
federica.pasini5@unibo.it

Since light, heat and oxygen are among factors determining the oils rancidity or deterioration, vegetable oils may lose their quality upon improper storage. With the presence of a photosensitizer, light can increase the rate of free radical formation and the fatty acid sensitivity to oxidation [1].

The aim of the study was to evaluate the effect of low intensity and low power ultraviolet light on the photo-oxidation of different vegetable oils during short exposure periods (maximum 8 hours).

Peroxide value, hexanal content and *p*-anisidine value were monitored in order to assess the oxidative status of the following samples: extra virgin olive oil (EVOO), grape seed oil (GO), high oleic sunflower oil (HOSO) and low oleic sunflower oil (SO). The evidence on the photo-oxidative stability of oils exposed to light in real shelf-life conditions are numerous, but most of these studies use fluorescent lamps or high intensity UV rays, whereas few researches focus on the effect in the first moments of exposure.

Results showed GO as the refined oil with the highest peroxide value and hexanal content. The higher peroxide concentration compared to other refined oils may depend on an incomplete removal of polar compounds (such as phospholipids) during the degumming phase [2]. These polar compounds may be pro-oxidants in the final oil and promote the oxidation of free fatty acids, producing an increase in peroxides. For all treatment times considered, SO had a lower and constant peroxide value compared to the other refined oils, despite the high content in polyunsaturated fatty acids (PUFA). This trend could be due to a stronger refining of the SO, eliminating most of the pigments that catalyze the oil photo-oxidation. Furthermore, the peroxide and hexanal contents of SO was not so different from those of HOSO, who is richer in monounsaturated fatty acids (MUFA). *p*-Anisidine values were not different among the three refined oils.

EVOO was the sample with the highest peroxide value, probably justified by the fact that it does not undergo refining process and the final oil present both antioxidant (tocopherols and phenols) and pro-oxidant compounds such as chlorophylls and pheophthins, active promoters of lipid photo-oxidation. Hexanal was also present in high amount in EVOO because it is one of the principal compounds of the typical aromatic profile of this oil and it is not removed by a deodorization process. Contrary to expectations, the EVOO *p*-anisidine values did not differ from those of the other oils.

Despite the different levels of unsaturation and of natural antioxidants concentration, the results of this study showed that the impact of light was quite similar on the different oil samples. In the future it could be useful to evaluate the impact of the treatment by light on the oils over time, for example with a shelf-life study or by adding a technological use of the treated oils.

References

- [1] Y. Zhou, W. Zhao, Y. Lai, B. Zhang, D. Zhang, *Frontiers in Plant Science*, **2020**, *11*, 1315.
- [2] S. Gharby, *The Scientific World Journal*, **2022**, *e6627013*, 1.

Homovanillyl oleate, a novel lipophenol from olive oil

Alessandra Cersosimo^{1,2}, Cinzia Benincasa², Pierluigi Plastina¹

¹Department of Pharmacy, Health and Nutrition, University of Calabria, Rende (CS)

²CREA Research Centre for Olive, Fruit and Citrus Crops, Rende (CS)

pierluigi.plastina@unical.it

The positive health effects of phenolic compounds found in olives and extra virgin olive oil (EVOO) are well known, especially after the European Food Safety Authority (EFSA) stated in 2010 that the consumption of polyphenols contributes to the protection of lipids from oxidative stress [1]. Furthermore, phenolic compounds such as hydroxytyrosol and oleuropein possess other positive properties including, anticancer and anti-inflammatory activity [2]. On the other hand, these molecules show low oral bioavailability and fast elimination in humans, mainly due to their hydrophilic character. Lipophenols are an emerging subclass of phenolic compounds characterized by the presence of a lipid moiety. They do not have some of the disadvantages described above due to their higher lipophilicity compared to polar phenolic compounds. Recently, we identified hydroxytyrosyl oleate (HtyOle) and tyrosyl oleate (TyOle), derivatives of hydroxytyrosol and tyrosol, respectively, in olive oil and by-products. Furthermore, we showed that HtyOle and TyOle possess anti-inflammatory, anti-diabetes, antioxidant, and tissue regenerative properties in *in vitro* cell models [3-7].

In this communication, the identification of another lipophenolic molecule, homovanillyl oleate (HvOle), in oil matrices including drupes, oils, leaves, pomace and paté is reported. The drupes and leaves of *Olea europaea* L., belonging to the Carolea cultivar, were collected in the CREA-OFA experimental field in Rende in October 2022. EVOOs were obtained from drupes of different cultivars and during different campaigns. By-products (paté and pomace) belonged to the Dolce di Rossano cultivar. The phenolic fraction was extracted from oil matrices with 8/2 (v/v) MeOH-water. The quantitative determination of HvOle was carried out by the external standard method in liquid chromatography coupled to mass spectrometry (LC/MS), in negative mode using multiple reaction monitoring (MRM) and the transition 431.3 [M-H]⁻ → 281.3. The data obtained demonstrate the presence of HvOle in all the matrices analyzed, differing only in quantitative terms. In particular, the amount of the analyte varies according to factors such as the cultivars, the stage of ripeness of the fruit and the techniques used to produce EVOOs. In conclusion, this work confirms the potential role of HvOle as a marker of quality as for other lipophilic derivatives. On the other hand, it opens a new scenario characterized by the presence and quantification of this compound also in matrices such as olives and leaves, in which other lipophenols were not detected.

References

- [1] EFSA, *Journal*, **2011**, 9, 2033.
- [2] F. Visioli, A. Davalos, M.-C. Lopez de las Hazas, M.C. Crespo, J. Tomé-Carneiro, *British Journal of Pharmacology*, **2020**, 177(6), 1316.
- [3] P. Plastina, C. Benincasa, E. Perri, A. Fazio, G. Augimeri, M. Poland, R. Witkamp, J. Meijerink, *Food Chemistry*, **2019**, 279, 105.
- [4] C. Benincasa, C. La Torre, P. Plastina, A. Fazio, E. Perri, M.C. Caroleo, L. Gallelli, R. Cannataro, E. Cione, *Antioxidants*, **2019**, 8, 233.
- [5] C. Benincasa, C. La Torre, A. Fazio, E. Perri, M.C. Caroleo, P. Plastina, E. Cione, *Antioxidants*, **2021**, 10(7), 1051.
- [6] M.C. Caroleo, P. Plastina, A. Fazio, C. La Torre, F. Manetti, E. Cione, *Pharmaceutics*, **2021**, 13(7), 1085.
- [7] P. Plastina, *Olives and Olive Oil in Health and Disease Prevention (Second Edition)*, Academic Press (USA), **2021**.

***Prunus domestica* L.: chemical characterization and biological activities**

Hammad Ullah¹, Daniele Giuseppe Buccato¹, Lorenza Francesca De Lellis¹, Alessandra Baldi¹, Anella Saviano¹, Alessandro Di Minno^{1,2}, Francesco Maione¹, Roberto Ciampaglia¹, Maria Daglia¹

¹Department of Pharmacy, University of Naples Federico II, Naples, Italy

²CEINGE-Biotecnologie Avanzate, Via Gaetano Salvatore 486, 80145 Naples, Italy

hammad.ullah@unina.it

Prunus domestica L. fruits with their high concentration of polyphenols and dietary fibers are characterized by low glycemic index. Their consumption in adequate amounts and on a regular basis could be a potential protective strategy against metabolic syndrome (MetS) risk factors [1–3]. In the present study, the hydroethanolic extract obtained from fruit pulp of *P. domestica* subsp. *syriaca* was subjected to chemical characterization via UHPLC-HRMS technique followed by *in vitro* and *in vivo* biological property assessment against MetS risk factors. The UHPLC-HRMS analysis showed the presence of hydroxycinnamic and quinic acid derivatives (46.7% of total peak area), procyanidins (37%), and flavonol glycosides (7.9%). Considering that the target physiological effect is represented by MetS risk factors, the sugar content of fruit pulp extract was eliminated, to obtain an extract with minimal sugar content for the assessment of the biological activities. The fruit extract inhibited α -amylase, α -glucosidase, HMG CoA reductase, and pancreatic lipase enzyme activities, with IC₅₀ values of 7.01 mg/mL, 6.4 mg/mL, 2.5 mg/mL, and 6.0 mg/mL, respectively. *In vivo* studies (performed in *Mus musculus*) revealed considerable hypoglycemic and hypoinsulinemic activities of *P. domestica* fruit pulp extract. BALB/c mice were challenged with oral sugars load followed by the supplementation of *P. domestica* fruit pulp extract (750 mg/kg of mouse body weight). The blood glucose and insulin levels were measured at T0, T10 min, T20 min, and T 1 h. *P. domestica* fruit extract showed significant effects at T10 min and T20 min in reducing blood glucose and insulin levels. In conclusion, this extract could be used to reduce MetS risk factors, and in turn to prevent the cardio-metabolic disorders.

References

- [1] H. Ullah, A. De Filippis, H. Khan, J. Xiao, M. Daglia, *Food and Chemical Toxicology*, **2020**, *144*, 111574.
- [2] H. Ullah, E. Sommella, C. Santarcangelo, D. D'Avino, A. Rossi, M. Dacrema, A.D. Minno, G. Di Matteo, L. Mannina, P. Campiglia, P. Magni, M. Daglia, *Nutrients*, **2022**, *14*, 340.
- [3] H. Boeing, A. Bechthold, A. Bub, S. Ellinger, D. Haller, A. Kroke, E. Leschik-Bonnet, M.J. Müller, H. Oberitter, M. Schulze, P. Stehle, *European Journal of Nutrition*, **2012**, *51*, 637.

Effects of fermentation with *Saccharomyces cerevisiae* on pomegranate peel; a study of phenolic compounds and polysaccharides

Mohamad Khatib¹, Beatrice Zonfrillo¹, Lorenzo Cecchi², Gemma Selleri¹,
Maria Bellumori¹, Marzia Innocenti¹, Nadia Mulinacci¹

¹Department of NEUROFARBA, University of Florence, Via Ugo Schiff 6, 50019 Sesto F.no (Florence), Italy

²Department of Agricultural, Food and Forestry Systems Management (DAGRI), University of Florence, Italy
beatrice.zonfrillo@unifi.it

The fruit of *Punica granatum* L. (Punicaceae) is widely used in popular medicine in many countries. Fruit juice and peel extracts of pomegranate have shown numerous health benefits, e.g. antimicrobial, antioxidant and antidiabetic effects [1]. Pomegranate peel is approximately 38-50% of fresh fruit weight and is the main by-product of juice production. Fermented foods play a significant role in the diets of many cultures and improve nutritional and nutraceutical properties, e.g. facilitating the absorption of nutritional and bioactive components [2]. Fermentation technology has been widely applied for the treatment of fruits and vegetables, but few studies on the fermentation of pomegranate peels are available so far [3]. Aim of the study was to evaluate the chemical changes determined by the *Saccharomyces cerevisiae* on the pomegranate peel from Wonderful and G1 varieties. Samples of dried peel were wet with water (1g/10 mL), and fermented as such in open and closed bottles under agitation in Thermo-Shaker. Some peel samples were boiled for 2 minutes before fermentation to reduce the activity of endogenous microorganisms. Commercial yeast (25 mg/g DW) was used to inoculate the samples, blank samples were yeast free. All samples were fermented at 25°C for 48 and 96 hours, then centrifuged twice to collect the supernatant which was successively treated and analyzed. In particular, the tannins were determined by HPLC-DAD and the polysaccharides by DLS and ¹H-NMR. The yields in dry extract were close to 50% of the weight of the dried peels. The results showed a large amount of ethanol produced by yeast fermentation, while alcohol was absent in the blank samples. The tannin content was similar in all samples and varied between 442 mg/g and 534 mg/g of dry extract. Polysaccharides (precipitated after ethanol addition) ranged from 7.4% to 10% for yeast-fermented and blank samples, respectively. The analyzes by DLS allowed to evaluate the molecular dimensions of the polysaccharides which were similar for blank samples and yeast added peels, indicating a partial hydrolysis of the polysaccharides even without the use of yeast. The predominant polymer (90-99% of the total polysaccharide pool) showed a hydrodynamic volume close to 8.2 *10⁵ kDa, with a molecular size ranging from 282-414 nm. It should be emphasized that after a 48-hour fermentation with "autochthonous" microorganisms and with the addition of yeasts, the size of the pectic polysaccharides was reduced of 50-70% compared to the native polysaccharides recovered after the decoction of the peel [4]. These preliminary results suggest that the aqueous extracts of fermented pomegranate peel can be suitable substrates to evaluate the prebiotic properties, already observed *in vitro* for the native polysaccharides of the fruit [5]. Pomegranate peel fermentation can be proposed as a natural and low-cost process to obtain new functional ingredients from this by-product potentially able to improve human microbiota health by providing both prebiotic compounds and a high amount of hydrolysable tannin.

References

- [1] T.P. Magangana, N.P. Makunga, O.A. Fawole, U.L. Opara, *Molecules*, **2020**, 25(20), 4690.
- [2] C. Wang, J. Zhang, F. Hu, S. Zhang, J. Lu, S. Liu, *Bioresource Technology*, **2020**, 308, 123272.
- [3] H.M. Liu, P. F. Xu, M.Y. Cheng, S.N. Lei, Q.L. Liu, W. Wang, *Molecules*, **2021**, 26(11), 3432.
- [4] L. Cecchi, M. Khatib, M. Bellumori, V. Civa, P. Domizio, M. Innocenti, N. Mulinacci, *Food Chemistry*, **2023**, 403, 134338.
- [5] M. Khatib, C. Giuliani, F. Rossi, A. Adessi, A. Al-Tamini, G. Mazzola, D. Di Gioia, M. Innocenti, N. Mulinacci, *Food Chemistry*, **2017**, 235, 58.

***In vitro* antimicrobial and antibiofilm properties of *Cistus x incanus* L., *Scutellaria lateriflora* L. and their combination**

Hammad Ullah¹, Daniele Giuseppe Buccato¹, Anna De Filippis², Lorenza Francesca De Lellis¹,
Alessandra Baldi¹, Alessandro Di Minno^{1,3}, Massimiliano Galdiero^{2,4}, Maria Daglia¹

¹Department of Pharmacy, University of Naples Federico II, Naples, Italy

²Department of Experimental Medicine, University of Campania “Luigi Vanvitelli”, Naples, Italy

³CEINGE-Biotecnologie Avanzate, Via Gaetano Salvatore 486, 80145 Naples, Italy

⁴UOC of Virology and Microbiology, University Hospital of Campania “Luigi Vanvitelli”, Naples, Italy

hammad.ullah@unina.it

The larger impact of periodontal diseases on host health draws the researchers' attention towards its prevention and treatment at the initial stages of the disease [1]. Traditional herbal medicines and plant-based food supplements can be considered alternative approaches to chlorhexidine to avoid the development of periodontitis and the adverse effects of bacteria resistance [2–3]. The present study evaluated the *in vitro* antimicrobial activity against *Porphyromonas gingivalis* (expressed as growth inhibitory percentage), protective effect against cellular invasion by *P. gingivalis* (expressed as CFU/mL relative to the bacteria that can invade the cell monolayer) and the antibiofilm activity (expressed as absorbance values recorded at 570 nm) of two commercial extracts obtained from *Cistus x incanus* L., *Scutellaria lateriflora* L. and their combination. Both the extracts showed a mild dose-dependent inhibitory activity against *P. gingivalis* growth (i.e., maximum inhibitory percentage of 45 for *C. incanus* and of 47 for *S. lateriflora*). The combination of the two extracts showed a greater bacterial growth inhibition than the sum of the percentages of bacterial growth inhibition recorded for *C. incanus* and *S. lateriflora* extracts when tested alone at the same concentrations. Thus, as the combination of these two extracts was more active than the extracts alone, we performed the subsequent experiments only on the combination of *C. incanus* and *S. lateriflora*. The reduction in the invasiveness of *P. gingivalis* in cells treated with the combination of these extracts compared to untreated cells was slightly greater at the highest concentration of the tested extracts (Cells + *P. gingivalis*: CFU/mL 6.4×10^7 ; Cells + *P. gingivalis* + *Cistus* + *Scutellaria* 30 mg/mL each: CFU/mL 6.4×10^5). Biofilm reduction resulted to be around 80% after *Cistus* + *Scutellaria* treatment. In conclusion, the combination of *C. incanus* and *S. lateriflora* showed the potential to decrease the growth and invasion of *P. gingivalis* and biofilm associated with periodontal disease, suggesting an alternative plant-based formulation intended to be used in the prevention of gingivitis.

References

- [1] M.A. Nazir, *International Journal of Health Sciences*, **2017**, *11*, 72.
- [2] C. Cruz Martínez, M. Diaz Gómez, M.S. Oh, *Pharmaceutical Biology*, **2017**, *55*, 1992.
- [3] S. Irani, *Journal of International Oral Health*, **2016**, *8*, 989.

An In Silico Framework to Mine Bioactive Peptides from Annotated Proteomes: A Case Study on Pancreatic Alpha-Amylase Inhibitory Peptides From Algae and Cyanobacteria

Florinda Perugino, Lorenzo Pedroni, Gianni Galaverna, Chiara Dall'Asta, Luca Dellafiora

Department of Food and Drug, University of Parma, 43124 Parma, Italy
florinda.perugino@unipr.it

Foodborne bioactive peptides may cover a wide range of presumed biological properties including anti-hypertensive activity, antioxidant effects, regulation of cholesterol level and glucose metabolism [1]. Concerning the capability to regulate glucose metabolism, the inhibition of pancreatic alpha-amylase is among the key early mechanisms underpinning the hypoglycemic properties of bioactive peptides [2]. Based on this assumption, the identification of alpha-amylase inhibitory peptides in food is fundamental both to better understand the effect of a given food on human health and to design functional foods, ingredients and food supplements.

Microalgae and cyanobacteria are gaining attention for their potential beneficial effects on living organisms and related health-promoting properties, including possible effects on glucose metabolism [3], although the underlying mechanisms still need further investigations.

Nowadays, the identification and characterization of bioactive sequences are mostly performed through *in vitro* studies, which are typically expensive and time consuming, although computational approaches already proved their multi-purpose advantageous applicability. In this context, this study aimed at developing an innovative and versatile *in silico* approach to support the identification of bioactive peptides.

Specifically, this work proposes a computer-driven workflow for a proteome-wide mining of alpha-amylase inhibitory peptides from the annotated proteome of *Chlorella vulgaris*, *Auxenochlorella protothecoides* and *Aphanizomenon flos-aquae* (AFA). In fact, based on their high protein content (up to 70% of algal biomass) [4,5] it is likely that they also contain alpha-amylase inhibitory peptides with possible effects on the modulation of glucose metabolism and bioavailability.

Firstly, an in-depth literature research was performed to identify a list of sequences annotated as alpha- amylase inhibitory peptides while considering for the sake of the study only short peptides (tri- and tetra-peptides) associated with individual IC₅₀.

This preliminary search was followed by a local sequence alignment to iteratively search each bioactive sequence within the selected proteomes. According to local sequence alignment outputs, a list of peptides was further investigated through molecular docking and molecular dynamic simulations and the stability of protein-peptide complexes were estimated.

The study: (i) highlighted the presence of alpha-amylase inhibitory peptides within the proteome under investigations (including ELS, which is among the most potent inhibitory tripeptides identified so far; (ii) mechanistically investigated the possible mechanism of interaction between the enzyme and the inhibitory sequences; and (iii) highlighted the importance of further investigation on proteome of *C. vulgaris* and AFA as valuable sources of alpha-amylase inhibitory peptides, and on CSSL and PGG sequences as strong candidates meant to mightily inhibit alpha-amylase.

References

- [1] N.P. Moller, K.E. Scholz-Ahrens, N. Roos, J. Scherezzenmeir, *European Journal of Nutrition*, **2008**, *47*, 171.
- [2] E. Valencia-Mejia, K.A. Batista, J.J.A. Fernandez, K.F. Fernandes, *Food Research International*, **2019**, *121*, 238.
- [3] C. Lauritano, A. Ianora, *Marine Drugs*, **2016**, *14*, 220.
- [4] G. Canelli, C. Tarnutzer, R. Carpine, L. Neutsch, C.J. Bolten, F. Dionisi, A. Mathys, *Frontiers in Nutrition* **2020**, *7*, 565996.
- [5] A.P. Batista, A. Niccolai, I. Bursic, I. Sousa, A. Raymundo, L. Rodolfi, N. Biondi, M.R. Tredici, *Foods* **2019**, *8*, 611.

Anti-glycative activity of northern Italy edible plant extracts

Giulia Moretto, Raffaella Colombo, Adele Papetti

Department of Drug Sciences, University of Pavia, 27100 Pavia, Italy (Italy)

giulia.moretto01@universitadipavia.it

Glycation is a non-enzymatic reaction between a reducing sugar and the side-chain amino groups of protein lysine or arginine residues, followed by further rearrangements, leading to the formation of advanced glycation end-products (AGEs). Given the various glycating agents involved, such as glucose, fructose and dicarbonyl compounds which are intermediates of the glycation reaction, AGEs are a heterogeneous group of compounds [1]. In *in vivo* AGEs accumulation is associated with many chronic disorders such as diabetes, retinopathy, nephropathy, cardiovascular and neurodegenerative diseases [2]. The development and discovery of new food ingredients with antiglycative activity among different plant species represents a potential approach to reduce and prevent AGEs-related disorders. Therefore, the aim of the study is to expand the knowledge about the anti-glycative properties of different plants present in the north of Italy, such as *Salvia pratensis*, *Althaea officinalis*, *Succisa pratensis*, *Sanguisorba officinalis*, *Diospyros kaki*, *Verbascum thapsus*, by an investigation about the *in vitro* capacity to inhibit the AGEs formation and to trap dicarbonyl compounds. NBT and BSA-MGO assays have been carried out to evaluate the inhibitory effect on the formation of Amadori products at the initial stage and the ability to interfere with the glycation process at the middle stage, respectively [3]. Finally, the direct glyoxal (GO) and methylglyoxal (MGO) trapping capacity of the extracts has been evaluated [4]. The extracts showed different capacity to act as antiglycative agents and *Salvia pratensis* and *Succisa pratensis* can be considered the most active. Further investigations are currently ongoing to identify the compounds responsible for the registered activities and to better understand the action mechanism.

References

- [1] A. Perrone, A. Giovino, *Oxidative Medicine and Cellular Longevity*, **2020**.
- [2] V. Prakash Reddy, P. Aryal, *Microorganisms*, **2022**, *10*, 1848.
- [3] I. Frosi, D. Vallelonga, *Foods*, **2023**, *12*, 529.
- [4] M. Maietta, R. Colombo, *Food Research International*, **2017**, *100*, 780.

***In Vitro* safety assessment of Phenylketonuria (PKU) dietary supplements exploiting Caco-2 intestinal cells**

Melissa Fanzaga¹, Carlotta Bollati¹, Lorenza d'Adduzio¹, Elvira Verduci², Carmen Lammi¹

¹Department of Pharmaceutical Sciences, University of Milan, 20133 Milan, Italy

²Department of Health Sciences, University of Milan, 20146 Milan, Italy

melissa.fanzaga@unimi.it

Phenylketonuria (PKU) is a rare genetic metabolic disorder that consists in the inability of patients to convert the amino acid phenylalanine to tyrosine, caused by mutations in the phenylalanine hydroxylase (PAH) gene, which lead to decreased catalytic activity of this hepatic enzyme [1]. PKU patients suffer from chronic hyperphenylalaninemia, toxic to cardiovascular and nervous system if left untreated, given the accumulation of this molecule in such tissues [2].

Currently, dietary restriction of phenylalanine intake (protein-rich foods) starting from the neonatal period remains the main PKU treatment [2].

The prescription of phenylalanine-free l-amino acid supplements having age-specific vitamins and minerals profile is essential for these patients, in order to ensure that their dietary needs are nevertheless met [3].

Phe-free protein substitute (L-AAAs) is a food for special medical purpose composed by a phenylalanine-free amino acid mixture along with other nutrients, representing a protein substitute for the dietary treatment of PKU [4].

Glycomacropeptide (GMP) is a whey-based natural protein low in phenylalanine, and it has been modified to provide an alternative protein source for PKU patients, showing probiotic, anti-inflammatory and nutraceutical properties as well [5].

It has been reported that peculiar PKU diet can lead to microbiota remodeling in the gastrointestinal (GI) tract, influencing GI homeostasis and predisposing to chronic inflammation [6].

