Società Chimica Italiana
Divisione di Spettrometria di Massa

Società Chimica Italiana
Gruppo Giovani

9th MS Jday

i giovani e la Spettrometria di Massa

2ND ONLINE EDITION - **24 GIUGNO 2021**

book of abstract

Committees

Scientific Committee

Donatella Caruso	Università degli Studi di Milano
Gianluca Giorgi	Università degli Studi di Siena
Valentina Lazazzara	Fondazione Edmund Mach (TN)
Fulvio Magni	Università degli Studi di Milano Bicocca
Flaminia Vincenti	Sapienza Università di Roma

Organizing Committee

Federico Fanti	Università degli Studi di Teramo
Raffaella Pascale	Gnosis by Lesaffre, Pisticci (MT)
Valentina Lazazzara	Fondazione Edmund Mach (TN)
Giovanni Ventura	Università degli Studi di Bari
Flaminia Vincenti	Sapienza Università di Roma

The 9th MS J-Day online conference is kindly supported and sponsored by:

Agilent Technologies

Scientific Program - *THURSDAY, JUNE 24 - MORNING SESSION*

9:00	*Welcome and opening ceremony - Fulvio Magni*
	PLENARY LECTURE - *CHAIRPERSON: V. LAZAZZARA*
9:15	*Characterization of bioactive secoiridoids in olive oil and leaves by advanced liquid chromatography -mass spectrometry techniques* R. ABBATTISTA – UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO
	ORAL SESSION - *CHAIRPERSONS: F. FANTI - R. PASCALE - G. VENTURA - F. VINCENTI*
9:45	*Characterization of quercetin derivatives in a crossing combination of Habanero white and Capsicum annuum peppers and evaluation of the encapsulation efficiency of peppers extract in liposomes by LC-MS/MS* M.A. ACQUAVIA– UNIVERSITÀ DEGLI STUDI DI BASILICATA & ALMAGISI S.R.L
10:00	*Phytochemical characterization of Goji berries (L. barbarum) and leaves by high-resolution mass spectrometry* A. LASALVIA– UNIVERSITÀ DI ROMA LA SAPIENZA
10:15	*High-resolution mass spectrometry for characterization of extracts from food by-products* K. MOROZOVA - FREE UNIVERSITY OF BOZEN-BOLZANO
10:30	*Optimization of a lipidomic LC-MS workflow for milk analysis on a Q-Exactive Orbitrap* S. IMPERIALE – FREE UNIVERSITY OF BOZEN-BOLZANO
10:45	*Supercritical fluid extraction with liquid chromatography-Orbitrap mass spectrometry for lipidomic analysis of mozzarella cheese* R. ZIANNI – ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA PUGLIA E DELLA BASILICATA
11:00	*Metabolic association of health relevant metabolites between peel and pulp in apples of old, commercial, and red-fleshed cultivars* A.T. CECI - LAIMBURG RESEARCH CENTRE & UNIVERSITY OF TRENTO
11:15	*Chemical characterization, antioxidant properties and enzyme inhibition of Rutabaga root's pulp and skin (Brassica napus L.)* A. STEFANUCCI - UNIVERSITY OF CHIETI-PESCARA "G. D'ANNUNZIO"
	break and poster session
11:45	*In silico food allergenic risk assessment of proteins extracted from microalgae spirulina and chlorella* M. BIANCO – UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO
12:00	*Mass spectrometry-based proteomics to investigate diagenetic effects on endogenous and environmental peptides of the Shandrin mammoth gut* A. CUCINA - UNIVERSITY OF CATANIA
12:15	*Proteomics profiling of FACS-sorted Leukocyte-derived Extracellular Vesicles as a "liquid biopsy" of immune response in untouched biofluids* M.C. CUFARO - UNIVERSITY "G. D'ANNUNZIO" OF CHIETI-PESCARA
12:30	*Post-translational modifications of the VDAC3 isoform purified from an ALS model cell line: a study using high resolution mass spectrometry* M.G.G. PITTALÀ - UNIVERSITY OF CATANIA
12:45	*The contribution of mass spectrometry as a magnifying glass for Covid-19 comprehension: what do peripheral lymphocytes conceal in their protein cargo?* S. VALENTINUZZI - CENTER FOR ADVANCED STUDIES AND TECHNOLOGY (CAST) & UNIVERSITY "G. D'ANNUNZIO" OF CHIETI-PESCARA
13:00	*A mass spectrometry study: postbiotic based ocular drug influences the proteomic profiles in the rabbits' tears* A. ZAMMATARO - UNIVERSITY OF CATANIA
13:15	*From micro- to non-invasive approaches to recognize painting binders on artworks* E.C.L. RIGANTE - UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO
	lunch break and poster session

Scientific Program - *THURSDAY, JUNE 24 - AFTERNOON SESSION*

ORAL SESSION - *CHAIRPERSONS: F. FANTI - R. PASCALE - G. VENTURA - F. VINCENTI*

14:30	*A novel MEPS-UHPLC-MS/MS analytical platform for the analysis of salivary oxylipins in heart failure patients* D. BIAGINI - UNIVERSITY OF PISA
14:45	*A metabolomic approach reveals hippuric acid as a possible hallmark of frailty* G. DE SIMONE - INSTITUTE FOR PHARMACOLOGICAL RESEARCH, MILAN
15:00	*Development of a method for the quantification of estrone and estradiol in serum by LC-MS/MS* V. FIORINI - DEPARTMENT OF EXPERIMENTAL AND CLINICAL BIOMEDICAL SCIENCES "MARIO SERIO"
15:15	*Mass Spectrometry Imaging as a tool to investigate region specific lipid alterations in symptomatic human carotid atherosclerotic plaques* F. GRECO - INSTITUTE OF LIFE SCIENCES, PISA & FONDAZIONE PISANA PER LA SCIENZA ONLUS
15:30	*An innovative UHPLC-MRM method for monitoring the cholesterol synthetic pathway in biological samples* A. LANNO - INSTITUTE FOR PHARMACOLOGICAL RESEARCH, MILAN
15:45	*Determination of salivary short chain fatty acids and hydroxy acids in heart failure patients by in-situ derivatization and Hisorb-probe sorptive extraction coupled to thermal desorption and gas chromatography-tandem mass spectrometry* A. LENZI - UNIVERSITY OF PISA
16:00	*A new LC-MS/MS method for the quantification of 1-Hydroxypyreneglucuronide in urine samples* C. MACCARI - UNIVERSITÀ DI PARMA
	break and poster session
16:30	*Phosphatidic acid methyl esters as markers of phospholipid profile alterations induced by phospholipase D during lipid extraction from microgreen crops: a hydrophilic interaction liquid chromatography – mass spectrometry study* A. CASTELLANETA – UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO
16:45	*Unambiguous positional assignment of double bonds in unsaturated fatty acyl chains of arsenosugar phospholipids (As-PL) in wakame seaweed by LC-ESI multistage mass spectrometry* D. CONIGLIO – UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO
17:00	*Accelerated dehydration of D-fructose performed in microdroplets by a commercial ESI Z-spray source* C. SALVITTI - UNIVERSITÀ DI ROMA LA SAPIENZA
17:15	*Determination of carbazole alkaloids in Murraya Koenigii by means of LC-MS/MS analysis with a predictive multi experiment approach* E. VITERITTI - UNIVERSITY OF TERAMO
17:30	*MS-based molecular networking: a modern strategy for the fast detection of new natural products* S. SCARPATO - UNIVERSITY OF NAPLES FEDERICO II
17:45	*Characterization of Rumex abyssinicus, a traditional Rwandan medicine* C. SPAGGIARI - UNIVERSITY OF PARMA & INES-RUHENGERI, INSTITUTE OF APPLIED SCIENCES, MUSANZE, RWANDA
18:00	*Detection of products of the reaction between 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) and common antioxidants by high resolution mass spectrometry* L. ANGELI – FREE UNIVERSITY OF BOZEN-BOLZANO
18:15	*closing remarks - Fulvio Magni, Donatella Caruso*

Summary

Plenary Lecture

Characterization of bioactive secoiridoids in olive oil and leaves by advanced liquid chromatography -mass spectrometry techniques

R. Abbattista[1], *I. Losito1*[,2], *A. Castellaneta*[1], *C. De Ceglie*[1], *C.D. Calvano*[1,3], *T.R.I. Cataldi*[1,2]

[1]Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", Bari, Italy
[2]Centro di ricerca interdipartimentale SMART, Università degli Studi di Bari "Aldo Moro", Bari, Italy
[3]Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari "Aldo Moro", Bari, Italy

Keywords: secoiridoids, olive oil and leaves, high resolution mass spectrometry

Secoiridoids are secondary metabolites unique of Oleaceae plants, including Olea europaea L., and belonging to the class of iridoids. Oleuropein and ligstroside, arising from an ester linkage between the glucosidic form of elenolic acid and hydroxytyrosol and tyrosol, respectively, are the most abundant secoiridoids in olive drupes and leaves [1]. Upon olive drupe crushing during extra virgin olive oil (EVOO) production, endogenous β-glucosidases lead to the respective aglycones, which, in turn, generate oleac(e)in and oleocanthal by methylesterase-mediated decarboxylation [2]. These compounds contribute to confer EVOO its bitterness and beneficial effects for human health, mainly due to their anti-inflammatory activity [3], but they also act as phytoalexins during olive plant defence following a pathogenic attack [4].

Due to their bioactivity, the cited secoiridoids have been the object of a deep structural investigation performed in our laboratories on EVOO, olive drupes and leaf extracts, based on reversed-phase liquid chromatography coupled to electrospray ionization Fourier-transform high resolution/accuracy mass spectrometry (RPLC-ESI-FTMS). As discussed in the present communication, extracted ion current chromatograms (XIC) emphasized the inherent complexity of secoiridoids, related to the presence of several isomeric forms and even of stable enolic tautomers, recognized by H/D exchange experiments integrated with RPLC separation [5]. The established analytical approach has been also effective for the detection of secoiridoids oxidative/hydrolytic by-products generated during EVOO storage, and for the evaluation of the influence of technological processes on the secoiridoid profile, crucial for shaping olive oil taste and quality [6,7]. Additionally, the identification of secoiridoids in olive leaves will be shown in this communication as an example of a comprehensive analytical approach to investigate the role of secoiridoids in the olive defense system.

References

1. D. Ryan, M. Antolovich, P. Prenzler, K. Robards, S. Lavee; *Scientia Horticulturae*, 92 (2002), 147-176
2. C. Romero-Segura, R. García-Rodríguez, A. Sánchez-Ortiz, C. Sanz, A.G. Pérez; *Food Research International*, 45 (2012),191-196
3. G.K. Beauchamp, R.S. Keast, D. Morel *et al.*; *Nature*, 437 (2005), 45–46.
4. I. Kubo, A. Matsumoto, I. Takase; *Journal of Chemical Ecology*, 11 (1985), 251-263
5. R. Abbattista, I. Losito, C. De Ceglie *et al.*; *Talanta*. 205 (2019), 120107
6. R. Abbattista, I. Losito, A. Castellaneta *et al.*; *Journal of Agricultural and Food Chemestry*, 68 (2020), 12310–12325
7. C. De Ceglie, R. Abbattista, I. Losito, *et al.*; *Journal of Agricultural and Food Chemestry*, 68 (2020), 3171−31

Oral Presentations

Characterization of quercetin derivatives in a crossing combination of Habanero white and *Capsicum annuum* peppers and evaluation of the encapsulation efficiency of peppers extract in liposomes by LC-MS/MS

M.A. Acquavia[1,2], *R. Pascale*[3], *C. Caddeo*[4], *A, Zizzamia*[1], *G. Martelli*[1], *G. Bianco*[1], *A. Vassallo*[1,5]

[1] Università degli Studi della Basilicata, Dipartimento di Scienze, via dell'Ateneo Lucano 10, 85100, Potenza, Italy [2]ALMAGISI S.r.l Corso Italia, 27-39100, Bolzano, Italy [3] Gnosis by Lesaffre, Pisticci, 75015 Matera, Italy; [4] Università di Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente, via Ospedale, 72, 09124 Cagliari, Italy; [5] Spinoff TNcKILLERS s.r.l., Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy.