In this context, we evaluated the safety of two dietary supplements for PKU patients, namely Phe-free protein substitute (L-AAAs), GMP and the mixture of the two (1:1), exploiting Caco-2 cells, a well-known immortalized cell line of human colorectal adenocarcinoma cells, widely used as a model of the intestinal epithelial barrier [7]

MTT experiments on Caco-2 cells were performed to assess the viability and metabolic activity of cells treated with increasing concentrations of L-AAAs and GMP and the mixture of the two (L-AAAs + GMP); while possible increased permeability of Caco-2 cells treated with dietary supplements under investigation has been assessed, analyzing tight junctions modulation and measuring the transepithelial electrical resistance (TEER) of differentiated Caco-2 in a Transwell system, in order to address possible intestine inflammation and damage caused by these food supplements intake.

References

- [1] T.T. Zhu, J. Wu, L. Y. Wang, X. M. Sun, *BMC Pediatrics*, **2021**, *21*, 126.
- [2] N. Al Hafid, J. Christodoulou, *Translational Pediatrics*, **2015**, *4*, 304.
- [3] A.M. Lammardo M. Robert, J.C. Rocha, M. van Rijn, K. Ahring, A. Bélanger-Quintana, A. MacDonald, K. Dokoupil, H. Gokmen Ozel, P. Goyens, F. Feillet, *Molecular Genetics and Metabolism*, **2013**, *110*, S1.
- [4] F.J. Rohr, A.W. Munier, H.L. Levy, *Journal of Inherited Metabolic Disease*, **2001**, *24*, 623.
- [5] M.J. Pena, A. Pinto, A. Daly, A. MacDonald, L. Azevedo, J.C. Rocha, N. Borges, *Nutrients*, **2018**, *10*, 1794.
- [6] G. Bassanini, C. Ceccarini, F. Borgo, M. Severgnini, V. Rovelli, G. Morace, E. Verduci, E. Borghi, *Frontiers in Cellular and Infection Microbiology*, **2019**, *9*, 101.
- [7] R. Glahn, *Designing Functional Foods*, **2009**, *13*, 340.

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Effect of a chemically characterized dietary fiber-based food supplement on postprandial glycemic and insulinemic response: a randomized, placebo-controlled, double-blind, cross-over, clinical trial

Lorenza Francesca De Lellis¹, Hammad Ullah¹, Daniele Giuseppe Buccato¹, Alessandra Baldi¹, Roberto Piccinocchi², Alessandro Di Minno^{1,3}, Gaetano Piccinocchi⁴, Roberto Sacchi⁵, Maria Daglia¹

¹Department of Pharmacy, University of Naples Federico II, Naples, Italy

²Level 1 Medical Director Anaesthesia and Resuscitation A. U. O. Luigi Vanvitelli, Via Santa Maria di Costantinopoli, 80138 Naples, Italy

³CEINGE-Biotecnologie Avanzate, Via Gaetano Salvatore 486, 80145 Naples, Italy

⁴Comegen S.c.S., Società Cooperativa Sociale di Medici di Medicina Generale, Viale Maria Bakunin 41, 80125 Naples, Italy

⁵Applied Statistic Unit, Department of Earth and Environmental Sciences, University of Pavia, Viale Taramelli 24, 27100 Pavia, Italy

lo.delellis2@libero.it

Dietary fiber possesses healthy effects exerted via the reduction of the risk of metabolic related issues, gastrointestinal ailments, cardiovascular diseases, cancer, and psychiatric issues [1–2]. The growing interest in food fiber (especially arabinoxylans and β -glucans) has led research towards the development of novel food supplement ingredients using by-products of the agri-food industry that are economically and environmentally sustainable [3]. This study aimed to demonstrate the efficacy of brewer's spent grain (BSG) extract-based food supplement in the reduction of postprandial glycemia and insulinemia in healthy subjects. The chemical characterization of BSG extract revealed the presence of resistant starch (14.64 g/100 g), β -glucans (1.92 g/100 g), arabinoxylans (7.50 g/100 g), and other soluble fibers (6.43 g/100 g), in addition to the bioaccessible ferulic acid (91.3 mg/100 g). Then, a randomized, placebo-controlled, double-blind, cross-over, clinical trial was performed, where 40 normoglycemic subjects were randomized into two groups, 1 and 2 ($n = 20$), receiving either BSG extract-based food supplement or placebo. At the baseline and in the first 60 min, the two glycemic curves overlapped substantially but after 90 and 120 min the postprandial blood glucose values were significantly lower in BSG group than the corresponding values in the placebo group. This improved clinical outcome was supported by the significant reductions in postprandial insulinemia with the supplementation of BSG extract. None of the subjects reported adverse effects. This study showed the improvement of glucose metabolism and insulinemic response with the tested BSG extract-based food supplement in normoglycemic subjects.

References

- [1] A. De Filippis, H. Ullah, A. Baldi, M. Dacrema, C. Esposito, E.U. Garzarella, C. Santarcangelo, A. Tantipongpiradet, M. Daglia, *International Journal of Molecular Sciences*, **2020**, *21*, 4929.
- [2] H. Xu, S. Li, X. Song, Z. Li, D. Zhang, *Nutrition*, **2018**, *54*, 48.
- [3] M. Jackowski, Ł. Niedźwiecki, K. Jagiełło, O. Uchańska, A. Trusek, *Biomolecules*, **2020**, *10*, 1669.

***Artemisia absinthium* L. extract microencapsulated into alginate-based beads**

Alessandro Candiani, Yassine Jaouhari, Vincenzo Disca, Federica Pollastro,
Marco Arlorio, Lorena Segale, Jean Daniel Coisson

Università del Piemonte Orientale, Dipartimento di Scienze del Farmaco,
Largo Donegani 2, 28100 Novara, Italy
alessandro.candiani@uniupo.it

Artemisia absinthium L. (Asteraceae) is rich in bitter compounds and among these, absinthin is a powerful hTAS2R46 receptor agonist. This latter receptor belongs to the TAS2Rs, a family of G protein coupled receptors (GPCRs) related to bitter taste, expressed not only in the mouth but also in many extraoral tissues [1, 2]. Particularly, the activation of hTAS2Rs in the gastrointestinal tract entails effects that go far beyond the perception of taste as the delay or inhibition of the gastric emptying, the modulation of endocrine hormones with the final result of favouring the sense of satiety [3]. Capitalizing to these effects, the aim of this work was to microencapsulate a dry ethanolic extract of *A. absinthium* into alginate-based beads to obtain a suitable product for food, nutraceutical and pharmaceutical applications, masking the intense bitter taste while preserving the integrity of the compounds to let them target the gastrointestinal bitter receptors exerting their potential activity.

To this purpose, an ethanolic dry extract of *A. absinthium* was used after complete dissolution in ethanol. This solution was added to a previously prepared polymeric solution, composed of sodium alginate and phospholipon[®] 90G (phosphatidylcholine) used as stabilizer, under magnetic stirring to obtain a homogeneous dispersion. Beads formed by dripping the dispersion through two fluid-nozzle into a 100 mM CaCl₂ solution, followed by curing, filtration and rinsing. Morphology and size of wet microparticles were investigated by stereomicroscopy and image analysis. Dry microparticles were obtained by fluid bed drying and characterized for morphology by stereomicroscope, SEM and image analysis, particle size distribution by sieving, flowability by the determination of the dynamic angle of repose, residual water by thermogravimetric analysis (TGA). Swelling behaviour in water, HCl pH 1.0 and phosphate buffer at pH 6.8 was evaluated and process recovery was calculated. Total polyphenols content (TPC) and antioxidant activity of the absinthe ethanolic extract were determined by Folin-Ciocalteu and ABTS test, respectively.

Wet microparticles were spherical in shape (shape factor SF = 0.890) and showed smooth surface, while dried ones were still sphere-like (SF = 0.886) but with an irregular surface. Mean diameter of wet and dried microparticles was 2.163 mm and 0.909 mm, respectively; the angle of repose value indicated a discrete flowability attitude; TGA results on the dried microparticles showed an 8.80% of residual water; the swelling test showed a mild microparticle weight increase without disaggregation in water and HCl for 24 hours and a rapid/ great weight increase with subsequent microparticle disaggregation in phosphate buffer in less than 2 hours. ABTS essay revealed an IC₅₀ of 4.80 ± 0.24 mg TE/mL extract; TPC was 11.44 ± 0.34 mg CE/mL extract.

Microencapsulation of *A. absinthium* ethanolic extract was successfully achieved. The outcomes obtained suggested a suitable utilisation of these alginate beads in food and pharmaceutical fields. Further studies are ongoing to evaluate microparticles behaviour through *in vitro* simulated digestive process.

References

- [1] S.S. An, S.B. Liggett, *Cellular Signalling*, **2018**, 41, 82.
- [2] M. Behrens, W. Meyerhof, *Physiology & Behavior*, **2011**, 105(1), 4.
- [3] K. Tuzim, A. Korolczuk, *Journal of Translational Medicine*, **2021**, 19, 440.

Optimizing the extraction of Antioxidant and Anti-cholinergic polyphenols from *Arctium lappa* L. roots by Pressurized Liquid Extraction

Enrico Romano¹, Gloria Domínguez-Rodríguez^{2,3}, Fabrizio Masciulli¹, Donatella Ambroselli¹,
Mattia Spano¹, Giacomo Di Matteo¹, Cinzia Ingallina¹, Andrea Salvo¹,
Elena Ibáñez², Alejandro Cifuentes², Luisa Mannina¹

¹Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma,
Pizzale Aldo Moro 5, 00185 Roma (Italy)

²Laboratory of Foodomics, Institute of Food Science Research, CIAL, CSIC,
Nicolás Cabrera 9, Madrid, 28049, Spain

³Facultad de Ciencias, Departamento de Química Analítica e Ingeniería Química, Universidad de Alcalá,
Ctra. Madrid-Barcelona Km 33.600, 28871 Alcalá de Henares, Madrid, Spain
e.romano@uniroma1.it

Arctium lappa L., also called Burdock, is a genus of dicotyledonous angiosperm plants of the *Asteraceae* family. In particular, Burdock root is widely known as potential source of phenolic compounds with healthy properties [1]. Particularly, phenolic acids and their derivatives, flavonoids and lignans are the most abundant phenolic compounds in Burdock roots [2-7].

Phenolic compounds are commonly extracted by solid-liquid extraction (SLE). However, conventional extraction techniques require high volume of solvents and extraction times. In addition, these techniques provide low selectivity and reproducibility. Thus, advanced extraction techniques have been proposed to extract phenolic compounds, such as pressurized liquid extraction (PLE), trying to overcome these aspects [8].

Aim of this work was to optimize the PLE conditions to obtain high content of phenolic compounds with antioxidant and neuroprotective/anticholinergic properties from Burdock roots. Three different extraction temperatures (50°C, 100°C and 150°C) were employed using ethanol/water (70:30, v/v) as solvent extraction at 1500 psi with an extraction time of 20 min. In addition, SLE by maceration was carried out using the same extraction solvent for PLE, at room temperature for 24 h.

In order to determine the influence of the extraction technique on the recovery of bioactive phenolic compounds, PLE extracts were compared with SLE extracts in terms of total polyphenol content determined by Folin-Ciocalteu assay, antioxidant capacity by DPPH and ORAC assays, and the neuroprotective/anticholinergic capacity by AChE and BuChE tests. In addition, a characterization per family of phenolic compounds of PLE and SLE extracts was performed by HPLC-DAD.

The results show that PLE made it possible to extract a higher content of phenolic compounds with greater bioactive properties than SLE.

References

- [1] D.D. Herrera-Balandrano, T. Beta, Z. Chai, X. Zhang, Y. Li, W. Huang, *Food Chemistry*, **2021**, 358, 129897.
- [2] R. Ferracane, G. Graziani, M. Gallo, V. Fogliano, A. Ritieni, *Journal of pharmaceutical and biomedical analysis*, **2010**, 51(2), 399.
- [3] R. Jaiswal, N. Kuhnert, *Food & Function*, **2011**, 2(1), 63.
- [4] L.-Z. Lin, J.M. Harnly, *Journal of Agricultural and Food Chemistry*, **2008**, 56(21), 10105.
- [5] Y. Maruta, J. Kawabata, R. Niki, *Journal of Agricultural and Food Chemistry*, **1995**, 43(10), 2592.
- [6] Z. Wang, Y. Yan, T. Nisar, L. Zou, X. Yang, P. Niu, L. Sun, Y. Guo, *Food Chemistry*, **2018**, 246, 233.
- [7] T.M. Moro, M.T. Clerici, *Food Research International*, **2021**, 141, 109889.
- [8] R.M. Alonso-Salces, E. Korta, A. Barranco, L.A. Berrueta, B. Gallo, F. Vicente, *Journal of Chromatography A*, **2001**, 933(1-2), 37.

Tomato fruits metabolite and chemical-biological hazards through NMR and MS methodologies

Valeria Vergine, Maria Elisa Crestoni, Cinzia Ingallina, Luisa Mannina

Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma,
P.le Aldo Moro 5, 00185 Roma (Italy)
valeria.vergine@uniroma1.it

Tomatoes and tomato-based food are worldwide diffused foodstuffs, whose nutritional importance is related to their contents of many antioxidant compounds, like carotenoids, polyphenols, and vitamins. [1-3] However, tomatoes are a food category extremely exposed to safety risks that can be related to the presence of chemical residuals, like pesticides [4], and microbial contaminants among which bacteria and fungi. [1,5-6]. In this scenario, the present study was carried out in the frame of ONFOODS consortium [7] (Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods) stemming from the National Recovery and Resilience Plan (NRRP), and is aimed at the metabolite profiling and characterization of new and (re)-emerging hazards of tomato fruit varieties, such as Torpedino di Fondi (TF) and San Marzano (SM), for the improvement of tomato quality that emerges as a matter of priority for customer's safety.

Torpedino di Fondi (TF) is a hybrid tomato landrace developed in Sicily and recently introduced in the south Lazio area along with the classical San Marzano (SM) cultivar. TF tomatoes and traditional SM tomatoes were compared through a multidisciplinary approach consisting of morphological, chemical (FT-ICR MS, NMR, HPLC, and spectrophotometric methods), and biological (antioxidant and antifungal *in vitro* activity) analyses. [3,8] The high mass accuracy typically achieved with FT-ICR MS implies that elemental formulas of many metabolites and harmful compounds present in trace amounts, like pesticides, agrochemical derivatives and metals can be determined. In addition, advanced NMR and MS techniques will be applied to provide a broad chemical and metabolomic profile to monitor traceability and quality of tomatoes. In particular, the chemico-biological hazards related to phytosanitary treatments, manufacturing processes and incorrect storage practices will be characterized. So, the combined application of both targeted and untargeted methodologies allowed to outline the chemical profile of both TF and SM tomatoes, especially it is crucial to identify in tomatoes the hazards and microbial contaminants to improve their healthy quality, and to determine the molecular effects of processing and storage on food safety and genuineness.

References

- [1] I. Concina, M. Falasconi, E. Gobbi, F. Bianchi, M. Musci, M. Mattarozzi, M. Pardo, A. Mangia, M. Careri, G. Sberveglieri, *Food Control*, **2009**, 20(10),873.
- [2] A. T. Diplock, J. L. Charuleux, G. Crozier-Willi, F. J. Kok, C. Rice-Evans, M. Roberfroid, W. Stahl, J. Vina-Ribes, *British journal of nutrition*, **1998**, 80(S1), S77.
- [3] C. Ingallina, A. Maccelli, M. Spano, G. Di Matteo, A. Di Sotto, A. M. Giusti, ..., L. Mannina, *Antioxidants*, **2020**, 9(10), 1027.
- [4] G. Gambacorta, M. Faccia, C. Lamacchia, A. Di Luccia, E. La Notte, **2005**, *Food control*, 16(7), 629.
- [5] R. H. Bishop, C. L. Duncan, G. M. Evancho, H. Young, **1982**, *Journal of Food Science*, 47(2), 437.
- [6] W. Jiang, J. Sandahl, J. Dubois, M. Flavin, S. Reddy, A. Neigh, ..., A. Gore, **2023**, *Bulletin of Environmental Contamination and Toxicology*, 110(2), 45.
- [7] Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Award Number: Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title “ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods”.
- [8] A.P. Sobolev, L. Mannina, D. Capitani, G. Sanzò, C. Ingallina, B. Botta, A. Di Sotto, **2018**, *Food Chemistry*, 255, 120.

Development of ^{13}C qNMR and ^1H -NMR/PLS-R methods to determine the content of boswellic acids in *Boswellia serrata* Roxb. raw extracts

Danny Vincenzo Piazza, Eleonora Truzzi, Davide Bertelli

Department of Life Sciences, University of Modena and Reggio Emilia, via G. Campi 103, 41125 Modena, Italy
eleonora.truzzi@unimore.it

Boswellic acids (BAs) are pentacyclic triterpenoids extracted from the oleogum resin of the genus *Boswellia* with marked anti-inflammatory and potential antitumor effects. *B. serrata* Roxb. is native to India and Pakistan and its oleogum resin contains several organic acids and up to 12 different types of BAs. Among these, the α - and β -BAs, acetylated β -BA (ABA), 11-keto- β -BA (KBA), and 3-O-acetyl-11-keto- β -BA (AKBA) are the most concentrated and pharmacologically active compounds. Currently, the extracts of *B. serrata* oleogum resin are used to produce food supplements and medicated feeds for the treatment of chronic inflammations. The content of BAs in these extracts is often determined using a not selective method based on an acid-base titration. Consequently, the concentration of BAs in the extracts is overestimated. Without a proper quality control of the imported extracts, the food supplements could contain low amounts of BAs, leading to the commercialization of inefficacious products in the market.

The aim of the present project was the development of fast analytical methods based on a ^{13}C -qNMR or ^1H -NMR coupled to chemometrics approaches. The here presented strategies could be convenient compared to conventional separative methods because do not require calibration curves or the employment of expensive standards for determining the BA content.

Thirty samples of raw extract purchased from Indian producers were dissolved in methanol-d₄ and analyzed by NMR. The content of total BAs was reported on the label of the samples and expressed as percentage. ^1H - and ^{13}C -NMR spectra, and 2D HSQC and HMBC spectra of BA standards were acquired using a Bruker FT-NMR Avance III HD 600 MHz (BBO H&F 5mm cryo-probe) to assign each spectral signal. ^1H - and ^{13}C -NMR spectra of the samples were acquired under quantitative conditions (delay time equal to 5-fold the nuclei relaxation time). The qNMR method was built using the ^{13}C -NMR spectra due to the complexity of the proton spectra with an extensive signal overlapping of BAs in the extracts. The content of the α - and β -BAs, ABA, KBA, and AKBA were determined using pyridoxine as an external standard. The qNMR method was validated in terms of intra- and inter-day precision and recovery. The ^1H -NMR spectra were employed for building a Partial Least Squares Regression (PLS-R) method. The spectra were preprocessed by baseline correction, followed by Pareto scaling and mean-centering and used to generate x-matrix. The y-matrix was created by using the qNMR results expressed as percentage of total BAs in the extracts. The predictive performance of the PLS-R model was assessed in terms of root mean square error (RMSE) and coefficient of regression R^2 on unknown samples (30% of the total number).

The ^{13}C -qNMR method demonstrated a high precision and recovery percentages demonstrating the reliability of the approach. The ^1H -NMR/PLS-R model showed good R^2 and RMSE in calibration, cross-validation, and prediction, suggesting the high predictive capability in determining the total content of BAs in the extracts.

The BA contents were proved to be overestimated in all the thirty samples highlighting the presence on the market of low-quality imported extracts used for producing the food supplements or medicated feeds. Moreover, one analyzed food supplement did not show any trace of BAs, demonstrating also the occurrence of frauds in the Italian market. The results underlined the urgency to impose stricter quality controls on the content of bioactive compounds within the food supplements or medicated feeds, to assure the safety of consumers and the reproducibility of the beneficial effects.

Development and application of a novel analytical method for the determination of 8 plant sterols/stanols in 22 legumes samples

Agnese Santanatoglia^{1,2}, Giovanni Caprioli¹, Sauro Vittori¹, Gianni Sagratini¹

¹School of Pharmacy, University of Camerino, 62032, Camerino, Italy

²Research and Innovation Coffee Hub, Via Emilio Betti 1,
62020, Belforte del Chienti, Italy
agnese.santanatoglia@unicam.it

Pulses represent one of the traditionally most important dietary components worldwide, supplying proteins, dietary fibres, minerals, and vitamins. Legumes represent a very important source of nutrients such as proteins and complex carbohydrates, as well as dietary fibre and minerals, including magnesium and potassium. They also have a low-fat content, mainly of the unsaturated type [1,2]. They also contain numerous bioactive molecules such as polyphenols (flavanols, anthocyanins, phenolic acids, etc), and soyasaponins, exerting hypocholesterolemic action. Moreover, legumes are a rich source of phytosterols, a group of chemical compounds of plant origin able to reduce the intestinal absorption of cholesterol, blood levels of it and of low-density lipoprotein or "LDL", reducing also the consequent risk of atherosclerosis [3,4]. However, the daily intake (300 mg/day) is not sufficient to achieve the cholesterol-lowering effect; in fact, 2 g are needed to reduce blood cholesterol level by 10% [5]. In this work, an HPLC-DAD method was developed for the quantification of cholesterol and 7 main PSs (β -sitosterol, campestanol, campesterol, fucosterol, sitostanol, stigmasterol, and stigmastanol) in legumes samples. The method was validated, and good results were obtained in terms of linearity, repeatability, LOD and LOQ. Then, a process of extraction for phytosterols from legumes was optimized by modifying a standard method reported in the literature. The developed new procedure, through ultrasound-assisted extraction (U.A.E), allowed a reduction of extraction time by half. Then, the developed HPLC-DAD method, after being validated, was applied to 22 legume samples. Six phytosterols were observed in legumes and the highest total phytosterols content was found in Black Chickpeas (699.9 mg/kg) followed by Brown Lentils, Whole Fava Beans, and Corona Beans. The developed extraction and analytical method could thus be considered a reference method for PSs extraction in legumes. Moreover, the obtained results confirm that legumes should be included in the diet as great sources of cholesterol-lowering agents.

References

- [1] Food and Agriculture Organization (FAO), International Year of Pulses, **2016**.
- [2] C.A. Adebamowo, E. Cho, L. Sampson, M.B. Katan, D. Spiegelman, W.C. Willett, M.C. Holmes, *International Journal of Cancer*, **2005**, *114*, 628.
- [3] F.K. Nzekoue, G. Khamitova, S. Angeloni, A. Nacher Sempere, J. Tao, F. Maggi, J. Xiao, G. Sagratini, S. Vittori, G. Caprioli, *Food Chemistry*, **2020**, *325*, 126836.
- [4] F.K. Nzekoue, G. Borsetta, L. Navarini, D. Abouelenein, J. Xiao, G. Sagratini, S. Vittori, G. Caprioli, *Food Chemistry*, **2022**, *372*, 131188.
- [5] C.E. Cabral, M.R.S.T. Klein, *Arquivos brasileiros de cardiologia*, **2017**, *109*, 475.
- [6] S. Feng, L. Wang, T. Belwal, L. Li, Z. Luo, *Food Chemistry*, **2020**, *315*, 126217.
- [7] A. Santanatoglia, F.K. Nzekoue, G. Sagratini, M. Ricciutelli, S. Vittori, G. Caprioli, *Journal of Food Composition and Analysis*, **2023**, *118*, 105195.

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Target screening method for the quantitative determination of 118 pyrrolizidine alkaloids in food supplements, herbal infusions, honey and teas by liquid chromatography coupled to quadrupole Orbitrap mass spectrometry

Serena Rizzo¹, Rita Celano¹, Simona Serio¹, Valentina Santoro¹, Mariateresa Russo²,
Anna Lisa Piccinelli¹, Luca Rastrelli¹

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

²Department of Agriculture Science, Food Chemistry, Safety and Sensoromic Laboratory,
University of Reggio Calabria, Reggio Calabria, Italy
apiccinelli@unisa.it

An analytical procedure for the screening of 118 pyrrolizidine alkaloids (PAs) [1] was successfully validated and applied to their quantitative determination in food supplements, herbal infusions, honey, and teas. It provides the reliable analyte identification by high-resolution tandem mass spectrometry (HRMS/MS), the accurate determination of 21 regulated PAs, and broad contamination profiles. 10% of 281 analyzed samples resulted contaminated at levels above the maximum levels (MLs) of European legislation. The contamination of herbal infusions of mixed plants can represent a possible health concern (23%; mean of PA sum above ML). A high number of PAs not included in the regulation was detected in honey and herbal food supplements, but their contribution was only relevant to the overall level in honey. The results indicate the need to continue collecting contamination data in food supplements and infusions of mixed herbs and to expand the PA-pool to be monitored in honey and related products.