Keywords: *white peppers; liposomes; LC-MS/MS*

Common peppers (*Capsicum*) are an important source of phytochemicals, including polyphenols, flavonoids and bioactive capsaicinoids [1]. Capsaicinoids are responsible for the hot, spicy flavour presented by chilli peppers. Due to the broad range of biological activities (antioxidant, anti-inflammatory, antimicrobic, anticancer, cardioprotective) covered by *Capsicum* metabolites, many peppers-based nutraceutical products are available on the market [2]. Recently, novel delivery systems for enhancing the oral bioavailability of bioactive molecules are being evaluated, such as liposomes, micelles, micro/nano-emulsions, colloidal capsules and solid nanoparticles [3]. In this study, we investigated the liposomal encapsulation of a methanolic extract of white chilli peppers, obtained from an original crossing combination between *Capsicum chinense* (also known as Habanero white) and *Capsicum annum* species. An LC-MS/MS method was developed to conduct a preliminary screening of the metabolic profile of the white peppers. In addition to capsaicinoids, a deep qualitative profile of the quercetin derivatives was provided. Nine glycoconjugates were identified, *i.e.* quercetin-6-*C*-hexoside-8-*C*-pentoside/apioside, quercetin-4′-rhamnoside-7-hexoside, quercetin 7-*O*-pentoside/apioside, quercetin-3-*O*-pentosyl/apiosyl-rhamnosyl-hexoside, quercetin 7-*O*-glucoside, quercetin-3-*O*-rhamnoside-7-*O*-hexoside, quercetin-3-*O*-rutinose, quercetin 7-*O*-rhamnoside and quercetin rhamnoside-(feruloyl-hexoside). The developed LC-MS/MS method was applied to quantify the liposomial encapsulation efficiency of the white peppers extract. High encapsulation efficiency values were obtained.

References

1. R. Pascale, M. A. Acquavia, T. R. I. Cataldi *et al.*, *Anal. Bioanal. Chem.* 412 (2020), 3005.
2. K. Singletary, *Nutr. Today* 46 (2011), 33.
3. A. Vassallo, V. Santoro, I. Pappalardo *et al.*, *Nanomaterials* 10 (2020), 2080.

Phytochemical characterization of Goji berries (*L. barbarum*) and leaves by high-resolution mass spectrometry

A. Lasalvia[1], *A. Maccelli*[1], *C. Ingallina*[1], *L. Mannina*[1], *S. Fornarini*[1], *M.E. Crestoni*[1]

[1]Dipartimento di Chimica e Tecnologie del Farmaco, Università di Roma "La Sapienza", P.zzle Aldo Moro 5, 00185, Roma, Italy

Keywords: *Goji berries, FT-ICR MS, Food analysis.*

Goji berries (GBs) (*Lycium* species) are an excellent source of macro- (carbohydrates, dietary fiber, protein and fat) and micro-nutrients (vitamins and minerals). Traditionally used as medicinal herb and food supplement in East Asia, GBs have gained increasing interest in western world justifying the naming as "superfood". Nowadays, GBs have achieved a wide employment as functional food and are marketed in many dietary, cosmetic, and pharmaceutical preparations and supplements due to the wide variety of pharmacological functions, including antioxidant, immunomodulatory and anticancer properties[1]. Not only berries, but also Goji leaves hold a high amount of antioxidant and bioactive compounds, thus making them promising in nutraceutical and cosmetic field. Usually used as tea and spices, they are often considered as waste material in industrial processes. The characteristic composition has shed new light on this raw material, since waste material can be renovated into a good source of ingredients useful in different purposes. The present contribution reports on an untargeted metabolic profiling of the hydroalcoholic and organic extracts of fruits and leaves of two different GBs cultivars: Sweet Rose (SR) and Big Life (BL) of L. *barbarum*, from south Lazio (Italy), at red (ripe) and green (unripe) ripening stages. In addition, a sample of BL grown with less sun exposure (LE) was analysed. Direct infusion electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), a fast and sensitive method, provides an untargeted profiling of complex matrices and has been recently demonstrated to be a powerful means for a comprehensive quality and safety assessment of essential oils and foodstuffs[2]. The ultrahigh resolving power of FT-ICR MS can resolve a spectrum with a thousand of signals allowing to eliminate chromatographic separation. In addition, the extremely high mass accuracy enables to assign each signal to a candidate metabolite by querying specific metabolomic databases. This approach has provided a qualitative overview of different classes of metabolites, such as lipids, flavonoids, organic and fatty acids, amino acids and carbohydrates. Graphical visualisation tools (van Krevelen diagrams (vKds) and elemental composition histograms) and multivariate statistical analysis (PCA) were accomplished. vKds diagrams have shown a higher density of entries in the lipids, polyketides, amino acids and polyphenols areas, and, less represented, carbohydrates, tannins and nucleic acids, similar in fruits and leaves. The relative frequency distribution of molecular formulas reveals that the most abundant metabolites in all extracts are CHO (alcohols, carbohydrates, lipids and polyphenols) and CHNO (amino acids and alkaloids) species. Lastly, PCA has shown the cultivars to be clearly differentiated by the first component, whereas the harvest stage by the second one. An exception is represented by LE fruits, closer in composition to the unripe samples.

References

1. Ma, Z.F.; Zhang, H.; Teh, S.S.; Wang, *et al.*; *Oxid. Med. Cell. Longev.* Jan 9 (2019), 2437397.
2. C. Ingallina, A. Maccelli; M. Spano *et al.*; *Antioxidants,* 9 (2020), 1927.