References

[1] S. Rizzo, R. Celano, A. L. Piccinelli, S. Serio, M. Russo, L. Rastrelli, *Food Chemistry*, **2023**, *406*, 135058.

Development of a green extraction method for the valorization of Wild Artichoke by-product using Pressurized Liquid Extraction, UPLC-MRMS and multivariate data analysis

Stefania Pagliari¹, Ciro Cannavacciuolo¹, Rita Celano², Sonia Carabetta³, Mariateresa Russo³,
Massimo Labra¹, Luca Campone¹

¹Department of Biotechnology and Biosciences, University of Milano-Bicocca,
Piazza Della Scienza 2, 20126 Milano, Italy

²Department of Pharmacy, University of Salerno,
Via Giovanni Paola II 132, Fisciano, 84084 Salerno, Italy

³Safety and Sensoromic Laboratory (FoCuSS Lab), Department of Agriculture Science, Food Chemistry,
University of Reggio Calabria, Via dell'Università 25, 89124 Reggio Calabria, Italy

stefania.pagliari@unimib.it

The valorization of food by-products is a key issue in modern society to improve the economic and environmental sustainability of the food production chain [1]. Artichoke (*Cynara cardunculus L.*), is a herbaceous plant native to the Mediterranean region, widely used in the agri-food field. In fact, large quantities of inedible parts of the artichoke plant, such as leaves, stems, roots, bracts, and seeds are discarded each year during industrial processing. These by-products can be a source of numerous phytochemicals such as dietary fibers, phenolic acids, and flavonoids, with interesting properties for human health [2]. However, one of the most relevant problems concerns the recovery of high-value components from these by-products [3,4]. The aim of this work is to develop a new valorization strategy for the sustainable utilization of artichoke leaf waste by combining green pressurized liquid extraction (PLE), spectrophotometric assays, and UPLC-HRMS phytochemical characterization to obtain a bioactive-rich extract with high antioxidant capacity. Finally, multivariate analysis of the main selected metabolites was used to compare the different solvent extractions used in PLE and to assess the optimal instrumental conditions for their recovery.

References

- [1] N. Mirabella, V. Castellani, S. Sala, *Journal of Cleaner Production*, **2014**, 65, 28.
- [2] M. Palermo, G. Colla, G. Barbieri, V. Fogliano, *Journal of Agricultural and Food Chemistry*, **2013**, 61, 7960.
- [3] C. Picot-Allain, M.F. Mahomoodally, G. Ak, G. Zengin, *Current Opinion in Food Science*, **2021**, 40, 144.
- [4] I. Pagano, L. Campone, R. Celano, A.L. Piccinelli, L. Rastrelli, *Journal of Chromatography A*, **2021**, 1651, 462295.

Ginsenosides quantified by HPLC-MS in ginseng root extracts and biological activity

Simone Angeloni¹, Cinzia Mannozi¹, Oliviero Marinelli², Cristina Aguzzi², Massimo Nabissi², Laura Bordoni³, Irene Petracci³, Rosita Gabbianelli³, Valentina Cecarini⁴, Mauro Angeletti⁴, Anna Maria Eleuteri⁴, Huimin Liu⁵, Giovanni Caprioli¹, Gianni Sagratini¹

¹Chemistry Interdisciplinary Project (ChIP), University of Camerino,
Via Madonna delle Carceri, I-62032, Camerino, MC, Italy

²School of Pharmacy, University of Camerino,
Via Madonna delle Carceri, I-62032, Camerino, MC, Italy

³Unit of Molecular Biology and Nutrigenomics, School of Pharmacy, University of Camerino,
Via Madonna delle Carceri, I-62032, Camerino, MC, Italy

⁴School of Biosciences and Veterinary Medicine, University of Camerino,
Via Gentile III da Varano, I-62032, Camerino, MC, Italy

⁵College of Food Science and Engineering, Jilin Agricultural University,
Changchun, Jilin 130118, China

cinzia.mannozi@unicam.it

Several papers demonstrated that ginseng and ginsenosides exert a potential anti-obesity effect [1], however, just a limited number of studies deeply focused on the potential antioxidant activity of ginsenosides and ginseng root extract [2]. Therefore, the aim of the present work was to evaluate the effect of different extraction methods on ginsenosides content quantified by HPLC-MS in ginseng root extracts and their biological activity (inflammatory and oxidative state). Moreover, the extract and individual ginsenosides were evaluated for their skills to hinder inflammation in macrophage cells. Thus, with the scope to obtain an extract rich in ginsenosides, three types of extraction methods, such as solid-liquid extraction (SLE), ultrasound-assisted extraction (UAE) and SLE assisted by Naviglio extractor, using different solvents (methanol, ethanol, and mixtures with water such as methanol:water 70:30, 50:50 and 30:70 (v/v) and ethanol:water 70:30, 50:50 and 30:70 (v/v)), have been investigated. Four ginsenosides (Rb1, Rb2, Rg1 and Rg2) were quantified by HPLC-MS instrument. The method that showed less performance in terms of total ginsenoside was Naviglio extractor, apart from the tested solvents. The ethanol:water 50:50 (v/v) mixture has been selected to prepare the dried extract of ginseng roots and it was tested for anti-inflammatory and antioxidant activities.

The most abundant ginsenosides in the dried extract were Rg1 (13.07 ± 0.29 mg/g) and Rb1 (7.64 ± 0.03 mg/g), while the extract contained a total concentration of ginsenosides of 25.39 ± 0.32 mg/g. Biological studies demonstrated that ginsenosides and the extract strongly reduced the expression of interferon- γ and the extract was able to down-regulate the mtDNAcn at levels similar to control. In addition, the extract and the tested ginsenosides exerted antioxidant effects in macrophages decreasing the amount of ROS/RNS, markers for protein oxidation and the activity and expression of enzymes involved in oxidative processes such as NOS and NOX.

Such extract as well as some ginsenosides demonstrated to possess interesting antioxidant properties such as decreasing the content of markers for protein oxidation, the expression of NOX and the amount of ROS/RNS. In addition, tested ginsenosides and the extracts also possessed fascinating anti-inflammatory activities, i.e., reduction of IFN- γ expression, NOS activity and iNOS expression.

References

- [1] P. Mathieu, I. Lemieux, J.P. Després, *Clinical Pharmacology & Therapeutics*, **2010**, 87(4), 407.
[2] W. Chen, P. Balan, D. G. Popovich, *Molecules*, **2021**, 26(4), 1158.

In-depth molecular characterization by UPLC-MS and UPLC-IM-MS of oligosaccharides in complex mixtures from hydrothermal treatment of lignocellulosic biomasses

Andrea Fuso¹, Simona Scarpella², Laura Righetti^{1,3,4}, Massimiliano Rinaldi¹, Ginevra Rosso⁵, Franco Rosso⁵, Augusta Caligiani¹

¹Food and Drug Department, University of Parma, Via Parco Area delle Scienze 17/A, 43124 Parma, Italy

²Waters SPA, Viale T. Edison 110, 20099 Sesto San Giovanni, Milan, Italy

³Wageningen Food Safety Research (WFSR), Wageningen University & Research, P.O. Box 230, Wageningen 6700 AE, Netherlands

⁴Laboratory of Organic Chemistry, Wageningen University, Stippeneng 4, Wageningen 6708 WE, Netherlands

⁵Soremartec Italia Srl, Ferrero Group, 12051 Alba, CN, Italy
augusta.caligiani@unipr.it

Food research is increasingly focusing on dietary fibres since they have recognised health benefits and because the relationship between chemical structure and functionality is still in many respects unknown [1]. Indeed, characterizing their structure in detail is very complex, and even mixtures of oligo- and polysaccharides in the market of functional foods are often not thoroughly characterized.

In this study, hazelnut shells were subjected to hydrothermal treatment at 170 °C for 1 hour to extract partially degraded, water-soluble hemicellulose, to be re-used as a functional ingredient. After that, an enzymatic hydrolysis using xylanase was carried out to produce xylo-oligosaccharides (XOS), which have well-established prebiotic effects [2]. Molecular characterization of this mixture was attempted in the first instance by UPLC/ESI-MS: this technique allowed to identify XOS with a degree of polymerization (DP) between 2 and 10 and widely varying amounts of bound acetyl groups. However, most of the acetylated XOSs were neither separated chromatographically nor identified in detail, for example in terms of acetyl group position. Moreover, it is known that an even greater complexity can occur, since D-xylose and L-arabinose branching can be present as well [3]. The following step was therefore based on an ACQUITY I-Class UHPLC separation system coupled with a VION IMS QTOF mass spectrometer (Waters, Wilmslow, UK) equipped with an electrospray ionization (ESI) interface. Initially, a library of all possible deacetylated XOS (N = 9) was manually built uploading the structures (.jmol files) within UNIFI software (Waters, Milford, MA, USA), and then automatically generating all the possible combinations of acetylated structures (N = 126). After instrumental analysis, the acquired data were processed according to a targeted metabolomics/“reactomics” approach, using the in-house built UNIFI library. Results confirmed the UPLC-MS data, even expanding the identifications thanks to the additional dimension of separation provided by the use of ion mobility, which allows to further separate analytes according to their size and shape. Indeed, for each molecular structure of XOS, it was possible to experimentally acquire the collisional cross section (CCS) value, which is related to the molecule three-dimensional structure. In addition, CCS can also be predicted using machine learning algorithms, being an inherent molecular descriptor. The comparison of predicted and experimental CCS increases the confidence of the annotation process. With this approach, several XOS having the same retention time, DP, and number of acetyl groups were indeed separated.

This approach could be very useful from an untargeted perspective as well: hundreds of oligosaccharides and their derivatives (e.g., dehydrated sugars) containing different monomers, bond configurations, and substituents were found in the hydrothermal extracts, which could be considered to enrich the libraries, making it possible to thoroughly characterize complex mixtures of oligosaccharides by comparing mass spectra, retention time, and CCS. Considering that these molecules are often found in functional products, it is of utmost importance for research to accelerate in the ability to characterize every detail of their molecular structure by taking advantage of state-of-the-art technologies.

References

- [1] M.J. Amicucci, E. Nandita, C.B. Lebrilla, *Journal of Agricultural and Food Chemistry*, **2019**, *67*, 4418.
- [2] A. Fuso, D. Risso, G. Rosso, F. Rosso, F. Manini, I. Manera, A. Caligiani, *Foods*, **2021**, *10*, 1197.
- [3] R.D. Singh, J. Banerjee, A. Arora, *Bioactive Carbohydrates and Dietary Fibre*, **2015**, *5*, 19.

New methodological approaches in glyphosate's research in cereals and its derivatives

Licia Pantano¹, Gaetano Cammilleri¹, Maria Drussilla Buscemi¹, Francesco Giuseppe Galluzzo¹, Andrea Macaluso¹, Tiziana Bertuglia¹, Nicola Cicero^{2,3}, Mariarita Pisano¹, Vincenzo Ferrantelli¹

¹Istituto Zooprofilattico Sperimentale della Sicilia, via Gino Marinuzzi 3, 90129 Palermo

²Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)

University of Messina, Viale G. Palatucci, 98168 Messina, Italy

³Science4life S.r.l., start-up dell'Università di Messina, Messina, Italy

drussilla.buscemi@izssicilia.it

In this reaserch project we developed and applied a fast and reliable LC-HRMS method for the identification and quantification of glyphosate in plant origin samples [1].

The validation of the method was carried out according to the EC Decision 657/2002 [2], clearly demonstrating the suitability of this method for the quantitative determination of glyphosate and its metabolite (AMPA and glufosinate) with satisfactory recovery values (between 94 and 108% respectively for glyphosate, between 84 and 111% for AMPA and between 95 and 105% for glufosinate for the 4 concentration levels considered) and linearity values r^2 greater than 0.996 and an excellent repeatability (respectively between 3 and 92 for glyphosate, between 1.6 and 176 for AMPA and between 3.2 and 125 for glufosinate for all concentration levels considered). Regarding the sensitivity of the method, we revealed satisfactory LOD and LOQ values (between 7 and 17 for the LOD and 20 $\mu\text{g}/\text{Kg}$ for LOQ for all the analytes examined).

The method was subsequently applied to 48 real samples of flour and pasta belonging to various commercial realities. The method was not affected by any matrix effect linked to the type of product tested, detecting concentrations with mean values of $0.456 \pm 0.307 \mu\text{g}/\text{Kg}$. The method developed in this research project has therefore proved to be reliable and precise and, given the reduced analysis times, it is also faster than the methods reported in the literature for the field of official controls.

References

[1] V.K. Nandula, N.J. Hoboken, *Experimental Agriculture*, **2010**, 47(2), 415.

[2] European Commission (EC), Food Safety, Plants, Glyphosate.

Electrochemical biosensors for detection of glucose and fructose in honey

Luca Surace, Rosaceleste Zumpano, Mattia Spano, Giacomo Di Matteo,
Luisa Mannina, Franco Mazzei

Department of Chemistry and Drug Technologies, Sapienza University of Rome,
P.le Aldo Moro 5, 00185, Rome, Italy
luca.surace@uniroma1.it

Quality control plays a crucial role in the evaluation of authenticity, nutritional value, and safety of food products at industrial and individual level. As an example, when fermentation processes are involved in foodstuffs production, the control of sugar concentrations (e.g. glucose (G), fructose (F), sucrose) is used to optimize and improve the industrial processes as well as the economic efficiency. For instance, honey is a food matrix where glucose and fructose are predominant in the composition with percentages of 38% and 31% respectively [1] and the F/G result an important index for the evaluation of the sample botanical origin when is unifloral, its stability, its crystallization, its preservation over time and its chemical-physical properties [2]. Moreover, the sugar content can affect the honey density and its ability to be safely stored without fermenting. Therefore, accurate measurement of sugar levels in honey is essential to ensure its quality and safety for human consumption. Traditional methods for sugar analysis such as High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are highly specific but expensive and often require sample pretreatments. Biosensors in general and more specifically the electrochemical ones have shown significant advantages over other analytical methods, such as rapid response times, high sensitivity, possibility of miniaturization, and reduction of costs [3]. The aim of this work was the development of two easy-to-use electrochemical biosensors for the rapid detection of glucose and fructose in honey. The transducer is represented for both by a commercial carbon-based screen-printed electrode (SPE) modified with multi-walled carbon nanotubes (CNTs), where the employed bioreceptors (glucose oxidase from *Aspergillus Niger* (GOx); fructose dehydrogenase from *Aspergillus Niger* (FDH)) has been immobilized via glutaraldehyde (GA) cross-linking. Characterization of biosensors was performed by electrochemical techniques such as chronoamperometry (CA) and cyclic voltammetry (CV), determining the linearity range of response, lower detection limit (LOD) and sensitivity. The two analytical devices were compared with Nuclear Magnetic Resonance spectroscopy (NMR) methodology in terms of sensitivity and reproducibility. Both developed methods have been further employed for G and F determination in three single-flower honey samples (i.e. chestnut, sunflower and sulla) evaluating the most suitable one for rapid and accurate screening of these specific food matrices. HPLC technique was used as validation method, being both electrochemical biosensors and NMR in good agreement with the standard method (accordance of 90% and 89% respectively for F and G in the case of electrochemical biosensors, 85% and 84% respectively in the case of NMR). The ease of use and realization of the two electrochemical biodevices, together with the good analytical performance suggest the possible employment of these systems for glucose and fructose screening in honey matrices.

References

- [1] L.W. Doner, *Journal of the Science of Food and Agriculture*, **1977**, 28, 443.
- [2] M.M. Cavia, M.A. Fernández-Muiño, E. Gómez-Alonso, M.J. Montes-Pérez, J.F. Huidobro, M.T. Sancho, *Food Chemistry*, **2002**, 78, 157.
- [3] D. Grieshaber, R. Mackenzie, J. Vörös, E. Reimhult, *Sensors*, **2008**, 8, 1400.

Flow bioprocessing of citrus glycosides for high-value aglycone preparation

Agostina Colacicco¹, Giorgia Catinella¹, Christian Pinna¹, Alessandro Pellis³, Stefano Farris¹,
Lucia Tamborini², Sabrina Dallavalle¹, Francesco Molinari¹,
Martina Letizia Contente¹, Andrea Pinto¹

¹Department of Food, Environmental and Nutritional Sciences (DeFENS),
University of Milan, via L. Mangiagalli 25, 20133 Milan, Italy

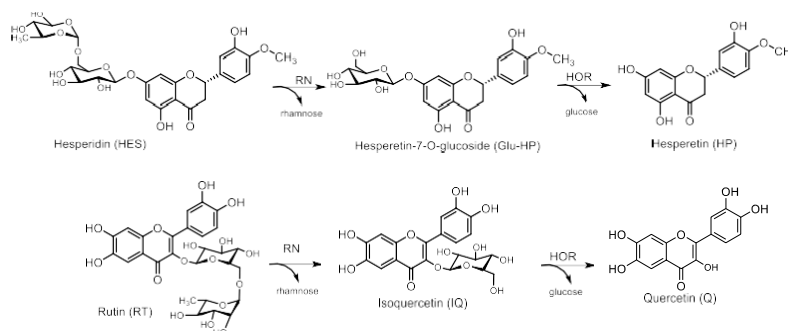
²Department of Pharmaceutical Sciences, via L. Mangiagalli 25, 20133 Milan, Italy

³University of Genova, Department of Chemistry and Industrial Chemistry,
via Dodecaneso 31, 16146 Genoa, Italy

agostina.colacicco@unimi.it

Agri-food waste is a significant environmental and economic problem as tons of residues (seeds, peels, leaves, branches, trunks, and roots) which are typically unexploited, need to be managed and disposed. Starting from these residues, green technologies, such as biocatalysis, can be used to obtain natural valuable compounds with attractive biological properties.

In this work we focused on hesperidin and rutin, glycosides typically found in citrus species especially in fruits and their by-products (e.g., peels and leaves) [1]. By employing a commercially available rhamnosidase (RN) and a home-prepared β -thermophilic glycosidase (HOR) from *Halothermothrix orenii* the corresponding aglycones, hesperetin (HP) and quercetin (Q), which are characterized by better bioavailability have been obtained [2].



Scheme 1. Obtaining of Quercetin and Hesperetin as aglycones from the corresponding natural rutinoides

After an in-depth study in batch mode for the determination of the best reaction conditions, a continuous process was developed through a combination of enzyme immobilization techniques and flow reactors, thus increasing the sustainability and the overall reaction yields (>99%, 5 min). To further reduce the process-related costs a co-immobilization of both the enzymes on the same matrix (i.e., glyoxyl-agarose) has been developed to prepare a high-performing multi-active biocatalyst [3-4].

To further increase the solubility of the starting material a green solvent 2,2,5,5-tetramethyloxolane (TMO) has been employed in a biphasic system (50:50 TMO-buffer) [5].

Due to the recovery and reuse of all the materials involved in the flow biotransformation this strategy can be considered a zero-waste process.

References

- [1] A. Gupta, A.K. Singh, R. Kumar, R. Ganguly, H.K. Rana, P.K. Pandey, G. Sethi, A. Bishayee, A.K. Pandey *Molecules*, **2019**, 24(6), 3399.
- [2] J. Xiao, *Critical Reviews in Food Science and Nutrition*, **2017**, 57, 1874.
- [3] M. Romero-Fernández, F. Paradisi, *Current opinion in Chemical Biology*, **2020**, 55, 1.
- [4] J. M. Bolivar, F. López-Gallego, *Current Opinion in Green and Sustainable Chemistry*, **2020**, 25, 100349.
- [5] A. Pellis, F.P. Byrne, J. Sherwood, M. Vastano, J.W. Comerford, T.J. Farmer, *Green Chemistry*, **2019**, 21, 1686.

Total Volatile Metabolome fingerprinting, target biochemical signatures and nutritional profiling of wild *Ulva* sp. grown in Northern Italian Adriatic sea

Natasha Damiana Spadafora¹, Tatiana Chenet², Mirco Cescon¹, Francesco Chiefa¹, Sofia Malcangi¹, Claudia Stevanin², Paola Tedeschi¹, Flavio Franchina¹, Alberto Cavazzini¹, Luisa Pasti²

¹Department of Chemical, Pharmaceutical and Agricultural Sciences,
University of Ferrara, 44121, Ferrara, Italy

²Department of Environment and Prevention Sciences,
University of Ferrara, 44121, Ferrara, Italy
damiana.spadafora@unife.it

Macroalgae have been consumed as food for decades in the Far East and Asia Pacific countries. In the last few years, the interest toward this food has also grown in North America and Europe [1]. Macroalgae have been highly priced for their economic potential and are also currently recognised as sustainable functional food [2,3]. Furthermore, macroalgae are considered an attractive avenue for their ability to produce secondary metabolites with potential bioactive properties, including antibacterial, antifungal, antiviral, and antioxidant effects [4,5]. However, the comprehensive biochemical fingerprint and nutritional value of macroalgae is still not fully understood. This study focuses on the characterization of wild seaweed (*Ulva* sp.) through total volatile metabolome fingerprinting and nutritional profiling. The seaweed samples were collected in two seasons from different anthropogenised locations in the geographical area of Sacca di Goro (Northern Italian Adriatic sea). To enhance the power of separation of the complex metabolome we used comprehensive two-dimensional gas chromatography coupled to mass spectrometry and flame ionization detectors (GCxGC-MS/FID) across a single analytical run. Both Headspace Solid Phase Microextraction (HS-SPME) and solvent liquid extraction were carried out prior to analysis. In parallel, the qualitative nutritional profiling, based on the bromatological measures of lipids, proteins and carbohydrates, was performed on the same samples. In this work, we focused on the metabolic signatures that deliver the flavour of the sea lettuce in an attempt to identify target compounds of potential interest for the agro-food industry. The biochemical profiling enabled the identification of compounds of the classes of aldehydes, alcohols and ketones among others. The biochemical profiles of geographical areas and collection seasons will be compared and correlated with results from the nutritional profiling. Furthermore, changes at nutritional level will be discussed in detail. Overall, the combination of metabolome fingerprinting, biochemical signatures and nutritional profiling could reveal relevant nutritional as well as flavour notes of high value for the food industry.

References

- [1] J. Cai, A. Lovatelli, J. Aguilar-Manjarrez, L. Cornish, L. Dabbadie, A. Desrochers, S. Diffey, E. Garrido Gamarro, J. Geehan, A. Hurtado, D. Lucente, G. Mair, W. Miao, P. Potin, C. Przybyla, M. Reantaso, R. Roubach, M. Tauati, X. Yuan, *FAO Fisheries and Aquaculture Circular No. 1229*, **2021**.
- [2] A. B. A. Ahmed, M. Adel, P. Karimi, M. Peidayesh, *Advances in Food and Nutrition Research*, **2014**, 73, 197.
- [3] S. Mohamed, S. N. Hashim, H. A. Rahman, *Trends in Food Science and Technology*, **2012**, 23, 83.
- [4] A.M.S.Mayer, A.D. Rodriguez, R.G.S. Berlinck, M.T. Hamann, *Comparative Biochemistry and Physiology C: Toxicology and Pharmacology*, **2007**, 145, 553.
- [5] M. Plaza, S. Santoyo, L. Jaime, F.J. Señoráns, A. Cifuentes, E. Ibáñez, *Journal of Pharmaceutical and Biomedical Analysis*, **2010**, 5, 2.

Discrimination between Mangalica pigs and commercial hybrids through bulk and compound specific isotopic analysis

Silvia Pianezze¹, Matteo Perini¹, Edi Piasentier², Jose Manuel Muñoz Redondo³,
José Manuel Moreno-Rojas³

¹Centro Trasferimento tecnologico, Fondazione Edmund Mach
Via Mach 1, San Michele All'Adige, TN

²Dipartimento di Scienze Agroalimentari, Ambientali e Animali,
Università degli Studi di Udine, Via Sondrio 2A, Udine, UD

³Department of Food Science and Health, Andalusian Institute of Agricultural
and Fisheries Research and Training (IFAPA), Alameda del Obispo,
Avda. Menéndez Pidal, SN, 14004 Córdoba, Spain
matteo.perini@fmach.it

Mangalica pigs are a Hungarian curly-haired swine breed characterized by a fatty meat and a relatively low reproductive performance. Due to this factor and to the request for higher meat/fat ratio products Mangalica pigs almost disappeared in the nineteen seventies [1]. This swine breed escaped from extinction thanks to their new economic exploitation and to the growing interest to breed endangered animals. Nowadays, features such as adaptivity to extensive housing conditions, stress and disease resistance, motherliness and high-quality meat are requested. Even if fatty, Mangalica pigs' meat meet all these requirements [1].

Based on the growing interest in this breed of pigs, the aim of this study was to provide a tool for the discrimination between Mangalica pork and other types of meat. A set of 37 pigs has been considered: 13 Mangalica pigs (M), 12 commercial hybrids bred outdoors (CA), and 12 commercial hybrids raised indoors (CC). For the first time, carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic ratios have been analysed in the defatted meat ($\delta^{13}\text{C}_{\text{PROT}}$, $\delta^{15}\text{N}_{\text{PROT}}$) and in the bulk fat ($\delta^{13}\text{C}_{\text{FAT}}$) of Mangalica pigs. Furthermore, a compound specific isotopic analysis of six fatty acids (including C14:0, C16:0, C16:1, C18:0, C18:1 and C18:2) in the *longissimus lumborum* fat was carried out.

The results show that $\delta^{13}\text{C}_{\text{PROT}}$, $\delta^{13}\text{C}_{\text{FAT}}$ and $\delta^{15}\text{N}_{\text{PROT}}$ are discriminating parameters, while the $\delta^{13}\text{C}$ values of the single fatty acids do not give additional supportive information.

References

[1] I. Egerszegi, J. Rátky, L. Solti, K-P. Brüssow, *Archives Animal Breeding*, **2003**, 46, 245.

NMR-Based automatic protocol for the assessment of honey authenticity

Elisabetta Schievano¹, Marco Tessari²

¹Department of Chemical Sciences, University of Padova, via Marzolo 1, 35131 Padova, Italy

²Magnetic Resonance Research Center, Radboud University, Nijmegen, the Netherlands

elisabetta.schievano@unipd.it

Chloroform extracts of honey contain plant nectar metabolites and compounds secreted by the bees that can provide information about its botanical, geographical, and entomological origin [1,2,3]. The ¹H NMR spectrum of such an organic extract can, therefore, be considered as a fingerprint of honey to confirm or disprove its authenticity [4]. Here we present a protocol to check honey authenticity based on the automatic analysis of the NMR spectrum of its organic extract. From the integrals of specific regions of the spectrum we demonstrate that it is possible to reveal the presence of honey manipulations or confirm its genuineness. In the case of non-genuine samples, the values of these integrals allow to formulate some hypotheses about the corresponding type of adulteration. This approach has proved able to unmask adulterations that had escaped official methods such as pollen analysis and Isotopic Ratio Mass Spectrometry. Importantly, the method requires only a single NMR measurement on a low amount of material, with reduced sample preparation time and can, in principle, be applied to high-throughput analysis of honey.

References

- [1] M. Kortensniemi, C. M. Slupsky, T. Ollikka, L. Kauko, A. R. Spevacek, O. Sjövall, B. Yang, H. Kallio, *Food Research International*, **2016**, 86, 83.
- [2] E. Schievano, C. Finotello, J. Uddin, S. Mammi, L. Piana, *Journal of Agricultural and Food Chemistry*, **2016**, 64, 3645.
- [3] E. Schievano, A. Dettori, L. Piana, M. Tessari, *Food Chemistry*, **2021**, 361, 130050.
- [4] E. Schievano, M. Stocchero, V. Zuccato, I. Conti, L. Piana, *Food Chemistry*, **2019**, 288, 96.

Calabrian Chili Pepper flavouring extracts: characterization and valorisation of this variety towards the PGI mark

Samanta Corsetti¹, Laura Alessandrini¹, Alberto Casale², Monia Floridi², Gianni Sagratini¹

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

²New Flavours srl, Via Dell'Artigianato 7, 06010, Monte Santa Tiberina, Italy

samanta.corsetti@unicam.it

Chili pepper is a relevant spice, which is widely used all over the world; it belongs to the *Capsicum* genus and the *Solanaceae* family. Considering not just its flavour and its colour but also its nutritional value, it is a suitable source of compounds (mainly capsaicinoids and carotenoids) with antioxidant and antimicrobial activity useful for functional food production. In fact, chili peppers were probably firstly used as medicinal plants before being exploited as spices. Indeed, the potential benefit of capsaicinoids is widely known, also for the treatment of pain, ischemic heart disease or for the anti-obesity [1] and the recently studied chemo-preventive effects [2]. However, the antioxidant activity of carotenoid compounds was proven to be even higher, if compared to capsaicinoids in hot chili peppers: that can explain their good anticancer activity, as well [3]. Furthermore, antidiabetic activity was also displayed by the phenolic content of *Capsicum* oleoresin obtained from different varieties of hot chili peppers [4]. These effects are promising, considering the side effects of synthetic anti-obesity drugs, for instance. Specifically, the characterization of Calabrian chili pepper extracts is a first result of our recent collaboration with New Flavours® company, which produces naturally derived flavours that can be exploited by food industries. Thus, this investigation aims to evaluate the possible presence of the bioactive compounds, particularly vitamins, carotenoids and polyphenols, to investigate whether those flavouring products could be also exploitable vehicles of the beneficial properties of the Calabrian Chili Pepper variety. In fact, a complete characterization exclusively of this specific variety is still missing in scientific literature and it could be also an interesting opportunity to contribute to the valorisation of this variety that will soon also obtain the PGI mark. In fact, it could be a possible inspiration for future functional foods [5]. A Soxhlet extraction of the pre-dried and powdered matrix of Calabrian Chili Pepper, provided by New Flavours® company, was performed, through a Universal Extractor in Soxhlet mode. Specifically, in total 24 extracts have been obtained varying the main parameters: solvent, matrix/solvent ratio and extraction time. Ethanol anhydrous and hydroalcoholic solutions at 70% and 50% were chosen as solvents. The Total Phenolic, Total Flavonoid and Total Carotenoid Content have been monitored, along with the possible and important antioxidant activity that was evaluated through DPPH spectrophotometric assays. Results were compared with the ones obtained analysing Calabrian Chili Pepper vegetable matrix. Moreover, 20 polyphenols, two capsaicinoids contributing to hot chili pepper pungency (capsaicin and dihydrocapsaicin), Vitamin C, E and the main carotenoids were quantified using an HPLC-DAD method. The quantification aimed to highlight possible differences in their concentrations between the Calabrian Chili Pepper vegetable matrix and the 24 extracts, as well. It is the beginning towards a future more accurate characterization and a possible valorisation of the Calabrian Chili Pepper.

References

- [1] Y. Wang, Y. Zhou, J. Fu, *Current Opinion in Pharmacology*, **2021**, 61, 1.
- [2] A. Azlan, S. Sultana, C.S. Huei, M.R. Razman, *Molecules*, **2022**, 27, 898.
- [3] M. Mokhtar, M. Russo, F. Cacciola, P. Donato, D. Giuffrida, A. Riazi, S. Farnetti, P. Dugo, L. Mondello, *Food Analytical Methods*, **2016**, 9, 1381.
- [4] C. Hsu, G. Yen, *Journal of Agricultural and Food Chemistry*, **2007**, 55, 1730.
- [5] A. De Nino, L. Di Donna, L. Maiuolo, F. Mazzotti, A. Napoli, E. Perri, A. Tagarelli, G. Sindona, *Journal of applied cosmetology*, **2006**, 24(1), 7.

Chemical composition and biological properties of the hydroalcoholic extract from the *Allium sativum* L. ecotype “Sulmona red garlic”

Fabrizio Masciulli¹, Donatella Ambroselli¹, Enrico Romano¹, Simonetta Cristina Di Simone², Alessandra Acquaviva², Maria Loreta Libero², Nilofar², Mattia Spano¹, Giacomo Di Matteo¹, Cinzia Ingallina¹, Andrea Salvo¹, Luisa Mannina¹

¹Department of Chemistry and Technology of Drugs, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

²Department of Pharmacy, G. d'Annunzio University of Chieti-Pescara, 66013 Chieti, Italy
fabrizio.masciulli@uniroma1.it

Garlic (*Allium sativum* L.), generally used as food taste-active enhancers, is also of great interest for therapeutic treatments and health benefits antioxidant [1]. Despite this evidence, in literature only few pilots are reported about the potential phytotherapeutic use of the “Sulmona red garlic” (ecotype traditionally cultivated in the Abruzzo region, Middle Italy) [2].

Here, the phytochemical profile of aerial red garlic bulbs and the evaluation of biological properties are reported. Both untargeted (NMR) and targeted (HPLC-DAD-MS) methodologies were used to provide complementary information on metabolite composition of the hydroalcoholic extract [3,4]. In particular, NMR analyses allowed sugars, organic acids, amino acids and the organosulphur compounds alliin and allicin to be determined whereas the HPLC analyses revealed the presence of phenolics compounds (catechin, chlorogenic acid, and gallic acid).

The biological activity of garlic hydroalcoholic extract was assessed towards HCT116 colon cancer cells. A chemopreventive effect was observed. This effect could be related to the inhibition of TRPM8 expression, a receptor possibly involved in inflammatory pathway and carcinogenesis [6]. Moreover, the same extract reduced the gene expression of TNF- α (tumor necrosis factor), HIF1- α (hypoxia-inducible factor) and VEGFA (vascular endothelial growth factor) indicating its ability to contrast cancer development through angiogenic pathway. These findings were supported by *in silico* experiment supported biological effects, according to its prediction, organosulphur compounds, especially alliin, may directly interact with TRPM8. The chemico-biological profile of Sulmona red garlic here reported suggests the potential use of this product in phytotherapeutic remedies for management colon inflammatory diseases.

References

- [1] L. Recinella, E. Gorica, A. Chiavaroli, C. Frascchetti, A. Filippi, S. Cesa, F. Cairone, A. Martelli, V. Calderone, S. Veschi, P. Lanuti, A. Cama, G. Orlando, C. Ferrante, L. Menghini, S. C. Di Simone, A. Acquaviva, M. L. Libero, Nilofar, L. Brunetti, S. Leone, *Foods*, **2022**, *11*, 3559.
- [2] A. Lasalvia, F. Cairone, S. Cesa, A. Maccelli, ME. Crestoni, L. Menghini, S. Carradori, B. Marinacci, M. Gallorini, O. Elsallabi, M. Pesce, A. Patruno, *Antioxidants*, **2022**, *11*, 2088.
- [3] C. Ingallina, A.P. Sobolev, S. Circi, M. Spano, C. Frascchetti, A. Filippi, A. Di Sotto, S. Di Giacomo, G. Mazzocanti, F. Gasparrini, D. Quaglio, E. Campiglia, S. Carradori, M. Locatelli, G. Vinci, M. Rapa, S. Ciano, A. M. Giusti, B. Botta, F. Ghirga, D. Capitani, L. Mannina, *Molecules*, **2020**, *25*, 1908.
- [4] M. Spano, G. Di Matteo, C. Ingallina, B. Botta, D. Quaglio, F. Ghirga, S. Balducci, S. Cammarone, E. Campiglia, A. M. Giusti, G. Vinci, M. Rapa, S. Ciano, L. Mannina, A. P. Sobolev, *Molecules*, **2021**, *26*, 2912.
- [5] A. Kopeć, J. Skoczylas, E. Jędrzeczyk, R. Francik, B. Bystrowska, J. Zawistowski, *Agriculture (Switzerland)*, **2020**, *10*, 40.
- [6] F. Borrelli, E. Pagano, B. Romano, S. Panzera, F. Maiello, D. Coppola, L. De Petrocellis, L. Buono, P. Orlando, A. A. Izzo, *Carcinogenesis*, **2014**, *35*, 2787.

Chemical artisanal cheese characterization from Comune di Sicilia goat ecotype

Luigi Liotta¹, Vincenzo Lopreiato¹, Annalisa Amato¹, Maria Elena Furfaro², Monica Greco², Nirey Sirio Velez Cervera², Andrea Letizia Bonsignore², Rossana Denaro², Mirela Raluca Folea², Carmelo Cavallo¹, Cristina Tomasella³, Vincenzo Chiofalo^{1,2}

¹Department of Veterinary Sciences, University of Messina, Messina, Italy

²CoRFilCarni, Consortium of Research for Meat Chain and Agrifood, Messina, Italy

³BIOGENE, Veterinary Diagnostic Center, Catania, Italy

luigi.liotta@unime.it

European goat milk production is mostly transformed into cheese and in the last decade the Italian goat cheese production has increased by 32% [1]. The interest in goat milk products is increasing due to their functional properties, high nutritional value, therapeutic value, and dietary characteristics, moreover, is considered part of its gastronomic, ethnological, and cultural heritage. Its importance becomes even more evident since the goats rearing allows utilization of marginal and less productive areas given the production is mainly extensive and semi extensive [2]. In Sicily, goats' production relies on rearing of the local breed, all these breeds are very well adapted to the local climate and geographic conditions of rearing, and they are very valuable from the aspect of preservation of genome of autochthonous breeds, too. The importance of goat production for rural development, sustainable utilization of natural resources has sensitized the regional government to implement different subsidies schemes to support this livestock sector to endangered, such as the case of Comune di Sicilia goat (BIOSAVE project, PSR Sicilia 2014–2020—Sub-measure 10.2b, CUP G49J21006760009). To preserve this Sicilian goat local ecotype and your relative dairy heritage, three types of artisanal cheeses were studied. This research effort aimed to detail the nutritional of these cheeses, with definite indication to the artisanal cheese-making technology. On 10 cheese samples for type, nutritional traits such as Calories (Reg. UE 1169/2011), Fat (ISTISAN 1996/34, Met B), Carbohydrates (ISO 1871:2009 and AOAC 985.29 1986), Protein (ISO 1871:2009), Salt (UNI EN 13805:2014 and UNI EN ISO 17294-2:2016) content were performed. The diversity of nutritional composition levels, reported in Table 1, validates that all artisanal cheeses display a high level of inconsistency and unicity.

Table 1. Nutrition Facts of Comune di Sicilia goat cheeses (values expressed in g/100g)

Traditional name	Coagulation	Seasoning	Calories	Total Fat	Saturated Fat	Total Carbohydrate	Protein	Salt
Capra Lattica	Lactic acid bacteria	24 hours	209 kcal 866 kj	15.72	10.56	2.04	11.37	0.26
Nuvola di Capra	Lactic acid bacteria	20 days	447 kcal 1854 kj	37.88	26.63	1.76	24.85	1.82
Caprella	Calf rennet	30 days	342 kcal 1419 kj	28.69	19.46	2.98	18.07	0.90

Our results may be of possible interest in revealing the typical characteristics of traditionally produced goat cheeses from Comune di Sicilia goat, which are linked to historical and cultural uniqueness, and decisive in safeguarding and enhancing local genetic resources that have economic relevance for the sustainability of marginal areas.

References

- [1] S. Currò, C. L. Manuelian, M. De Marchi, A. Goi, S. Claps, L. Esposito, G. Neglia, *Italian Journal of Animal Science*, **2020**, *19*, 593.
- [2] A. Pino, L. Liotta, C. L. Randazzo, A. Todaro, A. Mazzaglia, F. De Nardo, V. Chiofalo, C. Caggia, *Food Microbiology*, **2008**, *70*, 143.

Identification and quantification of ciceritol in green coffee beans by RI- HPLC

Elisabetta De Angelis¹, Elena Guercia¹, Elisabetta Schievano², Luciano Navarini¹

¹Illycaffè S.p.A., via Flavia 110, 34147, Trieste, Italy

²Dipartimento di Scienze Chimiche, Università di Padova,

via Marzolo 1, 35131, Padova, Italy

elena.guercia@illy.com

Ciceritol is the trivial name of a pinitol digalactoside isolated from chickpea (*Cicer arietinum*) and characterized by GC/MS analysis for the first time in 1983 by Quemener & Brillouet [1]. Ten years later, its chemical structure was confirmed by NMR spectroscopy to be O- α -D-galactopyranosyl-(1 \rightarrow 6)-O- α -D-galactopyranosyl-(1 \rightarrow 2)-1D-4-O-methyl-chiro-inositol [2]. In addition to chickpea, ciceritol was detected in other legumes such as lentils, white lupin and soybeans [1,3]. In green coffee beans, carbohydrates account for 59-61% of the dry weight and polysaccharides are by far the most abundant. Regarding low molecular weight carbohydrates, in addition to monosaccharides in very low amount, oligosaccharides are the major constituents being sucrose the most abundant (4-8%) followed by small amounts of raffinose and stachyose [4]. Regarding inositol derivatives, *myo*-inositol, bornesitol and D-pinitol have been detected in green coffee as well as in coffee substitutes [5-6]. As far as galactosyl cyclitols is concerned, galactinol is the only one found in green coffee [7]. In a previous preliminary investigation on green coffee oligosaccharides by RI-HPLC, an unknown compound was occasionally detected and tentatively identified as ciceritol by using a chickpea extract as a surrogate standard (unpublished results). Unfortunately, ciceritol standard is not commercially available and therefore in the present study, isolation from chickpea and subsequent ¹H-NMR characterization was performed to get reliable identification of ciceritol in a wide range of green coffee samples (*Coffea arabica*) from different geographical origins. The present study, in ascertaining for the first time the naturally occurring presence of small quantity of ciceritol in green coffee, in addition to open new horizon from a plant physiology point of view, it may be helpful in revealing possible coffee adulteration.

References

- [1] B. Quemener, J.M. Brillouet, *Phytochemistry*, **1983**, *22*, 1745.
- [2] M. Bernabè, R. Fenwick, J. Frias, J. Jimenez-Barbero, K. Price, S. Valverde, C. Vidal-Valverde, *Journal of Agricultural and Food Chemistry*, **1993**, *41*, 870.
- [3] M.M. Pedrosa, C. Cuadrado, C. Burbano, K. Allaf, J. Haddad, E. Gelencsér, K. Takács, E. Guillamón, M. Muzquiz, *Food Chemistry*, **2012**, *131*, 862.
- [4] S. Knopp, G. Bytof, D. Selmar, *European Food Research and Technology*, **2006**, *223*, 195.
- [5] A.I. Ruiz-Matute, A. Montilla, M. D. del Castillo, I. Martínez-Castro, M. L. Sanz, *Journal of Separation Science*, **2007**, *30*, 557.
- [6] A. Masek, M. Latos-Brozio, J. Kałużna-Czaplińska, A. Rosiak, E. Chrzescijanska, *Forests*, **2020**, *11*, 557.
- [7] J.H. da Silva Taveira, F.M. Borém, L.P. Figueiredo, N. Reis, A.S. Franca, S.A. Harding, C.-J. Tsai, *Food Research International*, **2014**, *61*, 75.

New Methodology based on E-eye for the detection of honey adulteration with Tunisian traditional fraud solution

Ben Tiba Amani¹, Francesca Accetta², Luigi Liotta², Haj Said Ayoub³, Ambra Rita Di Rosa²

¹Laboratory of Interfaces and Advanced Materials, Faculty of Science of Monastir, University of Monastir, Monastir, Tunisia

²Department of Veterinary Science, University of Messina, Italy

³Centre for Research on Microelectronics and Nanotechnology of Sousse. Tunisia
luigi.liotta@unime.it

The honey is considered as natural and based food product that is widely used for many reasons especially as antioxidant food [1]. Honey fraud is a major global problem, with honey being the third most adulterated food product and, given the implications for human health, it is important to detect any adulteration. Nowadays, the heightened interest on the part of consumers involves the importance to develop increasingly efficient analytical methods such as the E-senses (electronic nose, electronic tongue and electronic eye) for rapid analytical assessment [2]. The aim of this study was to assess the pattern of colour pigmentation of the pure Chestnut Honey and the mixture with adulterants, specifically Tunisian traditional fraud solution (sugar, water and lemon). For the trial, different percentages of addition of fraudulent solution (2%, 5%, 25%, 50% and 75%) at different temperature (20°C, 40°C, 50°C, 55°C, 70°C and 100°C) were used. The honey samples were placed on cylindrical container of 6g of product in a measurement chamber, applying a camera for high-resolution data acquisition (16 million colors) by using an E-eye (Iris Visual Analyzer 400-Alpha MOS). Three photos were taken for each honey sample, with a white background and lighting only from above. The application of the software available in the instrument (Alphasoft, version 14.0) allowed to group color spectra in the 16-bit range for each RGB coordinate by selecting those present over 1%. To assess the capabilities of this instrument for the discrimination of different color of pure chestnut honey and adulterated honey, the data collected from each group with different percentage of fraud solution at different temperature were submitted to PCA analysis and the Discrimination Index (DI) was calculated. Results showed the high ability of the E-eye to discriminate the different percentages of honey adulteration. At all temperatures, the DI varies from 95% to 98%. Overall, with the increasing temperature, the E-eye was able to identify specific color codes referable to the different percentages of adulteration. Specifically, at 40°C, two color codes were identified useful for detecting the 2% of fraud solution addition (1329 and 1330); at 50°C, one code identified the 5% of adulteration (1346) and one code the 25% (1602); at 55°C two codes were clearly highlighted for the 25% of adulteration (1602 and 1603), one code for 50% (1618) and two codes for 75% (1890 and 1891). The honey heating to 70°C and to 100°C did not produce useful results, indeed at 100°C the color differences related to the percentage of adulteration were reduced. These results determine that the use of the E-eye can be a valid method to identify the adulteration of the honey based on the change of the intensity of the color.

References

- [1] L. Sobrino-Gregorio, R. Bataller, J. Soto, I. Escriche, *Food Control*, **2018**, *91*, 254.
- [2] R. Calvini, L. Pigani, *Sensors*, **2022**, *22*, 577.

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Development and validation of a comprehensive liquid chromatography method coupled to tandem mass spectrometry for the quantification of target contaminants in cereals

Francesca Rigano¹, Katia Arena¹, Francesco Cacciola², Luigi Mondello^{1,3,4}

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

²Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy

³Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental
Sciences, University of Messina, Messina, Italy

⁴Department of Sciences and Technologies for Human and Environment,
University Campus Bio-Medico of Rome, Rome, Italy

francesca.rigano@unime.it

The present research focused on the development of a novel comprehensive liquid chromatography (LC×LC) method in order to enhance the chromatographic resolution and increase the number of trace contaminants to be detected and accurately quantified in cereal products. The latter were selected among vegetable products due to the ever increasing overuse of pesticides and fertilizer to maximize the crop yield. Contaminants were chosen due to their occurrence in cereals and because their maximum residue levels (MRLs) are strictly regulated by current legislation. The method was validated in terms of robustness, limit of detection and quantification, linearity, matrix effect, sensitivity, precision and accuracy, according to validation criteria of the SANTE guideline document.

The LC×LC technique offers the advantage to remove interfering signals from the matrix, thus reducing limit of detection and quantification, and minimizing the need for laborious, tedious and time-consuming sample preparation steps, which could reduce the overall method accuracy.

Complementary stationary phases were selected in each chromatographic dimension to properly exploit different retention mechanisms and achieve satisfactory separation in the two-dimensional space. Targeted multicomponent analysis through time-segmented multiple reaction monitoring (MRM) were used to achieve high selectivity and sensitivity for quantification at low-levels.

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Methodology development for the determination of multiple mycotoxins in dark chocolate bars from Italian markets

Sonia Lombardi¹, Ivana Ledenko¹, Alessandra Cimbalo², Luigi Castaldo¹,
Luana Izzo¹, Alberto Ritieni¹

¹Department of Pharmacy, University of Naples Federico II,
Via Domenico Montesano, 49, 80131 Naples, Italy

²Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy,
University of Valencia, Burjassot, 46100 Valencia, Spain
sonia.lombardi@unina.it

Chocolate represents a popular food around the world due to its pleasant taste and aroma. Nevertheless, some studies suggested that chocolate might be prone to fungus contamination, which could result in the production of potentially harmful mycotoxins [1, 2]. Therefore, this study aimed to develop a method for the simultaneous determination of mycotoxins ($n = 15$) through an UHPLC-Q-Orbitrap HRMS analysis. The proposed method was validated according to Commission Decision 2002/657/EC and applied for the analysis of 18 dark chocolate bars from Italian markets. The proposed procedure was validated after optimization in terms of selectivity and specificity (mass accuracy < 5 ppm), linearity was $r^2 > 0.990$, average recoveries were from 71 to 120 %, and the intra-/inter-day precision was below 20 and 14 %, respectively. Results showed that the most relevant mycotoxin found in chocolate samples was zearalenone, found in 100 % of analyzed samples, ranging from < LOQ to 12.48 ng/g. Moreover, 16.67 % of tested samples showed aflatoxin B2 occurrence, and the level of contamination was < LOQ. On the other hand, the findings highlighted that 44.4% of the samples showed the simultaneous presence of at least 2 studied mycotoxins per sample. Attending to the large percentage of samples that displayed co-occurrence of mycotoxins in dark chocolate bars, this work highlights the necessity of careful evaluation of mycotoxins in these food products.