High-resolution mass spectrometry for characterization of extracts from food by-products

K. Morozova[1], G. Ferrentino[1], S. Giampiccolo[1], O.K. Mosibo[1], F. Pompeo[1], M. Scampicchio[1]

[1] Free University of Bozen-Bolzano, Faculty of Science and Technology,
Piazza Università 1, 39100 Bolzano, Italy

Keywords: *high-resolution mass spectrometry, food by-products*

Reduction of food losses and by-products valorization is a challenge in food processing as the large amount of non-edible residues produced by the industries cause pollution, difficulties in the management and economic loss. Recent studies show that by-products derived from fresh fruits and vegetables are rich in antioxidants [1, 2]. The recovery of antioxidants from food by-products and their characterization is of paramount importance for reduction of food waste. Among other innovative green technologies, supercritical fluid extraction (SFE) is one of the most promising. In this work liquid chromatography coupled with high-resolution mass spectrometry was applied for characterization of the antioxidants in the extracts of food by-products obtained using SFE from apple [1], grape, carrot pomaces and apple seeds [2]. The results were then compared to those obtained by classical liquid-liquid extraction. The extracts were characterized for their antioxidants capacity with electrochemical techniques and spectrophotometric assays, such as the Folin-Ciocalteu, the 2,2-diphenyl-1-picryhydrazyl radical (DPPH). A characterization of the extracts was performed by high-performance liquid chromatography coupled to Q Exactive Orbitrap mass spectrometer.

References

1. G. Ferrentino, K. Morozova, O.K. Mosibo, M. Ramezani, M. Scampicchio; *Journal of Cleaner Production*, 186 (2018), 253-261.
2. G. Ferrentino, S. Giampiccolo, K. Morozova, N. Haman, S. Spilimbergo, M. Scampicchio, 64 (2020), *Innovative Food Science & Emerging Technologies*, 102428.

Optimization of a lipidomic LC-MS workflow for milk analysis on a Q-Exactive Orbitrap

S. Imperiale[1], *K. Morozova*[1], *M. Scampicchio*[1]

[1] Faculty of Science and Technology, Free University of Bozen-Bolzano, Piazza Università 5, 39100 Bolzano, Italy

Keywords: *high-resolution mass spectrometry, milk fat extraction, TAG*

The lipid fraction of milk is a complex biological matrix. It consists of several different lipid classes including a myriad of different species. Therefore, LC-HRMS analysis of milk fat results in complex data describing the whole lipid profile [1, 2].
The profile obtained from LC-HRMS analysis is typically used to characterize the milk lipidome, but it is only scarcely used for targeted analysis of specific lipids [2, 3]. For this reason, there is the need to optimize targeted data acquisition for milk lipids.
In this study we propose a workflow to optimize the LC-HRMS parameters on a Q-Exactive Orbitrap mass spectrometer to analyse lipids in milk. First, the parameters were optimized for an analytical standard of triheptadecanoin. For this, we assessed different LC-HRMS parameters such as flow rate, MS resolution and scan settings in selected ion monitoring mode. The best analytical performance to determine the analytical standard was achieved at a flow rate of 200 µL/min, a mass resolution of 35,000 and an automatic gain control target of $2x10^5$. Finally, the optimized LC-HRMS parameters were applied to analyse milk fat extracts. Exemplary triacyl glycerides were determined in selected ion monitoring mode as target lipids. For each target lipid the ideal number of scans for peak generation were acquired. This resulted in well resolved peaks with high sensitivity and accuracy.
The here presented approach represents an effective method for empirically optimizing MS-based lipidomic workflows for targeted milk lipid analysis. This approach can be further extended to additional food matrices and other research fields.

References

1. T. Cajka, O. Fiehn; *Trends in Analytical Chemistry*, 61 (2014), 192–206
2. Z. Liu, S. Rochfort, B.G. Cocks; *Progress in Lipid Research*, 71 (2018), 70–85
3. Z. Liu, P. Moate, V. Ezerniks, B.G. Cocks, S. Rochfort; *Journal of Dairy Science*, 98 (2015,) 8473–8485

We kindly thank the EU for the financial support of this study by the FESR-1129 funded Project Heumilch (CUP: H36H19000000007).

Supercritical fluid extraction with liquid chromatography-Orbitrap mass spectrometry for lipidomic analysis of mozzarella cheese

R. Zianni[1], *A. Mentana*[1], *M. Campaniello*[1], *V. Nardelli*[1]

[1] Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata - Laboratorio Nazionale di Riferimento per il trattamento degli alimenti e dei loro ingredienti con radiazioni ionizzanti - Via Manfredonia 20, 71121 Foggia, Italy.

Keywords: *Supercritical CO_2, soft-cheese, Lipidomics*

Supercritical fluid extraction (SFE) is an efficient, environmental-friendly and selective alternative to conventional solvent techniques for lipid extraction from food matrices. Supercritical fluids exhibit liquid-like density and gas-like viscosity and diffusion coefficients, so can penetrate more into the solid matrix inaccessible to liquids due to negligible surface tension and viscosity [1]. SFE technology employs mainly carbon dioxide (CO_2) as supercritical fluid above the critical temperature of 31 °C and critical pressure of 74 bars. In Lipidomics, the extraction is the first and fundamental step to isolate a subset of components and to remove interferences, so it requires continuous in-depth studies. In this work, an appropriate CO2-SFE extraction was employed for lipidomic characterization of mozzarella cheese. The SFE lipid extracts were analysed by means Ultra High liquid chromatography-electrospray ionization Orbitrap mass spectrometry (UHPLC-ESI-Orbitrap-MS). High-resolution MS technique had improved the molecular species assignment and their quantification [2]. In addition, the LipidSearchTm software was employed to carry out the lipid identification based on MS/MS data analysis in accordance with defined fragmentation rules. A total of 8 classes of lipids, including ceramides (Cer), diacylglycerols (DG), triacylglycerols (TG), monogalactosyldiacylglycerol (MGDG), bismethyl phosphatidic acids (BisMePA), cholesterol ester (ChE), zymosteryl ester (ZyE), phosphatidylethanols (PEt) were measured. Finally, a comparison with Folch extraction was carried out showing how different extraction techniques provide complementary lipid information.

References

1. S. D. Manjare, K. P. Dhingra; *Materials Science for Energy Technologies*, 2 (2019), 463-484.
2. T. Cajka, O. Fiehn; *Analytical Chemistry*, 88 (2016), 524−545.

Metabolic association of health relevant metabolites between peel and pulp in apples of old, commercial, and red-fleshed cultivars

A.T. Ceci[1,2], M. Bassi[1], W. Guerra[1], M. Oberhuber[1], P. Robatscher, F. Mattivi[2,3], P. Franceschi[3]

[1] Laimburg Research Centre, Laimburg 6, Pfatten (Vadena), IT-39040 Auer (Ora), South Tyrol, Italy
[2] Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Via Sommarive 9, Povo, 38123 Trento, Italy
[3] Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige, Italy

Keywords: *metabolomics; antioxidant activity, polyphenols.*

Apples are rich in bioactive compounds and the consumption of these fruits has been continuously linked to lower risk of the onset of chronic diseases and cardiovascular disorders. The production of free radicals and reactive oxygen species (ROS) is implicated in the outbreak of these long-term health negative conditions; however, the potential role of apples in preventing the formation of ROS is often attributed to the high content of antioxidant compounds such as polyphenols [1]. Another healthy polyphenolic class is anthocyanins, which are hypoglycaemic and decrease the risk of type II diabetes. Considering that the content of phytochemicals depends on several factors, *e.g.*, environmental, in the current study twenty-two apple cultivars grown in the same site under identical climatic and agricultural conditions were analysed on their content of health relevant metabolites in the peel and pulp [2]. The different apple cultivars are categorized on according to their phenotypic and commercial characteristics: old, commercial, and red-fleshed [3].
The aim is focused on the cultivar-specific association between pulp and peel (sunny and shady sides) polyphenolic profiles by applying a multivariate Partial Least Squares (PLS) regression approach. The parameters used to evaluate the prediction ability of the PLS was the coefficient of determination in prediction (Q2) and the regression coefficients. We noticed that the variability in the concentration of polyphenolic compounds is more pronounced in commercial and red-fleshed apple varieties. For the red-fleshed varieties, it is interesting to observe that there are some genotypes (Y102 and BAY) combining a significant concentration of anthocyanins and a high content of the other classes of polyphenols. The results of PLS model shows a good predictive power for almost all assays considered, with median values of Q2 ranging from 0.5 and 0.7, in the old apple group. The situation is different for the commercial and the red-fleshed groups, where the median of Q2 is extremely variable. Our findings show that the old apple varieties are the most reliable group and a metabolic "association" between peel and pulp of five different old varieties is found. Indeed, the antioxidant activity in the peel is of commercial and red-fleshed apples is poorly predicted, expect for the total anthocyanin content in the red-fleshed group and it is linked to the sign of the presence of a more diverse metabolism within these two macro groups.

References

1. Tsao, R.; Yang, R.; Young, J.C.; Zhu, H; *J. Agric. Food Chem*. 51 (2003), 6347–6353
2. Bassi, M.; Lubes, G.; Bianchi, *et al.*; *International Journal of Food Properties* 20 (2017), 2626-2634
3. Baric, S.; Storti, A.; Hofer, M.; Guerra, W.; Dalla Via, J; *Erwerbs-Obstbau* 62 (2020), 117–154
4. Granato, D.; Santos, J.S.; Escher, G.B.; Ferreira, B.L.; *Trends in Food Science & Technology*, 72 (2018), 83–90

Chemical characterization, antioxidant properties and enzyme inhibition of Rutabaga root's pulp and skin (*Brassica napus L.*)

A. Stefanucci[1],G. Zengin[2], E. LlorentMartinez[3], M. Dimmito[1], A. Della Valle[1], S. Pieretti[4], G. Ak[2], K. Sinan[2], A. Mollica[1]

[1]Department of Pharmacy, University of Chieti-Pescara "G. d'Annunzio", Via dei Vestini 31, 66100 Chieti, Italy.
[2] Department of Biology, Science Faculty, Selcuk University, Konya, Turkey.
[3]University of Jaén, Faculty of Experimental Sciences, Department of Physical and Analytical Chemistry, Jaén, Spain.
[4]National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy.

Keywords: *Rutabaga, antioxidants, phytochemicals*

Rutabaga (*Brassica Napus* L.) belonging to Brassicaceae family, is a rich source of polyphenols and glucosinolates. It's consumption in human diet is highly appreciated for nutritional contribution and health benefits. *Brassica napus* L. (*B. napus* L.) is recognized as the world's most widely grown temperate oilseed crop containing erucic acid for industrial applications and plants germition, animal feed and fuel.

In this work we prepared two different extracts of Rutabaga root's pulp and skin, *e.g.* ultrasound assisted extract (UAE) and homogenizer assisted extract (HAE). The four extracts have been analyzed by HPLC-MS to assess the phytochemical characterization and tested by antioxidant and enzyme inhibitor assays (Figure 1) [1].

Rutabaga pulp and skin extracts possess tyrosinase and glucosidase inhibitory activities together with a moderate antioxidant ability. Our results show a high level of glucosinolates, in particular neoglucobrassicin in the skin extract, which let us suppose a potential application as crop in industry and as supplement in human diet.

Peak	Compound	Pulp-HAE	Pulp-UAE	Peel-HAE	Peel-UAE	Average
1	Disaccharide	23.29	37.95	18.28	27.46	26.75
2	Progoitrin	2.06	0.00	1.77	0.00	0.96
3	Glucoerucin	2.83	2.48	0.00	0.00	1.33
4	Glucobrassicin	6.56	6.18	0.00	0.00	3.19
5	Gluconasturtiin	15.10	12.04	21.21	16.89	16.31
6	Glucoraphenin	18.94	17.01	5.58	3.64	11.29
7	Neoglucobrassicin isomer	1.52	0.96	1.79	1.37	1.41
8	Neoglucobrassicin	26.30	20.42	48.66	48.57	35.99
9	Sinapic acid	3.41	2.95	2.72	2.06	2.78

Figure 1. Relative peak areas and heat map obtained by HPLC-ESI-MS analysis of Rutabaga pulp and peel extracts.

References

1. A. Stefanucci, G. Zengin, E. J. Llorent-Martinez, M. P. Dimmito, A. Della Valle, S. Pieretti, G. Ak, K. I. Sinan, A. Mollica, *Arabian Journal of Chemistry* 2020, *13*, 7078-7086.