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References

- [1] M.V. Copetti, B.T. Iamanaka, J.I. Pitt, M.H. Taniwaki, *International journal of food microbiology*, **2014**, 178, 13.
- [2] D.C.P. Abreu, F.A. da Silva Oliveira, E.A. Vargas, F.D. Madureira, E.J. Magalhães, L.P. Da Silva, A.A. Saczk, *Analytical and bioanalytical chemistry*, **2020**, 412, 1757.

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A rapid LC-MS/MS after quechers method for the determination of pesticides in dead bees for honey production risk management

Gaetano Cammilleri¹, Maria Drussilla Buscemi¹, Francesco Giuseppe Galluzzo¹, Licia Pantano¹, Emanuela Bacchi¹, Veronica Fiore¹, Andrea Macaluso¹, Nicola Cicero^{2,3}, Giovanni Lo Cascio¹, Barbara Randisi¹, Vincenzo Ferrantelli¹

¹Istituto Zooprofilattico Sperimentale della Sicilia, via Gino Marinuzzi 3, 90129 Palermo

²Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy

³Science4life S.r.l., start-up dell'Università di Messina, Messina, Italy

gaetano.cammilleri@izssicilia.it

The European honeybee, (*Apis mellifera* L.), is the most widely bred species in the world for honey production. The impoverishment of natural habitats has reduced the bee population. In addition, honey has been affected by agricultural industrialisation, which can result in contamination by pesticides and pollutants. During the last 10 years, we assisted to an unusual depletion in bee numbers, with a consequent loss of colonies called Colony Collapse Disorder (CCD) [1]. CCD is characterised by the rapid loss of the adult worker bee population from a colony. Actually, there are not sufficient studies investigating the role of pesticides in CCD. The aim of this study was therefore to develop and validate a LC-MS/MS method after QuEChERS extraction for the rapid determination of pesticides in dead bees found in beekeeping in order to obtain a useful tool for managing CCD emergency. Several parameters were tested for the method validation including accuracy, LOD, LOQ, recovery and robustness. For the analytes under study (Acetamiprid, Clothianidin, Imidacloprid, Thiacloprid, Thiamethoxam), a LOD ranging from 2.7-4.55 µg/kg and a LOQ of 3.4-4.85 µg/kg were found, whereas the average recovery obtained was between 70%-120%. Repeatability was also evaluated revealing satisfactory values. The method carried out provides a rapid and reliable tool in managing the risk of CCD bee mortality.

References

[1] EFSA, 2008, 154, 1.

Mycotoxins and heavy metals co-occurrence in Eucharistic Communion

Sonia Lombardi¹, Alessandra Cimbalo², Juliane Lima da Silva³, Lidia Ciriaco¹,
Luana Izzo¹, Alberto Ritieni¹

¹Department of Pharmacy, University of Naples Federico II,
Via Domenico Montesano, 49, 80131 Naples, Italy

²Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy,
University of Valencia, Burjassot, 46100, Spain

³Laboratory of Mycotoxins and Food Science, School of Chemistry and Food,
Federal University of Rio Grande, Rio Grande, 96203900, Brazil

sonia.lombardi@unina.it

The Eucharistic Communion is a Christian ritual widespread in different churches belonging to Catholic, Orthodox, Anglican, Presbyterian and Lutheran religions. To reduce the chance of decomposition, the bread served during the celebration is unleavened, made entirely of wheat, and freshly prepared. However, being a grain-based matrix, it could be contaminated by naturally produced toxic compounds, such as mycotoxins, or by heavy metals which can be introduced through water, air or soil [1]. For this purpose, the aim of the study was to determine the contamination levels of mycotoxins and heavy metals in Eucharistic Communion samples from Italy, Poland, Indonesia and South Korea. To carry out the study, a multi-mycotoxin method was optimized by using QuEChERS approach, and the quantitative analysis was performed by ultra-high performance liquid chromatography coupled to quadrupole orbitrap mass spectrometry (UHPLC-Q-Orbitrap HRMS). Regarding heavy metals detection, a double-beam atomic absorption spectrometer (AAS-6300 Shimadzu) was employed. Results highlighted the presence of 9 mycotoxins in all analyzed samples and up to 7 mycotoxins co-occurrence. Among them, deoxynivalenol (DON) reported the highest levels of contamination with a maximum concentration of 118.406 µg/kg in the Polish samples, followed by Italian (47.328, 34.955 µg/kg) and Indonesian (19.971 µg/kg) samples. Likewise, the presence of Enniatin A₁ (ENNA₁), Enniatin B₁ (ENNB₁), and Zearalenone (ZEA) with 1.697, 7.205 and 2.127 µg/kg, respectively was registered in the samples from Poland. As for metals, heavy metals content was in most cases under the LMA, except for the Cd that reported contamination levels up to LMA (0.2 mg/Kg) mostly in Polish sample (0.764 mg/Kg) followed once again by two of the Italian ones (0.407, 0.645 mg/Kg) and South Korean (0.430 mg/Kg). Based on these findings, more studies are needed to assess mycotoxins and heavy metals contamination in order to compile a comprehensive human risk assessment.

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References

- [1] A. Nicolaides, *Pharos Journal of Theology*, **2021**, 102.
- [2] C. Juan, L. Covarelli, G. Beccari, V. Colasante, J. Mañes, *Food Control*, **2016**, 62, 322.

Trace elements composition of marine collagen powder food supplements

Gaetano Cammilleri¹, Marina Tortorici¹, Francesco Giuseppe Galluzzo^{1,2}, Andrea Pulvirenti², Stefano Vullo¹, Salvatore Seminara¹, Andrea Macaluso¹

¹Istituto Zooprofilattico Sperimentale della Sicilia,
Via Gino Marinuzzi 3, 90129 Palermo, Italy

²Dipartimento di Scienze della Vita; Università degli studi di Modena e Reggio Emilia,
Via Università 4, 41121 Modena, Italy
francescogiuseppe92@gmail.com

Marine collagen is a protein derived from fish and other marine sources widely used in dietary supplements for its potential health benefits [1]. Examining the trace elements in marine collagen supplements is essential, as they can contribute to their overall nutritional profile and potential health benefits. Furthermore, due to the origin, it is essential to investigate the presence of toxicological trace elements such as arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg). The traces elements composition of 30 marine collagen food supplements was investigated. An accredited inductively coupled plasma mass spectrometry (ICP-MS) method was used to analyze Mg, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, and Pb. The presence of Hg was investigated with a Direct Mercury Analyzed DMA-80 (AAS). The concentrations (ppb) in decrescent order were: Mg 126.33±58.168, Al 20.677±5.9788, Fe 15.579±4.0916, Zn 1.9263±1.7974, As 0.7000±0.2712, Cu 0.5590±0.1184, Mn 0.3434±0.1023, Pb 0.1262±0.0251, Cr 0.0542±0.0139, Ni 0.0404±0.0294, V 0.0220±0.0086, Co 0.0102±0.0036, Cd 0.0027±0.0025. No detectable amount of Hg was found. Only one sample had a Cd content under the LOD. Therefore, no samples were over the limit imposed by Regulation (EC) 1881/2006 [2]. This work showed that marine collagen is a safe ingredient for food supplements. However, more samples of different types need to be analyzed better to understand the composition of this type of food supplement.

References

- [1] Y.S. Lim, Y.J. Ok, S.Y. Hwang, J.Y. Kwak, S. Yoon, *Marine drugs*, **2019**, *17*(8), 467.
[2] European Commission Regulation (EC) N. 1881/**2006**.

Survey on the presence of heavy metals in the most commonly consumed mediterranean fish species

Gaetano Cammilleri¹, Emanuela Bacchi¹, Licia Pantano¹, Nicola Cicero^{2,3},
Maria Drussilla Buscemi¹, Maria Rita Pisano¹, Andrea Macaluso¹, Vincenzo Ferrantelli¹

¹Istituto Zooprofilattico Sperimentale della Sicilia,
via Gino Marinuzzi 3, 90129 Palermo

²Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy

³Science4life S.r.l., start-up of University of Messina, Messina, Italy
gaetano.cammilleri@izssicilia.it

Heavy metals are chemical elements derived from urban production activities that have a negative impact on the quality of the marine system and especially on the health of fish and their consumers. In this work we examined the presence of Cadmium, Lead and Mercury in the most commonly consumed fish species of the Mediterranean Sea. In particular, 1,256 fish samples of the following species were examined: *Engraulis encrasicolus* (Alice or Anchovy), *Sepia officinalis* (Cuttlefish), *Sardina pilchardus* (Sardine), *Todarodes sagittatus* (Squid), *Scomber scombrus* (Mackerel), *Thunnus thynnus* (Tuna), *Loligo vulgaris* (Squid).

The heavy metals Cd and Pb were quantified using an ICP-MS after microwave-assisted digestion method, whereas the determination of mercury levels was carried out using a direct mercury analyser.

The average concentration of cadmium, lead and mercury in all the samples analysed was 0.04 mg/kg, 0.044 mg/kg and 0.083 mg/kg, respectively which are below the maximum limits imposed by the EU Reg. 1881/2006 [1].

Regarding Cadmium, the highest concentration was found in Cuttlefish showing a maximum value of 0.94 mg/Kg. Nevertheless, the highest average value was found in bony fishes, especially in anchovies (0.047±0.03 mg/Kg).

The highest concentration of lead and mercury was found in tuna samples with maximum of 0.45 mg/Kg and 1.75mg/Kg, respectively and with mean values of 0.04±0.01 mg/Kg and 0.43±0.2 mg/Kg.

The results of this study show a highly endangered fish resource. The particularly high mercury and lead values found in tuna confirm the phenomenon of biomagnification, where tuna is at the top of the marine trophic chain and therefore capable of bioaccumulating these xenobiotics.

Even though the consumption of these species does not represent a risk to human health, as the intake of these metals has been found to be below the maximum doses recommended by FAO/WHO experts, it would be necessary to promote appropriate legislation in order to safeguard the Mediterranean Sea species and at the same time the health of consumers.

References

[1] Commission Regulation (EC) N. 1881/2006.

Influence of innovative packaging with metalized PET on the lipidic and volatile component of coffee pods and capsules

Giulia Basile¹, Lucia De Luca¹, Alessandra Aiello¹, Martina Calabrese¹, Gianfranco Lambiase²,
Fabiana Pizzolongo¹, Raffaele Romano¹

¹Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici (NA), Italy

²Flessofab s.r.l, Montemiletto (AV), Italy

lucia.deluca@unina.it

The growing demand for high quality coffee capsules and pods because of their easiness, speed and convenience requires packaging that guarantees an optimal coffee preservation since coffee is a susceptible matrix of chemical changes during its storage time. A traditional flexible packaging for coffee capsules and pods was constituted by a multilayer of polyethylene terephthalate-aluminum-polyethylene (PET-AL-PE). The aluminum foil acts as a barrier against light, gases and water vapor but it has also its cons such as high costs and low environmental performance [1].

In this scenario, the objective of this research was to evaluate the possible use of an alternative primary packaging with a minor weight in order to replace aluminum foil with metalized PET (PET-metPET-PE) used to prepare coffee capsules and pods. This packaging was compared with the traditional packaging (PET-AL-PE) during the coffee storage at 25°C and 40°C for 180 days.

The coffee samples (packaged in capsules and pods) at specific times of storage were analyzed for their fat content by gravimetric method, peroxide number (NP) by titration method and volatile organic compounds (VOCs) by GC/MS method.

At time 0 the coffee packaged in capsules showed a lipid content of 9.5% in PET-AL-PE and 9.5% in PET-metPET-PE, while at the end of storage at 25°C was 9.2% in coffee packaged in PET-AL-PE and 9.5% in coffee packaged in PET-metPET-PE, while in coffee packaged at 40°C the lipid content was 9.2% in coffee packaged in PET-AL-PE and 9.6% in coffee packaged in PET-metPET-PE at the end of storage.

The coffee packaged in the pods, the lipid content at time 0 was 10.6% in PET-AL-PE and 11.0% in PET-metPET-PE, at the end of storage at 25°C was 11.2% in PET-AL-PE and 11.3% in PET-metPET-PE, while at the end of storage at 40°C was 11.0% in PET-AL-PE and 11.2% in PET-metPET-PE.

The NP value (marker of primary lipid oxidation) in coffee stored in capsules increased at the end of storage (180 days) in both standard and alternative packing and at both storage temperatures (0.6 meq O₂/kg vs 1.7 meq O₂/kg in PET-AL-PE; 0.6 meq O₂/kg vs 1.8 meq O₂/kg in PET-metPET-PE at 25°C; 0.6 meq O₂/kg vs 1.9 meq O₂/kg in PET-AL-PE and 0.6 meq O₂/kg vs 1.8 meq O₂/kg in PET-metPET-PE at 40 °C). Also, in the coffee packaged in pods an increase in the NP value at both temperature and in both packaging was found (0.7 meq O₂/kg vs 1.9 meq O₂/kg in PET-AL-PE; 0.7 meq O₂/kg vs 1.8 meq O₂/kg in PET-metPET at 25°C; 0.7 meq O₂/kg vs 1.8 meq O₂/kg in PET-AL-PE and 0.7 meq O₂/kg vs 2.0 PET-metPET at 40°C). Anyway, no statistical difference between packaging at the two storage temperatures was found. Regarding the VOCs a total of 40 compounds in both type of coffee (capsules and pods) were detected which the majority belonging to the class of pyrazines (9), pyrroles (4), pyridines (3), furans (8), phenols (4), ketones (5) and aldehydes (2), acids (1).

In conclusion, the innovative packaging (PET- metalized PET-PE) showed the same performance of standard packaging (PET- AL- PE) with the advantage of reducing the weight of both packaging (capsules and pods).

References

[1] F. Cincotta, G. Tripodi, M. Merlino, A. Verzera, C. Condurso, *LWT*, **2020**, *118*, 108718.

ICP-MS analysis and elemental profile of Italian honeydew honeys: a preliminary study

Andrea Mara¹, Gavino Sanna¹, Gianni Zoccatelli², Marco Ciulu²

¹Dipartimento di Scienze Chimiche, Fisiche, Matematiche e Naturali, Università degli Studi di Sassari,
Via Vienna 2, 07100, Sassari, Italy

²Dipartimento di Biotecnologie, Università degli Studi di Verona,
Strada le Grazie 15, 37134, Verona (Italy)
marco.ciulu@univr.it

Honeydew honey is produced from the foraging by honey bees of the honeydew produced by certain insects, which is mainly found on plants such as conifers, firs, pines etc.

In Italy, these honeys are mainly collected in the north and include forest and fir honeydew in their main productions. Over the years, the collection of these honeys has been negatively affected by rising temperatures that cause overlapping of nectariferous blooms during the honeydew harvesting period and, above all, by the use of pesticides that have led to a progressive decrease in honeydew-producing insects such as *Metcalfa pruinosa*. Nevertheless, alongside the typical productions of northern Italy, new types of honeydew honeys, such as eucalyptus, oak, citrus and hazelnut honeydew, have begun to appear in central and southern Italian markets.

This work is part of a broader project aimed at examining the quality of Italian honeydew honeys and identifying authenticity markers for new productions, also in light of the alarm launched by the European Commission regarding the increasing cases of honey counterfeiting and the consequent need for reliable analytical strategies for the correct attribution of origin [1].

As a preliminary step, the multi-elementary profile of 37 Italian honeydew honeys was evaluated, including forest- (13), fir- (7), eucalyptus- (6), citrus- (3), oak- (4), hazelnut- (2) honeydew honeys and finally two samples indicated by the producers as nectar and honeydew mixes.

Although the elemental content is mainly linked to the geographical origin, an attempt was made to discriminate honeydew honeys based on their botanical origin. In fact, this could also affect the elemental profile.

A previously developed and validated ICP-MS method [2] was applied to the samples for the determination of trace and toxic elements including Li, Be, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Pb, Sr, Mo, Ag, Cd, Sn, Sb, Te, Cs, Ba, Hg, Tl, Pb and U. The resulting dataset was subjected to a number of dimension reduction algorithms including Uniform Manifold Approximation and Projection (UMAP) and Principal Component Analysis (PCA) in order to detect differences among the groups of samples and identify the main discriminant variables.

The results of the multivariate analysis showed a good separation of the groups formed by forest, fir and eucalyptus. Specifically, the elements whose content was found to be the main responsible for this discrimination were Cs and U. In fact, Cs content appeared to be tendentially higher in fir samples (24÷164 ppb) when compared to forest (2.8÷67.5 ppb) and to eucalyptus honeydews (0.5÷1.2 ppb). On the other side, U concentration was higher in forest honeydews (0.1÷1.1 ppb) compared to fir (0.004÷0.1 ppb) and eucalyptus honeydews (0.004÷0.7 ppb). As previously stated, the resulting outcomes of this study are promising but still preliminary. Further studies and a larger number of samples are required in order to assess whether Cs and U content could be used to discriminate among the aforementioned botanical origins.

References

[1] European Anti-Fraud Office (OLAF), N. 6/2023.

[2] A. Mara, S. Deidda, M. Caredda, M. Ciulu, M. Deroma, E. Farinini, I. Floris, I. Langasco, R. Leardi, M.I. Pilo, N. Spano, G. Sanna, *Molecules*, **2022**, 27, 1.

Use of terrestrial gastropods as bioindicators of contamination status environment of Sicilian natural parks

Maria Drussilla Buscemi¹, Gaetano Cammilleri¹, Francesco Giuseppe Galluzzo¹, Lucia Pantano¹, Emanuela Bacchi¹, Andrea Macaluso¹, Nicola Cicero^{2,3}, Vincenzo Ferrantelli¹

¹Istituto Zooprofilattico Sperimentale della Sicilia,
via Gino Marinuzzi 3, 90129 Palermo

²Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy

³Science4life S.r.l., start-up dell'Università di Messina, Messina, Italy
drussilla.buscemi@izssicilia.it

We analyzed the presence of heavy metals and PAHs in 270 samples of terrestrial gastropods belonging to two different species from natural parks (Monti Sicani and Cano Randello) and large industrial and urban complexes (Syracuse and Ragusa), to confirm the possible use of these organisms as environmental bioindicators. Heavy metals such as V, Cr, Cd, Pb, As, Mn and Fe were analyzed through the development and validation of a method based on the ICP-MS. The mercury levels were instead calculated using a direct analyzer. For the detection of PAHs, a GC-MS / MS with QuEChErS extraction was developed and validated. The validation results revealed the high reliability of the methods developed. The results verified significant differences in the values of V, Cr, Pb, As and Fe between urban/industrialized areas and natural parks ($p < 0.05$). No significant differences were found for the values of Cd ($p > 0.05$). The Ragusa samples found the highest average values of V, Pb and As (0.18, 0.14 and 0.04 mg/Kg) while the Syracuse samples showed the highest average values of Chromium. The lowest values of heavy metals were found in the samples of Cava Randello. The present study reports for the first time the levels of Fe in terrestrial gastropods highlighting high concentrations (up to 720 mg/Kg). No mercury and PAH levels were detected. The results of this study confirm the use of terrestrial gastropods as environmental indicators for some heavy metals present in the terrestrial environment. Further studies are necessary in order to add the snails among the bioaccumulative organisms into surveillance networks set up by the European territory [1].

References

[1] A. Amaral, H. Anselmo, M. Toste Tristão da Cunha, A. Rodrigues, *BioMetals*, **2004**, *17*, 625.

Augmented smelling to reveal Brazilian Extra Virgin Olive Oil aroma blueprint: accurate quantification of key-aroma compounds by comprehensive two-dimensional gas chromatography and parallel detection by MS and FID

Andrea Caratti¹, Nathalia Brilhante², Simone Squara¹, Carlo Bicchi¹,
Humberto Bizzo³, Chiara Cordero¹

¹Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Torino, Italy

²Post-graduation Program in Food Science, Federal University of Rio de Janeiro,
Rio de Janeiro RJ, 21941-909, BRAZIL

³Embrapa Agroindústria de Alimentos, Rio de Janeiro RJ, 23020-470, BRAZIL
andrea.caratti@unito.it

Extra virgin olive oil (EVOO) is a valuable food commodity that is widely consumed worldwide. The aroma of extra virgin olive oil is affected by various factors such as the type of olive tree, the conditions in which the trees are grown, the stage at which the olives are harvested, and the method of oil extraction [1]. Despite being the third-largest importer of olive oil in the world, Brazil's production of olives and oil is relatively new and small compared to its domestic market's size.

To assess characteristic patterns of odorants for different cultivars (*Arbequina* and *Koroneiki*), different harvest years (2021 and 2022), and production regions (Rio Grande do Sul and Serra da Mantiqueira), comprehensive two-dimensional gas chromatography coupled with parallel detectors (MS and FID) was chosen. In fact, GC×GC-MS/FID leads to a high-performance analysis strategy capable of fully exploit the information encrypted on the volatile fraction including also those key-analytes responsible of the EVOOs aroma blueprint. Moreover, the complementary characteristics of MS and FID open the possibility of performing multi-target quantitative profiling by predicted relative response factors with great accuracy [2].

Untargeted/targeted fingerprinting workflow was carried out combining template matching strategies on the 2D-patterns of volatiles. Quantification of target volatiles was achieved via Multiple Headspace SPME, external standard calibration and FID predicted relative response factors (PRRF).

The combination of HS-SPME with GC×GC-MS/FID and PRRF resulted to be a great tool in the quality assessment of EVOO samples. By effective exploration of the information encrypted in EVOOs *volatilome*, the impact of functional variables is reliably correlated to diagnostic patterns with great classification and *identification* attitudes [3]. By the accurate quantification of key-odorants, an Artificial Intelligence smelling machine is realized, an Augmented smelling with unique comparative possibilities for EVOOs aroma qualities.

References

- [1] G. Luna, M. T. Morales, R. Aparicio, *Food Chemistry*, **2006**, 98, 243.
- [2] F. Stilo, M.d.P. Segura Borrego, C. Bicchi, S. Battaglino, R.M. Callejòn Fernandez, M.L. Morales, S.E. Reichenbach, J. McCurry, D. Peroni, C. Cordero, *Journal of Chromatography A*, **2021**, 1650, 462232.
- [3] L. Cuadros-Rodríguez, C. Ruiz-Samblás, L. Valverde-Som, E. Pérez-Castaño, A. González-Casado, *Analytica Chimica Acta*, **2016**, 909, 9.

Metabolic plasticity in *Glycyrrhiza glabra* leaves shapes development and environment

Rita Celano¹, Teresa Docimo², Simona Serio¹, Sonia Carabetta³, Rosa Di Sanzo³,
Mariateresa Russo³, Anna Lisa Piccinelli¹, Luca Rastrelli¹

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

²Institute of Bioscience and BioResources, National Research Council, Portici, Napoli, Italy

³Department of Agriculture Science, Food Chemistry, Safety and Sensoromic Laboratory (FoCuSS Lab),
University of Reggio Calabria, Reggio Calabria, Italy
rcelano@unisa.it

The Calabrian *Glycyrrhiza glabra* is one of the most appreciated licorice varieties in the world for its botanicals, food and pharmacological properties [1]. In the licorice cultivation process, the apigee parts represent an abundant processing waste with a high content of bioactive compounds still understudied [2].

Quantitative leaf profiles of different ecotypes of Calabrian *G. glabra*, collected in three different phenological stages (vegetative, reproductive and senescence), were investigated by UHPLC-UV analysis. The leaves showed a mean total phytochemicals content of 18 g/100g DM. The most abundant class of detected secondary metabolites in leaves were flavanones (36-60%) and prenylated dihydrostilbenes (10-37%) followed by flavon-C-glycosides (17-32%) and flavonol-O-glycosides (4-10%). The quantitative variations of the leaf markers according to the ecotype and phenological stage are very marked. The data obtained showed that the soil and climatic conditions play a decisive role in influencing the accumulation of secondary metabolites and highlight the variations of the metabolic profile during plant development. This study provides insights into the metabolic plasticity of the Calabrian *G. glabra* leaves highlighting the high content of bioactive compounds and identifying the best ecotype and harvesting period to obtain a richer matrix.

References

- [1] G. Pastorino L. Cornara, S. Soares, F. Rodrigues, M.B.P.P. Oliveira, *Phytotherapy research*, **2018**, *32*, 2323.
- [2] R. Celano, T. Docimo, A. L. Piccinelli, S. Rizzo, L. Campone, R. Di Sanzo, S. Carabetta, L. Rastrelli, M.T. Russo, *Industrial Crops and Products*, **2021**, *170*, 113688.