In silico food allergenic risk assessment of proteins extracted from microalgae *spirulina* and *chlorella*

M. Bianco[1], G. Ventura[1], C.D. Calvano[2,3], I. Losito[1,3], T.R.I. Cataldi[1,3]

[1]Dipartimento di Chimica, [2]Dipartimento di Farmacia-Scienze del Farmaco, [3] Centro Interdipartimentale SMART, Università degli Studi di Bari Aldo Moro, via Orabona 4, 70126 Bari (Italy)

Keywords: *allergen, microalgae, novel food*

Food allergy is a serious health problem triggered by proteins or protein epitopes able to affect susceptible individuals by immune-mediated reactions [1]. Although the most allergenic foods have been included in a list named "the big 8", the European regulation n° 1169/2011 has extended to 14 foodstuffs, *i.e.* eggs, milk, fish, peanuts, crustaceans, soybeans, wheat, tree nut, lupin, shellfish, celery, mustard, sesame, and sulfur dioxide, due to the increasing number of immune-mediated reactions. Many studies have been focused on these major allergenic foods, often establishing the occurrence of proteins responsible for allergenicity by recurring to marker peptides for their identification and quantitation [2]. Around the world the population is constantly growing and there is a rising demand of food including novel nutrient sources. Among innovative food products with high protein content lately introduced in the eating habits of western countries we can mention yeast, fungi, bacteria, algae, and insects [3].

Microalgae and cyanobacteria, such as *Chlorella vulgaris* and *Arthrospira platensis* also known as spirulina, are considered potential "superfoods" with protein content similar to or greater than common food sources like egg, milk, meat, soybean, and others [4]. Currently, great attention is devoted to the possible side effects of these foodstuffs with allergenic reactions in sensitized populations. Some studies reported the adverse reaction against *C. vulgaris* and *A. platensis* food supplements of 6-17 years old children [5,6].

Our main aim was to assess the allergenic potential of *C. vulgaris* and *A. platensis* extracts using reversed-phase liquid chromatography coupled to electrospray ionization and high resolution/accuracy mass spectrometry (RPLC-ESI-FTMS) in combination with a software-based workflow to interrogate AllergenOnline database (http://www.allergenonline.org) searching for proteome sequence similarity declared as allergens. In this communication, the workflow for the putative identification of allergen proteins in novel foods, food supplements and/or nutraceuticals will be presented and discussed.

References

1. Sicherer, S.H.; Sampson, H.A. *Journal of Allergy and Clinical Immunology*, 125 (2010), S116–S125.
2. Pilolli, R.; Nitride, C.; Gillard, *et al. Food Research International* 128 (2020), 108747.
3. Becker, W. Microalgae in *Human and Animal Nutrition*. In *Handbook of Microalgal Culture*; Blackwell Publishing Ltd: Oxford, UK, 2004; 312–351
4. Koyande, A.K.; Chew, K.W.; Rambabu, K.; Tao, Y.; Chu, D.-T.; Show, P.-L. *Food Science and Human Wellness*, 8 (2019), 16–24.
5. Tieberg, E.; Dreborg, S.; Bjorksten, B. *Journal of Allergy and Clinical Immunology*, 96 (1995), 257–259.
6. Petrus, M.; Culerrier, R.; Campistron, M.; Barre, A.; Rougé, P. *Allergy*, 65 (2009), 924–925.

This work was supported by Progetto di Ricerca di Interesse Nazionale-PRIN 2017YER72K- "Development of novel DNA-based analytical platforms for the rapid, point-of-use quantification of multiple hidden allergens in food samples", financed by the Italian Ministero per l'Istruzione, l'Università e la Ricerca (MIUR).

Mass spectrometry-based proteomics to investigate diagenetic effects on endogenous and environmental peptides of the Shandrin mammoth gut

A. Cucina[1], V. Cunsolo[1], A. Di Francesco[1], R. Saletti[1], G. Zilberstein[2], S. Zilberstein[2], A. Tikhonov[3], A.G. Bublichenko[3], P.G. Righetti[4], S. Foti[1]

1 Laboratory of Organic Mass Spectrometry, Department of Chemical Sciences, University of Catania, Viale A. Doria 6, 95125, Catania, Italy 2 Spectrophon Ltd, Oppenheimer 7, Rehovot, 7670107, Israel.
3. Zoological Institute, Russian Academy of Sciences, Universitetskaya nab.1 Saint-Petersburg 199034
4. Department of Chemistry, Materials and Chemical Engineering ‘‘Giulio Natta’’, Politecnico di Milano, Via Mancinelli 7, Milano 20131, Italy

Keywords: *Proteomics, mammoth, gut*

Remains of a woolly mammoth (Mammuthus primigenius) were discovered in 1972 in the Shandrin river [1]. A partial reconstruction of the ancient flora was possible thanks to the analysis of monoliths of the gastrointestinal tract at a macro- level allowing the identification of plant remains [2]. Particularly, the EVA technology [3] coupled with the high-sensitivity, high-resolution tandem mass spectrometry, was applied. Metaproteomics analysis allowed the identification of traces of plants at a molecular level in the gut supporting the hypothesis of the presence of a palustris forest. Moreover, the composition of microbiota peptides showed the same prevalence at phylum and genus level between the mammoth and the modern forest elephant (Loxodonta cyclotis) [4]. The antiquity of the identified peptides was supported by the comparison of the deamidation rate of potentially original and c-RAP contaminants [5]. Then, the unassigned spectra were examined by “dependent peptide” Max Quant search [6] to investigate diagenetic effects [7]. Considering that diagenetic PTMs are related to aging, but also temperature, pH and UV irradiation, the analogies in the level of oxidation at Tyr, Trp, Cys and Met residues between plants, bacteria and endogenous mammoth peptides held up a temporal and geographical correlation among them. The investigation of such modifications represents a new potential method not only to characterize conservation and damage of a well-known archaeological remain, but also to correlate traces of environmental peptides which show a similar profile of peptides damage.

These results demonstrate that EVA technology, coupled with high-resolution tandem mass spectrometry, represents a new, formidable approach which does not contaminate nor damage any of the items under investigation, and can be applied also to analysis of fossil remains.

References

1. Ukraintseva V.V.; *Mammoths and the Environment*, 8 - Cambridge Core 2013 *pp.* 117-128.
2. Serdyuk N.V.; *Palaeontologia Electronica*, a 21.3.35A (2018), 1-15.
3. Righetti P.G., Zilberstein G. and Zilbertstein S.; *Journal of Proteome Research* 19 (2020), 2914-2925.
4. Budd K., Gunn J.C., Finch T., Klymus K., Sitati N., Eggert L.S; *Ecol. Evol.* 10 (2019) *pp.* 5637–5650.
5. Mackie M., Rüther P., Samodova D., Di Gianvincenzo *et al.*; *Angewandte Chemie International Edition* 57:25 (2018) *pp.* 7369-7374.
6. Tyanova S., Temu T., Cox J.; *Nature Prot. 11-12* (2016) 2301-2319.
7. Pattison D.I., Rahmanto, A.S., Davis M.J.; *Photochem. Photobiol. Sci.* 11 (2012) 38-53.

Proteomics profiling of FACS-sorted Leukocyte-derived Extracellular Vesicles as a "liquid biopsy" of immune response in untouched biofluids

M.C. Cufaro[1], I. Cicalini[1], M. Marchisio[1], P. Lanuti[1], D Pieragostino[1] and Piero Del Boccio[1]

[1] Analytical Biochemistry and Proteomics and Cytomorphology Laboratory, Center for Advanced Studies and Technology (CAST), University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy

Keywords: *Proteomics, Leukocyte-Extracellular Vesicles, immune response*

Extracellular Vesicles (EVs) are nano-sized membrane-enclosed particles released by cells in many pathophysiological processes [1,2]. Nowadays EVs molecular characterization is a hot topic due to their emerging role as potential biomarkers. However, their isolation from biological fluids is very difficult for many reasons [4-7]. Actually, proteomics of EVs is hampered by the presence of circulating abundant proteins that may influence the purity of isolated EVs, increasing the dynamic range of the proteome and impairing the quality of mass spectrometry (MS)-based proteomics results [1,2,7]. In such a dynamic and complex contest, we recently optimized an innovative protocol for the isolation and subsequent proteomics characterization of total EVs from untouched biofluids by Fluorescence- Activated Cell Sorting (FACS), taking advantage of a lipophilic cationic dye able to probe and purify EVs for shotgun proteomics applications [2,8]. This original protocol was already applied for shotgun proteomics analyses of total EVs purified from various biological samples in many different pathological conditions [8-12]. Here we reported an update of the FACS-proteomics workflow in the ability to purify specific EVs-subsets suitable for MS-based proteomics analysis. We provided a successful proof-of-concept of the isolation and characterization of Leukocyte-derived EVs (CD45+) from fresh and untouched biofluids, *i.e.* tears and peripheral blood (PB), demonstrating a high capability to recognize EVs-specific protein cargo. We highlighted, for the first time, that in both biological fluids Leukocyte-derived EVs carry an active protein cargo able to trigger specific cellular information related to the "leukocyte mediated immunity" according to the identification of specific proteins involved in the chemotaxis and adhesion of neutrophils, such as protein S100-A7, S100-A8 and S100-A9. Moreover, our data showed that the protein outfit of Leukocyte-derived EVs is programmed to insert detailed information into the target cells. As a matter of fact, both in tears and in PB expression analysis revealed that one of the most significant predicted Upstream Regulator was Oncostatin-M (OSM) that plays a key role in the biological response against infection. OSM was related to cytokine-mediated signaling pathway and immune response, so our data confirmed that proteomics characterization of specific EVs phenotype could be considered a platform for "liquid biopsy" useful in the assessment of EVs clinical significance.

References

1. M. C. Cufaro *et al.*; *Journal of Oncology*, Jun 9 (2019), 1639854.
2. M. Marchisio *et al.*; International Journal of Molecular Sciences, 22 (2021), 48.
3. D. Choi *et al.*; *Proteomic Profiling. Methods in Molecular Biology*, (2021).
4. L. Muller *et al.*; *Journal of Immunological Methods*, 411 (2014), 55-65.
5. J. Lotvall *et al.*; *Journal of Extracellular Vesicles*, 3 (2014), 26913.
6. O. Ruhen *et al.*; *Proteomics*, 19 (2019), e1800155.
7. H. Kang *et al.*; *Micro and Nano Systems Letters*, 5(2017), 15.
8. D. Pieragostino *et al.*; *Journal of Proteomics*, 204 (2019), 103403.
9. D. Brocco *et al.*; *Cancers*, 13 (2021), 4.
10. D. Brocco *et al.*; *Journal of Oncology*, (2019), 5879616.
11. K. Falasca *et al.*; *AIDS*, 35 (2021), 595604.
12. C. Rossi *et al.*; *International Journal of Molecular Sciences*, 20 (2019), 16.

Post-translational modifications of the VDAC3 isoform purified from an ALS model cell line: a study using high resolution mass spectrometry

M.G.G. Pittalà[1], S. Reina[2], A. Cucina[3], V. Cunsolo[3], V. De Pinto[2], S. Foti[3], A. Messina[1], R. Saletti[3]

1 Molecular Biology Laboratory, Department of Biological, Geological and Environmental Sciences, University of Catania, Via S. Sofia 64, 95123 Catania, Italy;
2 Molecular Biology Laboratory, Department of Biomedical and Biotechnological Sciences, University of Catania, Via S. Sofia 64, 95123 Catania, Italy;
3 Laboratory of Organic Mass Spectrometry, Department of Chemical Sciences, University of Catania, Viale A. Doria 6, 95125, Catania, Italy.