Detection of Histamine in fishery: a retrospective study 2018-2022

Sara Morello^{1,2}, Samantha Lupi¹, Marianna Marturella¹, Elena Torres¹, Marilena Gili¹,
Daniela Manila Bianchi^{1,2}, Lucia Decastelli^{1,2}

¹Istituto Zooprofilattico Sperimentale Piemonte, Liguria e Valle d'Aosta Turin, Italy

²National Reference Center for detection in food of substances causing food allergies or intolerances (CReNaRiA),
Turin, Italy
sara.morello@izsto.it

Histamine is a biogenic amines produced by a wide range of spoilage microorganisms from the enzymatic decarboxylation of the histidine, an essential amino acid naturally present in foods and beverages. The conversion rate of histidine to histamine is due to the availability of the free histidine and of bacteria with decarboxylation activity, under suitable conditions of temperature (20-37°C) and pH (4.0-5.5) [1]. High levels of this biogenic amine in food can be responsible of adverse effects similar to those of allergic reaction: symptoms include urticaria, nausea, vomiting, diarrhoea, and the severity of effects is related to dose and varies from individuals [2]. As fish and fish products can be rich in histamine, this amine is one of the indicators used to evaluate fishery freshness and hygienic quality, in the frame of Food Safety European Regulation. According to Regulation (EC) No 2073/2005 [3], a sampling plan of nine units forming the samples is required in official fishery collection: m and M levels for histamine are fixed respectively at 100 mg/kg and 200 mg/kg for some fishery products from fish species associated with high amount of histidine (*Scombridae*, *Clupeidae*, *Engraulidae*, *Coryfenidae*, *Pomatomidae*, *Scombrosidae*) and at 200 mg/kg and 400 mg/kg for Fishery products which have undergone enzyme maturation treatment in brine, manufactured from same species. Number of sample units giving values over m or between m and M is always fixed at 2. This work presents the results of a retrospective survey on detection of histamine in fishery in Northern Italy from January 2018 to December 2022. A total of 138 different food matrices, including fish products (n=95) and fish (n=43), were collected by National Health Services in the frame of Regional Monitoring Plan of Food Safety or in the contest of food poisoning. Totally, 1242 units forming the samples (138 x 9) were tested by Food Control Laboratories of Istituto Zooprofilattico Sperimentale del Piemonte Liguria and Valle D'Aosta. Screening analyses were performed by a sandwich enzyme immunosorbent assay (SENSISpec ELISA Histamine kit; Eurofins Technologies, Hungary). Confirmatory analyses were performed by HPLC-DAD method (LOQ=20 mg/kg), with range of quantification from 20 to 400 mg/kg. Both methods were previously fully validated on fishery food matrices and accredited. A total of 4.38% (samples n=6; units forming the sample n= 54) including samples of tuna (n=4), mackerel (n=1), herring (n=1) was found to be not negative at the screening analysis and thus analyzed by confirmatory tests. Judgement of compliance or non-compliance of the whole sample was conducted according to n=9 c=2. Quantitative amount of histamine according to confirmatory analyses ranged between 21 and 7122 mg/kg. Histamine is one of the most poisoning biogenic amines occurring in foods, especially in fish: a program of monitoring level of this amine is a powerful and suitable way to guarantee surveillance of high level of histamine in foodstuffs, avoiding unintended exposition to risk for consumers' health.

References

- [1] A. Durak-Dados, M. Michalski, J. Osek, *Journal of Veterinary Research*, **2020**, 64(2), 281.
- [2] M. Schirone, P. Visciano, R. Tofalo, G. Suzzi, *Handbook of Experimental Pharmacology*, **2017**, 241, 217.
- [3] Commission Regulation (EC) N. 2073/2005.

ONFOODS: Research and innovation network on food and nutrition Sustainability, Safety and Security

Roberta Tardugno¹, Maria Lisa Clodoveo², Filomena Corbo¹, Loreto Gesualdo³

¹Department of Pharmacy-Drug Science, University of Bari Aldo Moro, Bari, Italy.

²Interdisciplinary Department of Medicine (DIM), University of Bari Aldo Moro, Bari, Italy.

³Department of Regenerative and Precision Medicine and Ionic Area (DiMePRE-J),

University of Bari Aldo Moro, Bari, Italy.

roberta.tardugno@gmail.com

The concept of sustainability has only recently entered everyday use. Sustainability refers to patterns of production and consumption that respect natural resources and their usual rhythms, focusing on long-term resilience and avoiding depletion of resources and environmental degradation.

ONFOODS (Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods) has planned to act with a comprehensive approach, joining together and synergizing the strengths and competences of several different disciplines, ranging from agricultural economics, food chemistry, food technology and engineering, microbiology, to human nutrition, and many disciplines of medicine. Only through a holistic approach we could become able to preserve the environment, acting on the virtuous implementation of our food systems and, at the same time, improving the wellbeing of the population and extending the life lived in good health, by reducing avoidable and premature mortality and inequity, and putting the person at the centre of a more effective, safe, sustainable, and fair social security system.

ONFOODS will face this challenge acting through the coordinated activity of seven spokes, each one focusing on a very specific, although wide, matter related to food production, transformation, and effect [1, 2]. The objective of Spoke 6 is to develop nutritional strategies for vulnerable individuals. Regarding innovation, the identification of new bioactive molecules proven to directly impact nutritional status in specific target groups with malnutrition, which will ideally contribute to the development of new products, including food supplements and nutraceuticals, food ingredients and food fortification approaches to counteract malnutrition are the main goals [1, 2]. Innovation also includes new sustainable nutrition protocols developed and validated for specific target groups with malnutrition.

References

[1] M.L. Clodoveo, M. Muraglia, P. Crupi, R.H. Hbaieb, S. De Santis, A. Desantis, F. Corbo, *Foods*, **2022**, *11*, 1915.

[2] M.L. Clodoveo, L. Di Lorenzo, C. Sabbà, A. Moschetta, L. Gesualdo, F. Corbo, *International Journal of Food Sciences and Nutrition*, **2020**, *71*:4, 525.

SAFETY EVALUATION OF HONEYS FROM NORTH ALGERIA AND TUNISIA

Laura Messina¹, Patrizia Licata¹, Ylenia Marino², Gianluca Antonio Franco¹, Fabio Bruno¹

¹Department of Veterinary Science, University of Messina, Via Giovanni Palatucci, 98168 Messina, Italy

²Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,

University of Messina, 98166 Messina, Italy

laura.messina@studenti.unime.it fabio.bruno@studenti.unime.it

Honeybees produce a complex solution rich in simple sugars, such as glucose and fructose, proteins, vitamins, salts and aromatic compounds, which make up honey, together with other substances contained in varying proportions. Honey is an optimal monitor of the environmental quality and often it is accepted as food and medicine by all generations, traditions and civilizations, for its therapeutic properties, antibacterial and antioxidant, antitumour useful for human health [1]. To contribute to the knowledge of the properties of honey, in more details, we investigate the contaminants concentrations belonging to various classes. Some of these are polychlorobiphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organochlorine and organophosphorus pesticides (OCPs and OPPs), fungicides (Fs) and herbicides (Hs), pyrethroid insecticides (PYRs), synergists (SYN) and other insect growth regulators (IGRs). The analyzes were developed on 15 multifloral honey from different botanical species (wildflower, eucalyptus, eucalyptus red flowers, prickly pears, lemon blossom, thyme, almond, rosemary and jujube) from northern Algeria (Algiers, Bouria, Ghardaïa, Laghouat, M'sila, Naâma, Tlemcen and El Bayadth) and on 10 honey samples from different botanical species from Tunisia (Sidi Bouzid, Nabeul and Sfax), according to the guidelines of the AOAC and the European Honey Commission. Furthermore, the use of chemical and pharmacological treatments for disease control, inside the hives, is also a further source of direct contamination [2]. This increases the percentage of harmful substances found in honey, altering its nutritional and reducing its quality. The aim of this research was to characterize the honeys from Algeria and Tunisia regions by their physico-chemical properties, the presence of pesticides and to check if the requirements of the international standards (Codex Alimentarius Commission, 2001; European Commission, 2002; IHC website) are met to preserve costumers against fraud and for the possible toxicological risk for humans. Extraction of the different classes of contaminants was conducted using a QuEChERS method according to the matrix type and the simultaneous analysis were performed with a Shimadzu TQ8030 HRGC-MS/MS. It allowed recoveries in the range 90–125%, with limits of detection (LODs) between 0.10 and 5.21 ng g⁻¹ showing a good sensitivity and accuracy based on the limits set by Regulation (EU) n.37/2010 of the Commission and Regulation (EC) n. 396/2005 with subsequent amendments. Analyses were carried out using a Thermo Scientific Trace GC Ultra coupled with a triple quadrupole mass spectrometer TSQ Quantum XLS equipped with an autosampler TrisPlus RSH [3]. Analysis showed that many of our honey samples were contaminated by at least one compound. OPPs were present in numerous samples; OCP and other contaminants (Fs, PYR, SYN, IGR) were found. According to current legislation, Alachlor was found in almost all Algerian and Tunisian honeys and exceeded the MRLs. As regards the honey samples from Algeria, the concentrations of Cyromazine and Cyhalothrin exceeded the MRLs in all honey samples studied. 50% of the Tunisian honeys showed levels of Metalaxyl-M above the established limit.

References

- [1] M.I. Khalil, S.A. Sulaiman, L. Boukra, *The Open Nutraceuticals Journal*, **2010**, 3, 6.
- [2] P. Guillebeau, *Special Bulletin*, **2004**, 28.
- [3] M. Saitta, G. Di Bella, M. R. Fede, V. Lo Turco, A. G. Potortì, R. Rando, M. T. Russo, G. Dugo, *Food Additives & Contaminants: Part A*, **2017**, 34:5, 800.

Use of magnetic ionic liquid (MIL) and Loop-mediated isothermal amplification (LAMP) for the detection of microbial agents in food: a proof of concept

Domenica Mangraviti¹, Danilo Donnarumma¹, Emanuela Trovato¹, Giuseppe Arcoletto²,
Giulia Casini², Cosimo Manzo², Paola Dugo^{1,3}, Luigi Mondello^{1,3}

¹Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

²Enbiotech s.r.l, Palermo, Italy

³Chromaleont S.R.L., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, Messina, Italy

domenica.mangraviti@unime.it

Contamination in food and water sources intended for human and animal consumption represents a relevant risk factor, not only for the health of consumers, but also for the economy of agri-food companies. In this context, effective food safety management systems are essential to reduce microbial contamination and improve food safety. Those controls require generally the use of various molecular diagnostic techniques in order to extract and amplify exogenous DNA/RNA from food matrices, and to reveal possible microbial agents occurrence. Usually, nucleic acids amplification is performed by Polymerase Chain Reaction (PCR). This method is widely known for its reliability and flexibility, but requires the employment of specialized laboratories and competent personnel, the use of complex and expensive equipment and rather long analysis times.

In the recent years, an alternative technology named LAMP "Loop-mediated isothermal amplification" has been developed. Differently to classical PCR, this technique not requires a thermal cycler and enables the extracted DNA analysis directly from material collected in the field. A critical aspect of the procedure is the nucleic acid extraction due to the needed of a sample purification step from any reaction inhibitors and contaminants before carrying out the diagnostic test.

In the present study, a really promising extraction method based on the use of the Magnetic Ionic Liquids (MILs) was taken into consideration for *Legionella spp* DNA extraction from aqueous matrices. The optimized procedure, easy to be automatized, does not require the utilization of any organic solvents, in line with the Green Chemistry principles. The nucleic acid amplification was performed by means of an innovative all-in-one molecular biology system that take fully advantage of the LAMP technology (ICgene Plus). This portable equipment, coupled to a fluorescence detector, has been employed for the rapid isothermal amplification of *Legionella spp* DNA, real-time detection and automatic interpretation of obtained data.

The research aimed to the development of a rapid and efficient approach for the extraction of nucleic acids from food matrices, with a reduced environmental impact, coupled to the ICgene Plus technology for amplification and data detection.

Carotenoids, polyphenols and sugar content of pumpkin, pumpkin flour and pumpkin bread after High Pressure Processing (HPP)

Stefania Sut¹, Francesca Loschi¹, Rohini Dhenge², Denise Gambato¹, Irene Ferrarese¹, Saverio Santi³, Massimiliano Rinaldi², Stefano Dall'Acqua¹

¹Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via F. Marzolo 5, 35131 Padova, Italy

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

³Department of Chemistry, University of Padova, Via F. Marzolo 9, 35131 Padova, Italy
stefania.sut@unipd.it

The demand for high-quality, fresh-like, and nutritious plant-derived foods is increased in the modern society. High Pressure Processing (HPP) is considered one of the emerging non-thermal technologies able to inactivate microbial load while exerting rather minimal effects on nutritional and organoleptic properties of food products [1].

Pumpkin (*Cucurbita moschata*) is a vegetal largely consumed worldwide, has good nutritional properties and can be a source of phytochemicals with health-promoting features [2]. The aim of the study is to evaluate the effect of HPP process on secondary metabolites and sugar content in pumpkin products obtained from *Cucurbita moschata* cv. Duchesne Ex Poir. Pumpkin subjected to HPP process was used to prepare pumpkin flour and with this flour a bread enriched with pumpkin flour was prepared. Different pressures and times were used in HPP process, 200, 400 and 600 MPa and 1, 3 and 5 minutes, respectively. Carotenoids, polyphenols and sugar were monitored in the different pumpkin samples by LC-UV/VIS, polyphenols by LC-DAD-MS and sugars by LC-ELSD respectively.

Significantly higher amount of carotenoids and polyphenols were observed in pumpkin, pumpkin flour and pumpkin bread subjected to HPP with medium-high pressures. This indicate that HPP at medium-high pressure could have an influence on extractable carotenoid and polyphenol from the vegetal matrix. Not significance differences were observed in sugars.

Results revealed that HPP process could affect the influence on extractable healthy promoting compound from vegetable sources that could be applied for enriching food products.

References

[1] G.I. Denoya, Q. Xia, F. Xie, *Present and Future of High Pressure Processing*, **2020**, 12, 273.

[2] X. Men, S.I. Choi, X. Han, H.Y. Kwon, G.W. Jang, Y.E. Choi, S.M. Park, O.H. Lee, *Food Science and Biotechnology*, **2020**, 30(2), 171.

Biosynthesis and chemical characterization of carotenoids produced by a novel *Planococcus* sp. isolated from South Africa

Daniele Giuffrida¹, Anesu Conrad Moyo², Laurent Dufossé³, Leonardo Joaquim van Zyl², Marla Trindade², Giacomo Dugo^{1,4}

¹Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF), University of Messina, 98168 Messina, Italy

²Institute for Microbial Biotechnology and Metagenomics (IMBM), Department of Biotechnology, University of the Western Cape, Bellville, Cape Town 7535, South Africa

³Chemistry and Biotechnology of Natural Products, CHEMBIO- PRO, ESIROI Agroalimentaire, Université de La Réunion, 15 Avenue René Cassin, CS 92003, CEDEX 9, F-97744 Saint-Denis, France

⁴Science4life S.r.l., start-up of University of Messina, Messina, Italy
dgiuffrida@unime.it

Of the pigments produced by bacteria, carotenoids have diverse biological functions that include, coloration, photoprotection, light harvesting, and regulating the fluidity of the bacterial phospholipid bilayer membrane. Biotechnologically, carotenoids have been utilized as food colorants, antioxidants, animal feed supplements, nutraceuticals, cosmetics, and pharmaceuticals. Numerous bacterial species, including some from the *Planococcus* genus, are known to produce carotenoids. The genus *Planococcus* comprises of halophilic, aerobic, Gram-positive, motile cocci from various environments, including saltern ponds. Here, we report on the identification and characterization of the carotenoid biosynthetic gene cluster and the C₃₀-carotenoid compounds produced by *Planococcus* sp. CP5-4. Mass-spectrometry guided analysis of the saponified and unsaponified pigment extracts showed that methyl 5-glucosyl-5, 6-dihydro-apo-4, 4'-lycopenoate esterified to heptadecatrienoic acid (C_{17:3}) was the main compounds. Furthermore, through phylogenetic analysis of the core carotenoid BGCs of *Planococcus* species we show that various C₃₀-carotenoid product chemotypes, apart from methyl 5-glucosyl-5, 6-dihydro-apo-4, 4'-lycopenoate and 5-glucosyl-4, 4'-diaponeurosporen-4'-ol-4-oic acid, may be produced that could offer opportunities for a variety of applications, including food chemistry.

Use of non-*saccharomyces* yeasts for the innovation of wine production and the improvement of their quality

Diego De Filippi¹, Erika Marino¹, Antonella Valenti¹, Filippo Amato¹,
Daniele Oliva², Valeria Guarrasi³

¹HTS enologia, Contrada Amabilina, 218/A – 91025 Marsala (TP)

²Istituto Regionale del Vino e dell'Olio (IRVO), Via della Libertà, 66 – 90143 Palermo (PA)

³Istituto di Biofisica, CNR, Via Ugo la Malfa, 153 – 90146 Palermo (PA)

famato@hts-enologia.com

The fundamental event in wine production is alcoholic fermentation, in which grape sugar is converted into ethyl alcohol. This is a metabolic process carried out by microorganisms commonly referred to by the term "yeasts," which groups several genera of oenological interest. Yeasts are responsible not only for the alcohol in wine, but also for the production of other substances on which the final quality of the product depends, such as certain fermentation esters responsible for the "fruity" notes of the bouquet, or hydrogen sulfide, reminiscent of the smell of rotten eggs, which is negative descriptor of the aroma. In the initial stages of fermentation, yeasts of the *Hanseniaspora*, *Candida*, *Metschnikowia*, *Pichia*, *Kluyveromyces*, and *Torulaspota* genera act spontaneously but are usually displaced after a short time by yeasts of the *Saccharomyces cerevisiae* species, which is responsible not only for wine production but also for beer and bread production. Since the 1970s, the use of "selected" yeast strains, i.e. yeast strains that stand out compared to others for the production of substances that can improve wine quality, has become increasingly popular. Most of the selected yeast strains available on the market today belong to the *Saccharomyces cerevisiae* species. This is the only one capable of fermenting a must's sugars, while all other yeasts are grouped under the generic term "non-*Saccharomyces*" and have long been considered poor fermenters. In the last fifteen years, numerous scientific studies have shown that it is possible to find, within the populations of non-*Saccharomyces* yeasts, certain strains that develop particular enzymatic activities that can significantly improve the quality of the wine. And indeed, recently the first preparations based on non-*Saccharomyces* yeasts, in particular the species *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Totulaspora delbrueckii*, *Pichia kluyvery*, and *Schizosaccharomyces pombe*, have appeared on the yeast markets for oenological use [1]. The present study, carried out within the framework of a project funded by the P.O. FESR SICILIA 2014/2020 Action 1.1.3, investigated the possibility of industrial use of two new non-*Saccharomyces* yeast strains selected at IRVO in Sicily, namely *Kluyveromyces marxianus* Km L2009 [2], belonging to species not yet present in the oenological yeast market, and *Candida zemplinina* Cz26 [3]. These strains have been successfully used in the production of more aromatic white wines and rounder or smoother red wines compared to wines made from the same musts fermented with commercial *Saccharomyces cerevisiae* yeast strains, through sequential mixed fermentation. Strain Cz26 has shown extremely positive technical characteristics in red wines (reduced alcohol content and increased glycerol production), but it is not suitable for rapid commercialization because it presents some operational difficulties (excessive foaming). In contrast, the Km L2009 strain is an innovative tool for the production of modern and fresh white wines with an extremely intense aromatic profile. This yeast should meet technical and commercial interest from the 2023 harvest.

References

- [1] R. Vejarano, A. Gil-Calderón, *Fermentation*, **2021**, 7, 171.
 [2] E. Barone, G. Ponticello, P. Giaramida, M. Squadrito, T. Fasciana, V. Gandolfo, F. Ardizzone, M. Monteleone, O. Corona, N. Francesca, D. Oliva, *Fermentation* **2021**, 7, 79.
 [3] S. Di Maio, G. Genna, V. Gandolfo, G. Amore, M. Ciaccio, D. Oliva, *South African Journal of Enology and Viticulture*, **2012**, 33, 80.

Metabolomics approach coupled with multivariate analysis of different parts of *Prosopis cineraria* (Ghaf) plant for evaluation of their antioxidant activity

Chiara Maria Giustra¹, Ciro Cannavacciuolo¹, Stefania Pagliari¹, Federico Cerri³,
Miriam Colombo¹, Massimo Labra¹, Luca Campone^{1,2}

¹Department of Biotechnology and Biosciences, University of Milano-Bicocca,
Piazza Della Scienza 2, 20126 Milan, Italy

²NBFC, National Biodiversity Future Center, 90133 Palermo, Italy

³Department of Earth and Environmental Sciences, University of Milano Bicocca, 20126 Milan, Italy
chiara.giustra@unimib.it

Nowadays noncommunicable diseases (NCDs), also known as chronic diseases, are the cause of the 74% global deaths each year. Among them, the main types of NCDs are those related to cardiovascular disorders, cancer, chronic respiratory diseases, and diabetes. In a word where the non-communicable diseases are even more increasing their incidence and where the medicine struggles to keep up with effective pharmaceutical treatments or these are too expensive, the prevention could play a key role [1]. The 21st century is characterized by climate change, temperature rises, drought and lack of water. These factors impact on agronomical production hindering both economical and human health related aspects. In this context the investigation of tropical and subtropical plants as potential sources of high added values products can play a key role. Here we propose a study on *Prosopis cineraria* (L.) Druce, commonly known as Ghaf, a tropical plant from the Fabaceae family (sub. Mimosaceae) able to grow in arid and semi-arid environments; in 2008, this tree has drawn attention for its various uses, and it was declared as the national tree of the United Arab Emirates (UAE) [2]. These characteristics, along with its documented therapeutic properties make it a promising matrix for multiple bioprospecting applications for human health purposes [2]. Metabolomic analysis is an innovative approach to investigate natural matrices, integrating complex data from chemical identification as mass spectrometry profiling and biological response. The present study aimed to carried out comparative chemical composition of different part of Ghaf, such as leaves, roots, twigs and bark. All matrices have been extracted with different solvents and the chemical composition determined by GC-MS and UHPLC-HRMS QToF. The obtained data were also combined for their *in-vitro* antioxidant activity by Multivariate Data Analysis to define a comprehensive fingerprint of the Ghaf and to identify the most promising bioactive components of this plant that could be used for the formulation of nutraceutical or pharmaceutical products. Preliminary results suggest that *P.cineraria* can be considered an interesting source of bioactive compounds useful in disease prevention and health promoting effects.

References

- [1] O.D. Rangel-Huerta, A. Gil, *International Journal of Molecular Sciences*, **2016**, *17*(12), 2072.
- [2] M. Giustra, F. Cerri, A.Yaprak, L.Salvioni, T.A. Abella, D. Prospero, P. Galli, M. Colombo, *Frontiers in Sustainability*, **2022**, *3*.
- [3] J. Sharifi-Rad, F. Kobarfard, A. Ata, S. A. Ayatollahi, N. Khosravi-Dehaghi, A.K. Jugran, M. Tomas, E. Capanoglu, K.R. Matthews, J. Popović-Djordjević, A. Kostić, S. Kamiloglu, F. Sharopov, M.I. Choudhary, N. Martins, *Biomolecules*, **2019**, *9*(12), 777.

Novel food: pasta containing cricket flour nutritional characterization and toxicological aspects by GC/MS

Giuseppe Avellone^{1,3}, Fabio D'Agostino², Silvia Orecchio¹, Claudia Lino³, Aldo Todaro⁴

¹Dipartimento STEBICEF, Università di Palermo,
Via Archirafi 32, 90123, Palermo

²Istituto per lo studio degli impatti Antropici e Sostenibilità in ambiente marino (IAS-CNR)
Via del Mare, 3 91021 - Campobello di Mazara, (TP)

³AteN Center, Università di Palermo,
Viale delle Scienze, 90128 Palermo

⁴Dipartimento SAAF, Università di Palermo,
Viale delle Scienze, 90100, Palermo

beppe.avellone@unipa.it

Novel foods and new food ingredients, initially governed by Community food legislation with Regulation (EC) 258/97 [1], are all those food products and substances for which significant consumption in the European Union cannot be demonstrated at the 15 May 1997, date of entry into force of the same regulation. The placing on the market of these foods must be authorized in advance. One of the novelties of the European Regulation (EU) 2283/2015 [2] relating to novel foods is that the request for authorization must be presented directly to the European Commission, following the guidelines published by EFSA which recently authorized the following regulation (EU) no. 2023/5 (Partially degreased powder of *Acheta domestica* - house cricket) [3]. The latter specifies that the quality of these new products will be rigorously monitored and must not be absolutely harmful to the health of the consumer. Food consumption of insects (entomophagy) could greatly limit some environmental damage. The advantages promoted by FAO in the use of insects in food are numerous. The date of entry was set at 1 January 2018 for the new requests and for all foods belonging to the categories "novel food" but to date there has been an interruption; probably given the lack of literature data concerning novel foods, finally today there has been a recovery and a marketing of cricket oak [3].

This study aims to determine the composition of pasta containing cricket flour, focusing on the content of mineral salts, fatty acids, amino acids and chitosan. While for the toxicological aspect considering the possible effects of accumulation from the environment or from the food administered, Petrol Hydrocarbon (TPH), linear chain hydrocarbons (C12-C40) and Polycyclic Aromatic Hydrocarbons (PAH) were evaluated.

References

- [1] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January **1997** concerning novel foods and novel food ingredients.
- [2] Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods **2015**.
- [3] Commission Implementing Regulation (EU) 2023/5 of 3 January **2023** authorising the placing on the market of *Acheta domestica* (house cricket) partially defatted powder as a novel food and amending Implementing Regulation (EU) 2017/2470.

Cardoon sprouts as source of bioactive compounds for nutrition and health

Valeria Toscano, Claudia Genovese, Giuseppe Diego Puglia,
Pietro Calderaro, Salvatore Antonino Raccuia

CNR - Institute for Agriculture and Forestry Systems in the Mediterranean
Via Empedocle, 58 - 95128 Catania, Italy
claudia.genovese@cnr.it

The increase of interest in the alimentary use of sprouts is mainly due to the great nutraceutical value that sprouts provide thanks to their high content of antioxidant compounds [1,2]. It is well-known that cardoon plants (*Cynara cardunculus* var. *altilis*) are rich in these compounds [3,4], mainly polyphenols, which make these species an important functional food, but the dietary consumption of their sprouts is still not spread. Moreover, *C. cardunculus* has developed mechanisms of resistance to salt stress, which determine a greater synthesis and accumulation of secondary metabolites [5,6].

The aim of this work was to produce domestic cardoon sprouts with a high content of biologically active compounds and to enhance their use as functional food in human consumption. Two different tests have been carried out: in the first one, the seeds were germinated on filter paper in Petri dishes; in the second one, the seeds were germinated on cotton wool in grids suspended on hydroponic solution. Both experiments have been conducted using three different salt stress conditions: 0.0 mM (control), 60 mM and 120 mM of NaCl. The fresh and dry weight yield, the total phenols and flavonoids content and the antioxidant activity have been determined from the sprouts obtained.

Overall, this study shows the possibility of increasing the content of bioactive compounds in cardoon sprouts by exploiting this plant's tolerance to salt stress and their potential use as healthy food for human consumption.

References

- [1] R.-Y. Gan, W.-Y. Lui, K. Wu, C.-L. Chan, S.-H. Dai, Z.-Q. Sui, H. Corke, *Trends in Food Science and Technology*, **2017**, 59, 1.
- [2] P. Paško, H. Bartoń, P. Zagrodzki, S. Gorinstein, M. Fołta, Z. Zachwieja, *Food Chemistry*, **2009**, 115 (3), 994.
- [3] M.I. Dias, L. Barros, J.C.M. Barreira, M.J. Alves, P. Barracosa, I. Ferreira, *Food Chemistry*, **2018**, 268, 196.
- [4] P.A.B. Ramos, S.A.O. Santos, A.R. Guerra, O. Guerreiro, C.S.R. Freire, S.M. Rocha, M.F. Duarte, A.J.D. Silvestre, *Industrial Crops and Products*, **2014**, 61, 460.
- [5] G. Pandino, S. Lombardo, G. Mauromicale, G. Williamson, *Food Chemistry*, **2011**, 126 (2), 417.
- [6] H. Pappalardo, V. Toscano, G. Puglia, C. Genovese, S.A. Raccuia, *Frontiers in Plant Science*, **2020**, 11:240, 1.

Nutraceutical characteristics of Sicilian cardoon honey

Claudia Genovese, Valeria Toscano, Pietro Calderaro,
Giuseppe Diego Puglia, Salvatore Antonio Raccuia

CNR - Institute for Agriculture and Forestry Systems in the Mediterranean
Via Empedocle, 58 - 95128 Catania, Italy
claudia.genovese@cnr.it

Honey has an important place in human nutrition since the earliest times, not only as a sweetener, but also as a high-quality food, with curative properties [1]. Many scientific works demonstrated the effect of honey treatments on health [2,3]. Moreover, since honey quality and chemical content reflect the source of nectar, honeys with different botanical origin would show different characteristics and nutraceutical properties [4]. Cardoon (*Cynara cardunculus* L.) is a Mediterranean plant and belongs to the Asteraceae family that includes three botanical taxa: the wild cardoon (*Cynara cardunculus* var. *sylvestris* Lam.), globe artichoke (*Cynara cardunculus* subsp. *scolymus* (L.) Hegi), and domesticated cardoon (*C. cardunculus* L. var. *altilis* DC) [5]. Cardoon is an important source of bioactive compounds with pharmaceutical potential, and it is extremely nutritive due to its low lipid content and high content of minerals, vitamins, and phenolic compounds, which are powerful natural antioxidant substances [6]. Recent study indicates *Cynara* extract as a natural product with antitumoral activity on malignant hematological disease [7]. Cardoon is a good honeyed species and during its flowering season, a characteristic honey is produced in some areas of Sicily and Sardinia.

The aim of this study was to evaluate the nutraceutical characteristics and antioxidant activity of cardoon honeys produced in Sicily. The following analyses were carried out: pH, sugars (fructose, glucose and saccharose) by HPAEC-PAD, minerals (Na^+ , K^+ , Mg^{2+} and Ca^{2+}) by suppressed ion chromatography, total phenolics and flavonoids content, phenolic composition by HPLC-UV/Vis and antioxidant capacity (DPPH, FRAP and ABTS assays).

The results showed that the analysed honeys had high amounts of potassium, a good total content of phenols and flavonoids and a good antioxidant capacity, which give the Sicilian cardoon honey a high nutraceutical value.

References

- [1] Z. Yaniv, M. Rudich, *Bee Products*, **1997**, 9, 77.
- [2] J.M. Alvarez-Suarez, S. Tulipani, S. Romandini, E. Bertoli, M. Battino, *Mediterranean Journal of Nutrition and Metabolism*, **2010**, 3, 15.
- [3] S. Samarghandian, T. Farkhondeh, F. Samini, *Pharmacognosy Research*, **2017**, 9(2), 121.
- [4] G. Di Marc, A. Gismondi, L. Panzanella, L. Canuti, S. Impei, D. Leonardi, A. Canini, *Journal of Food Science and Technology*, **2018**, 55(10), 4042.
- [5] H. Pappalardo, V. Toscano, G. Puglia, C. Genovese, S.A. Raccuia, *Frontiers in Plant Science*, **2020**, 11(240), 1.
- [6] L.R. Silva, T.A. Jacinto, P. Coutinho, *Foods*, **2022**, 11(3), 336.
- [7] C. Genovese, M.V. Brundo, V. Toscano, D. Tibullo, F. Puglisi, S.A. Raccuia, *Acta Horticulturae*, **2016**, 1147, 113.

Oven-dried olive pomace: changes in secondary metabolite, lipids and carbohydrates levels at different temperatures

Francesca Loschi¹, Nicola De Zordi², Stefano Dall'Acqua¹, Stefania Sut¹

¹Department of Pharmaceutical and Pharmacological Sciences, University of Padova
Via Marzolo 5, 35131 Padova, Italy

²Società Agricola Moldoi—S.A.M, SrL, Loc. Maras Moldoi 151/a, Sospirolo, 32037, Italy
francesca.loschi@unipd.it

Olive pomace, the solid by-product obtained from olive oil milling process, can be considered a source of antioxidant and health ingredients and, its stabilization is a great challenge for the olive oil industry due its easily degradation. This work is part of a project founded by Veneto Region, OLIVARE, focused on the reuse of by-products of olive milling. Olive milling produce high amount of waste as olive pomace that can contain different amounts of water on the basis of the milling process applied. Up to now several projects have considered the olive pomace as starting materials for the extraction of valuable secondary metabolites or bioactive compounds as tyrosol, oleuropein and unsaturated fatty acids. In this poster the drying process applied on olive pomace was considered as strategy to add value to a by-product and obtain valuable material with significant nutritional properties and antioxidant activity.

The olive pomace obtained from the two-phase olive oil mill, WOP (Wet Olive Pomace) with a moisture content above 70 % and from the three-phase olive oil mill, SOP (Solid Olive Pomace) with a moisture content below than 60 % were considered.

Materials were dried in a thermostatic oven in three different conditions, 50° for 48 h, 75 ° for 24 h and 100 °C for 12 h to obtain a product with a moisture content below 1 %. The effect of the drying process at different temperatures were evaluated considering secondary metabolites, lipids and carbohydrates. Dried pomace were characterized by LC-DAD-MS for polyphenols, secoiridoids, by GC-MS for fatty acids, by LC-ELSD for monosaccharides. Furthermore, total dietary fibre (TDF) and Folin – Ciocalteu assays were used for the determination of fibers and the total phenolic compound respectively.

Analyses showed limited differences in bioactive and nutrients content at the different oven-drying conditions. Monosaccharides content have been detected in WOP but were not detectable in SOP being the most evident difference. A stable and storable dried material was obtained from the two pomaces at each used temperature. All the dried samples presented valuable nutritional profile with 17-21% of lipids (on basis of dried weight), with high percent of oleic acid as expected. Protein content ranged from 5-10% (on the basis of dried weight) while TDF ranged from 45-66%. Results showed that the temperature in the considered range is significantly changing the content of measured metabolites.

In conclusion the olive pomace baked at 100°C could be a suitable health promoting material with possible use in feed, nutraceutical and cosmetic applications.

Sensory and physicochemical profile of different cultivar of *Mangifera indica* L. cultivated in Calabria (South Italy)

Giovanni Manuel Brancati¹, Giuseppe Bruni Pagnotta¹, Fabrizio Crea¹, Francesco Ioppolo¹, Giulia Marrapodi¹, Martina Pizzimenti¹, Davide Puntorieri¹, Davide Ritorto¹, Domenico Siclari¹, Valeria Torino¹, Matteo de Blasio², Rosa Di Sanzo³, Sonia Carabetta³, Mariateresa Russo³

¹Food science and technology LM students, Department Agraria, Mediterranean University of Reggio Calabria, Italy

²Food science and technology L student, Department Agraria, Mediterranean University of Reggio Calabria, Italy and “Azienda Agricola Domenica Scopelliti”; ³Food Chemistry, Authentication, Safety and Sensoromic Laboratory - FoCuSS Lab, Department Agraria, Mediterranean University of Reggio Calabria, Italy

mariateresa.russo@unirc.it

Mangifera indica (mango) is one of the most widely traded fruits in the world, occupying the second position as a tropical crop due to the wide variety of cultivars that exist. Mango has an attractive flavor and fragrance and high nutritional value [1,2]. The objective of this study was to determine the physicochemical characteristics and sensory profile of five mango cultivars (Kent, Kensington, R2E2, Haden and Tommy Atkins) grown in southern Calabria (Reggio Calabria, Catona). The physicochemical analyses carried out included: pH, titratable acidity, soluble solids (°Brix) and ratio (Brix/titratable acidity), The sensory profile was studied through the volatile profile. The total phenolic content (TPC) of the lyophilized pulp extracts was analyzed using the Folin-Ciocalteu reagent and a specific UHPLC-UV-VIS method was developed for polyphenolic fingerprinting. The study of the antioxidant activity (AA) was carried out on the aqueous-methanolic extracts of the lyophilisates, through the release of free radicals and reducing power (IC50). Finally, the profiling of the pulp aroma was carried out by SPME-GC/MS. The detected pH was within a range of 3.82 (*Kent*) and 4.99 (*Kensington Pride*). Total soluble solids were in a range between 15.4 °Bx (*Kensington Pride*) and 21 °Bx (*Tommy Atkins*). The relative moisture content of the 5 varieties analyzed ranges between 78.64% (*Kent*) and 83.90% (*Kensington Pride*). The cv *Kensington pride* showed the lowest ash content (0.35%), while the cv *Tommy Atkins* the highest (0.52%); titratable acidity ranges from 0.35% *Kensington Pride* to 1.75% *Kent*, the cv *Kensington* and *Tommy Atkins* were found to be the mango varieties with, respectively, the least and most reducing sugars and total carbohydrates. In detail, *Kensington Pride* has 0.39% reducing sugars and 7.38% total carbohydrates, while cv *Tommy atkins* has 1.04% and 8.78%. The fat content ranges from 0.02% of the cv *Kensington Pride*, to 0.19% of the cv *Tommy Atkins*, while the protein is between 0.7% of the *Kensington Pride* and 1.4% of the *Tommy Atkins*. From the TPC analysis it emerged that the cv *Kensington Pride* is the one with the highest percentage of polyphenols while the cv *Tommy Atkins* the lowest. The radical scavenger activity IC 50 varies from 91.78 g/L in cv *Haden*, to 154.85 g/L in *R2E2*. Aroma profiling of the pulps was carried out by SPME-GC/MS. The major compound of cv *Kensington*, *R2E2* and *Tommy Atkins* was 4-carene; 3-carene was the principal compound in the other three cultivars. *Tommy Atkins* is also characterized by a high amount of D-limonene (25%). Total data were processed by XLSstat software (19.01). PCA analysis showed similarity between cv *Kent* and *Haden*, different from cv *Tommy Atkins*. Being cv *R2E2*, a hybrid of *Kent* and *Kensington*, a further group, represented by cv *Kensington* and *R2E2*, was discriminated.

References

- [1] S. Agatonovic-Kustrin, E. Kustrin, D.W. Morton, *South African Journal of Botany*, **2018**, *116*, 158.
 [2] L. Veeranjanya Reddy, W. Young-Jung, Y. Weibing, *International journal of environmental research and public health*, **2021**, *18*, 741.

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Metabolite profiling of cv *Kensington pride* mango (*Mangifera indica* L.) fruits cultivated in Catona (Calabria, Reggio Calabria) during the ripening stages

Giovanni Manuel Brancati¹, Giuseppe Bruni Pagnotta¹, Fabrizio Crea¹, Francesco Ioppolo¹, Giulia Marrapodi¹, Martina Pizzimenti¹, Davide Puntorieri¹, Davide Ritorto¹, Domenico Siclari¹, Valeria Torino¹, Matteo de Blasio², Rosa Di Sanzo³, Sonia Carabetta³, Mariateresa Russo³

¹Food science and technology LM students, Department Agraria, Mediterranean University of Reggio Calabria, Italy

²Food science and technology L student, Department Agraria, Mediterranean University of Reggio Calabria, Italy and “Azienda Agricola Domenica Scopelliti”; ³Food Chemistry, Authentication, Safety and Sensoromic Laboratory - FoCuSS Lab, Department Agraria, Mediterranean University of Reggio Calabria, Italy

mariateresa.russo@unirc.it

Mango is one of the most commercialized fruits around the world and it has a high nutritional value an attractive taste and flavour [1].

As known, the ripening of fruit is a genetically programmed event characterized by a series of biochemical and physiological processes which modify the colour, aroma, flavour, consistency and nutritional value. The mango ripening process also involves a series of physiological, biochemical and organoleptic changes [2]. The effect of the compositional changes with the organoleptic implications induced by ripening were studied on mangoes of the cv *Kensington pride* grown in Calabria (Reggio Calabria, Catona locality) and harvested at the three stages of ripening: ripe, semi-ripe and unripe. The evolution of the physico-chemical parameters and the volatile profile was studied. The total phenolic content (TPC) of the freeze-dried pulp extracts was analyzed using the Folin-Ciocalteu reagent and tests were conducted on the aqueous-methanolic extracts of the freeze-dried pulp for antioxidant activity (AA) via free radical release and power reducing agent (IC₅₀). Polyphenolic fingerprinting was obtained via UHPLC-UV-VIS. The aroma profiling of the pulps was carried out using SPME-GC-MS. The pH and TPC analysis showed a progressive increase, from unripe to mature samples as well as the IC₅₀ radical scavenger activity ranging from 65.33 g/L to 79.05 g/L. The acidity decreased dramatically. The protein content was higher in medium ripe fruit (1.1%) and lower in ripe fruit (0.5%). Reducing sugars ranged from 1.12% in unripe mango to 1.87% in ripe mango, while soluble solids ranged from 15.45% to 14.62%. The results indicated that during maturation, reducing sugar content and pH tend to increase, while titratable acidity, ash, protein and fat decrease. During maturing, the aromatic profile intensifies.

References

- [1] L. Veeranjanya Reddy, W. Young-Jung, Y. Weibing, *International journal of environmental research and public health*, **2021**, *18*, 741.
 [2] S. Malkeet Padda, V.T. Cassandro do Amarante, M.R. Garcia, C. D. Slaughter, J. Elizabeth, *Postharvest Biology and Technology*, **2011**, *62*, 267.

Acknowledgments

This work was supported by FFM (Innovation Pole - Future Food Med), and by the by Research InfraStructure Diata Lifestyle. We thank Italian Society of Food Chemistry (ItaChemFood) for the sponsorship.

We also, thanks the “Azienda Agricola Domenica Scopelliti” which supplied the mango samples, and for the support in the selection, sampling and treatment of plant material. The students’ names follow the alphabetical order.

Evaluation of antioxidant and protective activities on human serum albumin of gallotannin

Giuseppe Tancredi Patanè, Maria Chiara Violante, Antonella Calderaro, Stefano Putaggio,
Carlo Maffei, Ester Tellone, Silvana Ficarra, Davide Barreca, Giuseppina Laganà

Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy
vlnmch98l41f112j@studenti.unime.it

Gallotannin is a type of polyphenol belonging to the sub-class of tannin, that is found in many foods such as red wine, dark chocolate, berries, and nuts [1]. Gallotannin has been shown to have beneficial effects on health in several studies, including the ability to reduce inflammation, prevent oxidative stress, and improve gastrointestinal health [2]. Gallotannin has also been studied for its antimicrobial and antibacterial properties, making it a potential therapeutic agent against infections [3]. Its chemical structure is characterized by the presence of a molecule of gallic acid that is bound to five glucose molecules through ester bonds. Starting from experimental evidence of interesting biological properties of gallotannin, we have decided to test the antioxidant potential of the molecule. The obtained results have showed the potentiality of gallotannin, in the range of 4.0-0.1 μM against some radicals in ABTS, FRAP and ORAC assays. Then, we tested the total antioxidant activity of gallotannin by relating it to the antioxidant activity of Trolox. The data showed that 1 μM of gallotannin is equivalent to 4.88 μM microequivalents of Trolox. The titration data, between a known solution of 10 μM gallotannin and increasing concentrations of human serum albumin (0-10 μM), showed that these two substances bind in a 1:1 ratio. The interaction between the two molecules has been analysed by UV-visible differential spectroscopy highlighting changes in the maximum of absorbance at around 278 nm. So, we have decided to test the antioxidant potential of the molecule on human serum albumin under stress conditions, reproducing *in vitro* some radicals, such as the superoxide anion and the hydroxyl radical. Gallotannin can protect HSA against hydroxyl radical, but not against superoxide anion. The obtained results showed interesting biological potentials of gallotannin, that makes it useful as nutraceutical for the fortification of food to increase its nutritional quality and increase its the shelf-life.

References

- [1] M. raga-Corral, P. García-Oliveira, A.G. Pereira, C. Lourenço-Lopes, C. Jimenez-Lopez, M.A. Prieto, J. Simal-Gandara, *Molecules*, **2020**, 25, 614.
- [2] H.F. He, *Frontiers in Nutrition*, **2022**, 9, 888892.
- [3] R. A. Youness, R. Kamel, N. A. Elkasabgy, P. Shao, M.A. Farag, *Molecules*, **2021**, 26, 1486.

Effect of olive cake on the mineral profile of Ragusan provola cheese

Federica Litrenta^{1,2}, Luigi Liotta², Vincenzo Lopreiato², Annalisa Amato²,
Angela Giorgia Potortì¹, Giuseppa Di Bella¹

¹Department of Biomedical, Dental and Morphological and Functional Imaging Sciences (BIOMORF),
University of Messina, Viale Palatucci, 13, 98168 Messina, Italy

²Department of Veterinary Sciences, University of Messina,
Viale Palatucci, 13, 98168 Messina, Italy

felitrenta@unime.it

The animal diet is an effective way to improve the mineral profile of dairy products and, consequently, human health [1]. Olive cake has attracted the attention of the scientific community due to its high concentration of macro- and micro-nutrients [2,3], including minerals, which allows it to be used as a mineral supplement in the diets of farm animals. The aim of the present study was to evaluate the effects of the mineral content of Provola Ragusana cheese obtained from dairy cows fed with dried and pitted olive cake.

The experiment was carried out during the period from March 2021 to July 2021 on 460 healthy multiparous dairy cows divided into two homogeneous groups named Control and Biotrak. The cows were farmed in a commercial dairy farm located in Ragusa (Sicily, Italy). The Biotrak group received a concentrate supplemented with 8% pitted olive cake (OC) of the drattile matter dose; the Control group received a concentrate with no olive cake supplementation. Four representative Provolas samples were analysed monthly for each group. Twenty elements (Ca, Na, K, Mg, Zn, Ti, Sr, Fe, Ni, Ba, Cr, Mn, Cu, Se, Cd, Mo, B, V, As and Pb) were determined by single quadrupole inductively coupled plasma mass spectrometry (ICP-MS). The results evaluated by consistent statistical analysis (ANOVA and Tukey's test) and principal component analysis (PCA) showed that the Provola cheeses from the Biotrak group had higher values for almost all the essential elements analysed. The Se content in the Biotrak Provolas was of particular relevance, as it was found to be almost twice as high as in the control Provolas, covering on average 17.18% of the Recommended Dietary Allowance (RDA). Also, the intake of Ca, Na, K, Fe, Zn, Mn, Cu, Se and Mo was higher in Biotrak, than Control Provolas thus indicating greater nutritional benefits for the human diet. In addition, Pb, As and Hg were not quantified in both Provolas Biotrak and Provolas Control, while Cd and Ni were found at very low levels. Exposure to the toxic elements Cd and Ni is not of concern compared to Tolerable Daily Intake (TDI) and Tolerable Weekly Intake (TWI) of the respective metals. The PCA of the 40 provola samples showed that the two groups are clearly distinguishable: the Biotrak provolas were separated from the Control provolas on the first component (PC1), which explains 55.837% of the total variance.

It can be concluded that the consumption of these Provolas has no negative effects on consumer health, confirming that the use of unconventional feeds in animal nutrition could be a sustainable and cost-effective way to improve the mineral profile of dairy products.

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References

- [1] E. Molina-Alcaide, D.R. Yáñez-Ruiz, *Animal Feed Science and Technology*, **2008**, 147, 247.
- [2] S. Hachicha, F. Sallemi, K. Medhioub, R. Hachicha, E. Ammar, *Waste Management*, **2008**, 28, 2593.
- [3] M. Ibourki, S. Gharby, D. Guillaume, A. Lakinfli, A. El Hammadi, Z. Charrouf, *Chemical Data Collections* **2021**, 35, 100772.

Evaluation of mercury content in processed meat and fish products of different geographical origins

Vincenzo Nava¹, Federica Litrenta¹, Antonio Cambria¹, Giovanni Bartolomeo², Rossana Rando¹, Angela Giorgia Potortì¹, Vincenzo Lo Turco¹, Giuseppa Di Bella¹

¹Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF), University of Messina, Viale Annunziata, 98122 Messina, Italy

²Science4life S.r.l., start-up of University of Messina, Messina, Italy
vnav@unime.it

Processed foods are now an integral part of the food system [1]. Processing makes it possible to extend the shelf life of a product while maintaining its quality. However, it can also be a source of contamination of various kinds (chemical, process, environmental) [1,2].

There are many different varieties of processed food, but the main food matrices that are processed are certainly meat and fish, which are important in the diet as a source of protein and other nutrients. It is therefore important to monitor these processed products to safeguard consumer health [2,3].

This study focused on the monitoring of Hg levels in various processed food products (beef, pork, poultry, shellfish, and fish) of EU and non-EU origin purchased in supermarkets and ethnic food shops in Messina (Italy). A direct mercury analyzer (DMA-80) was used to determine the mercury content. The results showed variable mercury levels depending on the type of food analyzed and the different fat contents.

Aquatic products had higher Hg levels than land-based products (9.249–290.211 µg/Kg vs <LOQ-3.727 µg/Kg). Among the aquatic species tested, the average mercury content increased in the following order: crab < shrimps < salmon < mackerel < sardines < tuna. However, all samples were within European legislative limits (Regulation No 915/2023).

In addition, estimated weekly intake values of Hg were calculated according to EFSA guidelines for each species analyzed and in relation to different dietary habits. The tolerable weekly intake (TWI) was fixed by EFSA in 2012 at 4 µg/kg body weight/week [4]. The TWI percentages were obtained for a 70 Kg adult using FAOSTAT data on average weekly consumptions of both fish and meat products in different countries of the world (Africa, America, Asia, Europe, Oceania). These percentages related to the consumption of land-based products range from n.d. to 5.78, whereas those related to aquatic products range from n.d. to 108.83%. More specifically, there was only one case in which a TWI percentage value of more than 100% was obtained: this was the case for the canned tuna in olive oil (*Katsuwonus pelamis*) sample when the average consumption was equal to that of the Oceania region.

In addition, an assessment was made using an average consumption of 200 g of each processed product. In this case, the mercury TWI was exceeded in only two fish samples of the genus *Thunnus thynnus*.

However, it is necessary to continue to monitor the processed food categories in order to protect the health of consumers. This is due to the high consumption of these products.

References

- [1] V. Nava, G. Di Bella, F. Fazio, A.G. Potortì, V. Lo Turco, P. Licata, *Applied Sciences*, **2023**, *13*, 793.
- [2] G. Kowalska, U. Pankiewicz, R. Kowalski, *Journal of Analytical Methods in Chemistry*, **2020**, 2148794.
- [3] S. Collado-Lopez, L. Betanzos-Robledo, M.M. Tellez-Rojo, H. Lamadrid-Figueroa, M. Reyes, C. Rios, A. Cantoral, *International Journal of Environmental Research and Public Health*, **2022**, *19*, 8651.
- [4] EFSA Journal, **2012**, *10*, 2985.

P100

The authenticity of honey obtained from Sicilian black bees by multi-element analysis with a chemometric approach

Angela Giorgia Potortì, Maria Aurora Arrigo, Antonio Cambria, Rossana Rando, Laura Messina, Vincenzo Lo Turco, Giuseppa Di Bella

Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy
agpotorti@unime.it

Honey is the result of many factors, and its composition and properties can vary greatly depending on the type of flowers and plants, the environment in which the plants grow, and even the insect itself.

The Sicilian black honeybee (*Apis mellifera* ssp. *sicula*) is a subspecies of the common honeybees (*Apis mellifera* ssp. *ligustica*) from which it can be distinguished by its darker color and its smaller wings [1]. Honey produced by black bees is a product with high therapeutic potential due to its content of antioxidants and antimicrobial agents against multi-resistant strains [2-3].

In this study, 18 samples of *Apis mellifera* ssp. *sicula* honey produced in the area of Sant'Agata di Militello (Messina, Sicily, Italy) from three different botanical origins (chestnut, orange-blossom, and wildflower) together with 16 samples of *Apis mellifera* ssp. *ligustica* honey from the same botanical and geographical origins were analyzed for their elemental (K, Ca, Na, Mg, Fe, Zn, Mn, Cu, Se, Pb, Ni, Cr, Cd and As) contents using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

Mann-Whitney U and Kruskal-Wallis tests, followed by Principal Component Analysis (PCA), were applied to the results obtained in order to compare the honey varieties produced by Sicilian black bees with those of the common bees according to their elemental content.

The results of the significance of the differences between the honeys of black and common bees showed that the *p*-level was less than 0.05 for K, Ca, Mg, Zn, Mn, Cu, Se and Cr: the honeys of black bees were characterized by a higher level of Zn, Cr and K. The discrimination between these honeys was achieved by PCA.

Subsequently, the results of the black bee honey samples were checked to evaluate significant differences among honeys from different botanical origins. The results for all the elements analyzed, except for Ca, Na, Mn and As, showed a *p* level lower than 0.05: chestnut honey has the highest content of K and Zn, while orange blossom has Mg and Fe, while wildflower has Cu, Ni and Pb. Again, PCA was able to discriminate among the samples. No significant differences among samples of different botanical origin were found in the analogous evaluation of honey samples from common bees.

The proposed study seems to offer the possibility of guaranteeing the authenticity of honey obtained from the Sicilian black bee.

References

- [1] P. Franck, L. Garnery, G. Celebrano, M. Solignac, J.M. Cornuet, *Molecular Ecology*, **2000**, 9, 907.
- [2] G.C. Tenore, A. Ritieni, P. Campiglia, E. Novellino, *Food and chemical toxicology*, **2012**, 50, 1955.
- [3] G.M. Lo Dico, A. Ulrici, A. Pulvirenti, G. Cammilleri, A. Macaluso, A. Vella, V. Giaccone, G. Lo Cascio, S. Graci, M. Scuto, A. Trovato Salinaro, V. Calabrese, R. Lo Dico, V. Ferrantelli, *Journal of Food Composition and Analysis*, **2019**, 82, 103225.

Effect of the filtration technology on the oxidative stability and bioactive compounds of the cold-pressed hempseed oil

Vincenzo Lo Turco, Ambrogina Albergamo, Federica Litrenta, Michelangelo Leonardi, Angela Giorgia Potorti, Giuseppa Di Bella

Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy
vloturco@unime.it

The cold-pressed hempseed oil (HO) successfully fits with the growing consumer interest in those natural cold-pressed oils which are often considered as functional foods, for the various health benefits provided over the refined counterparts [1,2].

However, in addition to antioxidants effective not only toward consumer health but also against the oxidative deterioration of the oil, HO contains also pro-oxidants, including a large content of polyunsaturated fatty acids (PUFAs) and photosensitizer chlorophylls [3], which inevitably accelerate its oxidative deterioration, especially in presence of light. In this scenario, the filtration technology may ameliorate the oxidative stability of HO, with positive effects on its shelf-life. Additionally, filtration may be an effective and sustainable alternative to the severe refining procedure, in view of keeping the good nutritional standards typical of cold-pressed oils [4,5]. Within this context, aim of the study was to monitor the oxidative stability and minor compounds of non-filtered and filtered HO (NF-HO and F-HO) over 12 weeks of storage in transparent glass bottles.

Filtration significantly ameliorated the hydrolytic and oxidative status of HO during storage. In fact, F-HO showed free fatty acids, peroxides and extinction coefficients K_{232} and K_{270} more stable than NF-HO and always within the guide values set by the Codex Alimentarius Standard 210-1999. As a result, F-HO better preserved the level of MUFAs, such as C18:1n-9, and PUFAs, such as C18:2 n-6, C18:3 n-6 and C18:3 n-3, than NF-HO from the autoxidation process, over the entire study period. Filtration consistently reduced chlorophylls, thus, producing a lighter and brighter colored oil. Accordingly, F-HO not only revealed an increased resistance to photooxidation but was also suitable for storage in clear bottles within 12 weeks, with a positive impact on consumer acceptance. F-HO predictably showed lower contents of carotenoids, tocopherols, polyphenols, and squalene compared to NF-HO. However, filtration appeared to play a “protective role” for these antioxidants, which had lower degradation rates in F-HO than in NF-HO during storage.

Overall, findings from this study may be of practical use to both producers and marketers of cold-pressed HO.

References

- [1] N. Cicero, A. Albergamo, A. Salvo, G.D. Bua, G. Bartolomeo, V. Mangano, A. Rotondo, V. Di Stefano, G. Di Bella, G. Dugo, *Food Research International*, **2018**, *109*, 517.
- [2] A. Albergamo, R. Costa, G. Dugo, Cold Pressed Oils, **2020**, *Academic Press*, 159.
- [3] B. Matthäus, L. Brühl, *European Journal of Lipid Science and Technology*, **2008**, *110*, 655.
- [4] J. Liang, A.A. Aachary, A. Hydamaka, N. M. Eskin, P.Eck, U.Thiyam-Holländer, *European Journal of Lipid Science and Technology*, **2018**, *120*, 1700349.
- [5] CODEX-STAN 210-1999.

Valorization of products and by-products from the Tunisian *Opuntia ficus-indica* (L.) Mill.

Ambrogina Albergamo¹, Hedi Ben Mansour², Nawres Ben Amour², Asma Beltifa²,
Laura Messina³, Giovanni Bartolomeo⁴, Giuseppa Di Bella¹,
Angela Giorgia Potortì¹, Vincenzo Lo Turco¹

¹Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF)
University of Messina, Viale G. Palatucci, 98168 Messina, Italy

²National Institute of Applied Sciences and Technology (INSAT), University of Carthage, Carthage 1054, Tunisia

³Department of Veterinary Sciences (SCIVET), University of Messina, Viale Annunziata, Messina, 98100, Italy

⁴Science4life S.r.l., start-up of University of Messina, Messina, Italy

aalbergamo@unime.it

Opuntia ficus-indica (L.) Mill., commonly known as the prickly pear cactus, is native to Mexico and has subsequently propagated in Latin Americas, South Africa, and the Mediterranean area, where many ecotypes, cultivars, and clones of the species have been established especially in Tunisia. In this country, 600,000 ha of *O. ficus-indica* have been planted in the last 50 years, and the cactus species has proved to be an important food and forage crop principally in the central and southern regions [1]. Alongside the seasonal and profitable production of tasty fruits (i.e., prickly pears) [2,3], however, conspicuous agricultural and processing waste, such as nopal (cladodes), fruit peels, and seeds, may impair the sustainability and eco-efficiency of the supply chain of *Opuntia* [4]. In line with most Sustainable Development Goals (SDGs), the optimal and responsible usage of agricultural and food resources for purposes leading to an effective transition to a circular economy is highly desired [5]. Within this context, aim of this study was to chemically characterize various products and by-products from the Tunisian *O. ficus-indica*, to make the most of its potential in the Mediterranean supply chains. Specifically, nopal and pulp, peel, and seeds of fruits were thoroughly elucidated for their proximate composition, fatty acid (FA) composition, inorganic elements, sugars, and polyphenols by well-established analytical approaches.

Nopal and prickly pear peel and seeds were abundant in fiber (respectively, 28.39, 12.54, and 16.28%). Seeds had also high protein (17.34%) and may be source of an edible oil, due to lipids (9.65%) poor in saturated FAs (14.12%) and rich in linoleic acid (61.11%). Nopal and peel showed the highest levels of Mg (493.57 and 345.19 mg/100 g), K (6949.57 and 1820.83 mg/100 g), Mn (59.73 and 46.86 mg/Kg) and Fe (23.15 and 15.23 mg/Kg), while the fruit pulp was predominantly constituted of sugars, glucose and arabinose being predominant (42.57 and 13.56 g/100 g). Total polyphenols widely varied among the *Opuntia* products (108.36–4785.36 mg GAE/100 g), being mainly represented by hydroxycinnamic and hydroxybenzoic acids, and flavonoids as well. In particular, peel may be re-valorized for these valuable bioactives, including 4-hydroxybenzoic acid (484.95 mg/100 g), cinnamic acid (318.95 mg/100 g), rutin (818.94 mg/100 g), quercetin (605.28 mg/100 g), and several isorhamnetin and kaempferol glycosides. Overall, this study highlights the peculiarity of the Tunisian *Opuntia* in terms of nutritional composition and phytochemicals, thus, providing a solid background to further elucidate its technological, functional and health attributes and, consequently, research new potential applications.

References

- [1] A. Nefzaoui, H. Ben Salem, *FAO Plant Production and Protection Paper*, **2001**.
- [2] A. Albergamo, A.F. Mottese, G.D. Bua, F. Caridi, G. Sabatino, G. Dugo, *Journal of Food Science*, **2018**, *83*, 2933.
- [3] A. Albergamo, G. Bartolomeo, L. Messina, R. Rando, G. Di Bella, *Opuntia spp.: Chemistry, Bioactivity and Industrial Applications*, Springer (Germania), **2021**, 457.
- [4] G. Di Bella, G. Lo Vecchio, A. Albergamo, V. Nava, G. Bartolomeo, A. Macrì, L. Bacchetta, V. Lo Turco, A.G. Potortì, *Journal of Food Composition and Analysis*, **2022**, *106*, 104307.
- [5] P. Ghisellini, C. Cialani, S. Ulgiati, *Journal of Cleaner Production*, **2016**, *114*, 11.

Preliminary chemical characterization of the Moroccan carob pods

Yasmine Mttougui Ben Amar¹, Giuseppa Di Bella², Vincenzo Lo Turco², Rossana Rando²,
Mouad Lahkim Bennani³, Jamal Brigui⁴, Nour-Eddine Chouaibi¹

¹Research Team: Materials, Environment, and Sustainable Development (MEDD), Faculty of Sciences and Techniques of Tangier (FSTT), Abdelmalek Essaâdi University, Tangier BP 416, Morocco

²Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF) University of Messina, Viale G. Palatucci, 98168 Messina, Italy

³Research Team of Biotechnology and Biomolecular Engineering (ERBGB), Faculty of Sciences and Techniques of Tangier (FSTT), Abdelmalek Essaâdi University, Tangier BP 416, Morocco

⁴Laboratory of Valorization of Resources and Chemical Engineering, Faculty of Sciences and Techniques of Tangier (FSTT), Abdelmalek Essaâdi University, Tangier BP 416, Morocco
vloturco@unime.it

The carob tree (*Ceratonia siliqua*) is a plant species of great value owing due to its numerous potential applications in medicine and food. Recent research has focused on characterizing the chemical composition of carob to gain insight into its health benefits and to uncover new opportunities for the plant. The chemical composition of carob varies depending on various factors such as genetic variability, environmental conditions, cultivars, cultivation practices, geographical origin and harvest time [1-3].

The aim of this study is to determine the initial chemical composition of mature ecotype carob pods collected from six different regions of Morocco during the period from July to October 2022. The carob fruit was manually separated into pulp, seeds, and total (pod). Samples were dried in an oven at 105°C for 24 hours and then ground (less than 0.5 mm in diameter) using a hand mill. Ash content was determined by AOAC official method 972.15; total crude protein by the Kjeldahl method; lipids content by the Folch method; dietary fiber content by AOAC official method 991.43; total sugars content by phenol-sulfuric acid method. Tocopherols were evaluated by high-performance liquid chromatography coupled to a fluorescence detector (HPLC-FD).

Overall, ash content ranged from 2.5% to 4%, lipids from 1.2% to 3.8%, and tocopherols from 8.7 mg/100 g of fat to 74.2 mg/100 g of fat. Furthermore, the pods were found to have the highest sugar content, whereas the seeds contained up to 40% fiber. Protein content ranged from 2% to 5% in the pulp and from 11% to 17% in the seeds.

This study provides valuable information about the chemical composition of carob and highlights the importance of further research in this area to fully realize the potential of this plant.

References

- [1] S.M. Nasar-Abbas, Z.-e-Huma, T.-H. Vu, M. K. Khan, H. Esbenshade, V. Jayasena, *Comprehensive Reviews in Food Science and Food Safety*, **2015**, *15(1)*, 63.
- [2] L. Iipumbu, *Thesis (Msc Food Sc (Food Science), Stellenbosch University*, **2008**, 3.
- [3] A. Richane, H.B. Ismail, C. Darej, K. Attia, N. Moujahed, *Tropical Animal Health and Production*, **2022**, *54*, 58.

Plant-based drinks as milk substitutes: mineral elements intake and fatty acids profile

Benedetta Sgrò¹, Vincenzo Nava¹, Angela Giorgia Potortì¹, Vincenzo Lo Turco¹,
Miriam Porretti², Giuseppa Di Bella¹

¹Department of Biomedical, Dental Sciences and of Morphological and Functional Images (BIOMORF),
University of Messina, 98168 Messina, Italy

²Department of Chemical, Biological, Pharmaceutical and Environmental Sciences,
University of Messina, 98166 Messina, Italy
benedetta.sgro@studenti.unime.it

In the last decade, cow's milk is often replaced plant-based drinks for different reasons as lactose intolerance, cow's milk proteins allergy, vegan lifestyle and other health and environmental reasons [1]. "Vegetal milks" are lactose and cholesterol free, the amount of protein is often limited, and nutrients found in milk, such as calcium, are lacking [2]. Plant-based drinks don't have a quality standard, the nutritional composition is different from milk and can change among different beverages, even within the same group [3]. The beverages composition in fatty acids (FA) and micro- and macro-elements depends on plants, production process, agricultural technologies, geological sources, and industrial and environmental pollution [4]. In this work, FA profile and micro- and macro-elements content were evaluated in 12 different types of plant-based drinks (walnut, almond, soy, rice, oat, rice and coconut, buckwheat, rice and walnut, sorghum, spelt, millet and coconut) available on the Italian market in order to characterize the beverages from a health and toxicological point of view. FA analysis was carried out by gas-chromatography coupled to flame ionization detector (GC-FID). Na, Mg, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, Cr, Al, B, Se, Mo, As, Ba, Li, Ag, V, Sb, Cd and Pb analysis was performed using inductively coupled plasma mass spectrometry (ICP-MS) and Hg content was determined through direct mercury analyzer (DMA-80). Following the quantification of the mineral elements, The Estimated Daily Intakes (EDIs) of minerals related to the consumption of one cup (200 mL) of these beverages were compared with the available values of Recommended Dietary Allowance (RDA), Reference Intake for Population (PRI) or Adequate Intake (AI) for essential elements and Tolerable Daily Intake (TDI), Tolerable Weekly Intake (TWI), Benchmark Dose Lower Confidence Limit (BMDL01), Acceptable Daily Intake (ADI), Reference Dose (RfD) or Tolerable Upper Intake Level (UL) for potentially toxic elements. The results, expressed as a percentage of these values, showed that the beverages are safe for adult consumers in terms of toxic and potentially toxic elements. Only the soy drink exceeded the RDA for molybdenum of 68%. FA profile of plant-based drinks was compared with FA profile of whole cow's milk in order to show differences and similarities. Plant-based drinks show less saturated FA, except for lauric acid (C12:0) which value depends on coconut drink and rice & coconut drink. The amount of saturated FA in whole bovine milk is more than 60%, while in plant-based drinks is less than 30% of total FA. The amount of monounsaturated and polyunsaturated FA in plant-based drinks is higher than whole bovine milk. In plant based-drinks, oleic acid (C18:1) and linoleic acid (C18:2 n-6) are the main source of FA with average value of 35.47% and 33.16%, respectively. In whole bovine milk, palmitic acid (C16:0) and oleic acid are the main source of FA with average value of 30.48% and 18.93%, respectively. In conclusion, the exposure to mineral elements through the daily consumption of one cup of plant-based drinks is not worrisome. In addition, plant-based drinks are a good source of unsaturated fatty acids, and their consumption would contribute to achieving the required intake.

References

- [1] D. Angelino, A. Rosi, G. Vici, M. Dello Russo, N. Pellegrini, D. Martini, SINU Young Working Group, *Foods*, **2020**, 9(5), 682.
- [2] P.E. Muneke, R. Domínguez, S. Budaraju, E. Roselló-Soto, F.J. Barba, K. Mallikarjunan, S. Roohinejad, J. M. Lorenzo, *Foods* **2020**, 9(3), 288.
- [3] A. Drewnowski, C.J. Henry, J.T. Dwyer, *Frontiers in Nutrition*, **2021**, 8, 796.
- [4] N. Manousi, G.A. Zachariadis, *Food Analytical Methods*, **2021**, 14, 1315.

Compared analyses of Nocellara Olive Oils for a thorough chemical composition

Giovanni Bartolomeo¹, Archimede Rotondo², Giovanna Loredana La Torre², Rossana Rando²,
Michelangelo Leonardi², Irene Maria Spanò², Giacomo Dugo^{1,2}

¹Science4life S.r.l., start-up of University of Messina, Messina, Italy

²Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF),
University of Messina, 98168 Messina, Italy

gbartolomeo@unime.it

Extra-virgin olive oil (EVOO) is the most used fat in the Mediterranean diet with the known beneficial effects upon the human health. Among the blessed features of the green gold, we point out the fatty acids peculiar composition and the specific phenolic fraction [1]. Indeed, the high content of mono-unsaturated fatty acid is evidenced as possible regulator of the HDL (high-density lipoprotein) and LDL (low-density lipoprotein) in human blood. The presence of the so-called saturated fatty acids (Palmitates and Stearates) and of the ω_6 and ω_3 are as well very important for the whole fat intake necessary for an healthy life. On the other hand, phenols are antioxidant agents preventing cardiovascular, anti-inflammatory diseases and aging [2].

These considerations triggered our careful analysis of 100 monovarietal samples all coming from the west Sicilian valleys where the most important discrimination factor is the micro-environmental condition determined by climate, soil, distance from the coast, altitude and so on. The panel of such micro-conditions can be reported as terroir and is in some way affecting the overall composition. The Nuclear Magnetic Resonance (NMR) spectroscopy with its quick and holistic approach [3] was compared with the traditional methods including the gas chromatography (GC-FID) [4-5]. Some conclusions are drawn about the fatty acids composition and the crucial phenolic profile, which is also providing a specific fingerprinting for the analyzed samples.

The composition analysis of Nocellara EVOOs reveals the wealth of the local production driving possible chances to enhance the quality and the specificity of the product.

References

- [1] L. Schwingshackl, M. Christoph, G. Hoffmann, *Nutrients*, **2015**, *7*, 7651.
- [2] J. Klikarová, A. Rotondo, F. Cacciola, L. Česlová, P. Dugo, L. Mondello, F. Rigano, *Food Analytical Methods*, **2019**, *12*, 1759.
- [3] A. Rotondo, L. Mannina, A. Salvo, *Food Analytical Methods*, **2019**, *12*, 1238.
- [4] A. Rotondo, A. Salvo, V. Gallo, L. Rastrelli, G. Dugo, *European Journal of Lipid Science and Technology*, **2017**, *119*, 1700151.
- [5] C. Naccari, R. Rando, A. Salvo, D. Donato, G. Bartolomeo, V. Mangano, V. Lo Turco, *La Rivista Italiana delle Sostanze Grasse*, **2017**, *94*, 230.

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Effect of dietary enrichment with flaxseed, vitamin E and selenium, and of market class on the broiler breast meat: technological and sensorial traits

Rossella Vadalà¹, Ambrogina Albergamo¹, Michelangelo Leonardi¹, Giovanni Bartolomeo², Emiliano Gurreri³, Giorgio Giardina³, Francesco Ruggeri³, Roberto Gualtieri⁴, Nadia Colombo⁴, Massimiliano Petracci⁵, Nicola Cicero^{1,2}

¹Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali
Università di Messina, Messina, Italia

²Science4life S.r.l. start-up dell'Università di Messina, Messina, Italia

³Leocata mangimi S.p.A, C.da Pennino Catanzaro, 97015 Modica (RG) Italia

⁴Avimecc Spa, C.da Fargione, Agglomerato Industriale ASI, 97015 Modica, Italia

⁵Dipartimento di Scienze e Tecnologie Agroalimentari (DISTAL), Alma Mater Studiorum
Università di Bologna, Bologna, Italia

n.cicero@unime.it

The impact of enriching the diet with flaxseed, selenium, and vitamin E, as well as the market class, on the quality of breast meat was investigated in terms of its technological and sensory attributes [1,2]. A randomized complete block design was employed, with a total of 6000 broilers assigned to either a standard diet or an enriched diet. The broilers were then slaughtered at three different ages: 37 days (light class), 47 days (medium class), or 57 days (heavy class). Subsequently, the breast muscles from each market class, both in the enriched and standard groups, were analyzed for their technological and sensory characteristics. These analyses were conducted at 24 hours post-mortem and after one month of frozen storage using a statistical multiple linear model.

The results revealed that the redness and yellowness of the muscles significantly increased and decreased, respectively, with advancing market age ($p < 0.05$). Additionally, the yellowness of the muscles increased significantly ($p < 0.05$) after frozen storage. However, the collected data consistently indicated a normal meat color. The water holding capacity of the muscles improved as a result of the enriched diet but significantly deteriorated after frozen storage ($p < 0.05$).

Regarding the sensory analysis, the juiciness and tenderness of the meat significantly improved with increasing slaughtering age and diet enrichment, as well as their interaction ($p < 0.05$). However, these attributes deteriorated after frozen storage. Overall, the fresh and enriched muscles from the heavy broilers exhibited the most favorable technological and sensory traits. This finding underscores the importance of considering market size and diet in order to achieve breast meat that enjoys greater consumer acceptance.

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References

[1] M. Petracci, M. Betti, M. Bianchi, C. Cavani, *Poultry Science*, **2004**, 83, 2086.

[2] A. Miezeliene, G. Alencikiene, R. Gruzauskas, T. Barstys, *Biotechnology, Agronomy, Society and Environment*, **2011**, 15, 61.

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