Keywords: *high resolution mass spectrometry, Voltage Dependent Anion Channel, post-translational modifications*

Voltage-Dependent Anion Channel isoforms (VDAC1, VDAC2, VDAC3) are a quantitatively relevant component of the outer mitochondrial membrane (OMM) proteome and play crucial role in cellular processes, in the regulation of metabolism and in survival pathways.
VDAC3 is the least abundant isoform and, consequently, the least known: in fact itis difficult to isolate it from other VDAC isoforms and other hydrophobic mitochondrial proteins.
Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease caused by progressive degeneration of the motor neurons in the brainstem and spinal cord. In ALS patients VDAC1 represents the docking site on the OMM for ALS-linked SOD1 mutants [1]. Recently, we have found in VDAC1, purified from a suitable cellular model of ALS motor neurons (NSC34-SOD1G93A cells), specific asparagine and glutamine deamidations responsible for a serious conformational changes in channel structure that can alter the physiological pool of interactors [2]. The involvement of the other VDAC isoforms in ALS is unknown.
In this work we aimed to evaluate whether oxidative stress in ALS induces post-translational modifications (PTMs) in VDAC3 isoform purified from NSC34 cell line expressing human SOD1G93A.The specific PTMs of VDAC3 isoform were analyzed by means of "in solution" tryptic and chymotryptic proteolysis and nUHPLC/High Resolution nESI-MS/MS, a procedure originally developed by us [3,4,5]. The results demonstrated the presence of selective deamidations of asparagine and glutamine in VDAC3 from ALS-related NSC34-SOD1G93A cells but not from NSC34-SOD1WT or NSC34 cells. Furthermore, we identified differences in the over-oxidation of methionine and cysteine residues between VDACs purified from ALS model and non-ALS NSC34 cells.
Our data indicate that also VDAC3 may be involved in mitochondrial dysfunction associated with ALS pathogenesis. Moreover, a possible role for VDAC3 as potential marker of malfunctioning mitochondria in ALS can be envisaged.

References

1. A. Magrì, *et al. Sci. Rep.* (2016), 6, 34802.
2. M. G. G. Pittalà *et al.*, *Antioxidants*, 2020, 9, 1218.
3. R. Saletti, *et al. Biochim. Biophys. Acta –Biomembr.* (2016), 1859, 301-311.
4. R. Saletti, *et al. Biochim. Biophys. Acta – Bioenergetics* (2018), 1859, 806-816.
5. M. G. G. Pittalà, *et al. Int. J. Mol. Sci.* (2020), 21, 1468.

The contribution of mass spectrometry as a magnifying glass for Covid-19 comprehension: what do peripheral lymphocytes conceal in their protein cargo?

S. Valentinuzzi[1,2], I. Cicalini[1], P. Lanuti[1,3], P. Simeone[1,3], V. Damiani[1,4], V. De Laurenzi[1,4], P. Del Boccio[1,2], D. Pieragostino[1,4]

[1]Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara, Italy;
[2]Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Italy;
[3]Department of Medicine and Aging Sciences, "G. d'Annunzio" University of Chieti-Pescara, Italy;
[4]Department of Innovative Technologies in Medicine & Dentistry, "G. d'Annunzio" University of Chieti-Pescara, Italy

Keywords: *Covid-19; Proteomics; Lymphocytes.*

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a highly transmissible and pathogenic beta-coronavirus that caused a pandemic of pneumonia named Covid-19 from late 2019 on [1]. Given the striking variability of symptoms, relying on marked reduction in lymphocyte subset counts, cytokine storm, neurodegeneration and hypercoagulopathy, a proper characterization of the molecular networks involved in Covid-19 must be done. In this context, peripheral lymphocytes bring the cellular basis of adaptative immune responses, thus playing a considerable role as predictors of Covid-19 outcomes. A SORTomics approach [2] was applied to purify by fluorescence activated cell sorting (FACS) and analyzed by shotgun proteomics 100,000 CD3+ T cells and 100,000 CD19+ B cells from plasma of hospitalized patients during infection (I), recovered (R) and healthy subjects (H). Proteins were digested by filter-aided sample preparation (FASP) and peptides were separated on an EASY Spray C18 analytical column before Orbitrap-Fusion-Tribrid-MS analysis. Data were processed through MaxQuant and Perseus software; quantified proteins underwent Ingenuity Pathway Analysis (IPA) and STRING for the functional enrichment analysis of the interacting networks. Results. 221 (I), 165 (R) and 234 (H) proteins were quantified in CD3+ T cells, while 205 (I), 118 (R) and 161 (H) proteins in CD19+ B cells. Intriguingly, I pool is devoid of effective lung healing markers (CD44) and expresses markers of cytoskeleton remodelling (MMP9), inflammation (ORM2), proteasomal activity (PSMB6), virus entry (HSPA9 and glycophorins), coagulation (PF4) and metabolic switch (SLC2A1). Moreover, iron homeostasis is affected in Covid-19 patients, as shown by upregulated levels of transferrin (TF), that is a known procoagulant. IPA Core Analysis suggests the interplay between inflammation, viral infection and the coagulation cascade by confirming the acute phase response signalling, the vascular dysfunction and the infection by RNA-viruses. These data are in agreement with the highlighted upstream regulators related to coagulation, inflammation, oxidative stress and neuronal homeostasis, thus providing useful tools in the assessment of Covid-19 infection response and to predict the outcomes.

References

1. Hu B, Guo H, Zhou P, Shi ZL. *Nat Rev Microbiol*. Mar (2021);19(3):141-154
2. Pieragostino D, Lanuti P, Cicalini I, *et al.*; *J Proteomics*. 2019 Jul 30;204:103403.

A mass spectrometry study: postbiotic based ocular drug influences the proteomic profiles in the rabbits' tears

A. Zammataro[1], R. Saletti[1], I. Abbate[2], A. Di Francesco[1], V. Cunsolo[1], S. Foti[1]

[1] Laboratory of Organic Mass Spectrometry, Department of Chemical Science, University of Catania
[2]Ricerca,Sviluppo Preclinico e Brevetti, SIFI S.p.A

Keywords: *Proteomic profile, rabbit tear fluid, postbiotic drug*

Postbiotics are functional bioactive compounds, generated by microorganisms in a matrix during a fermentation process (1). Recent works indicate that postbiotic-based drugs can have clinical effects and a direct immunomodulatory role. Experimental evidences demonstrate that these drugs produce improvement of health and relief of symptoms in a range of ocular diseases, like the dry eye syndrome (2).

In this work we present a comparison of the proteomic profile in rabbits' tears after a tolerability study. These trials were performed by SIFI S.p.A. on 10 rabbits (5 males and 5 females), in which left eyes were treated with a placebo, whereas right eyes were treated with a postbiotic drug.

All the samples were collected with sterilised glass microcapillaries and subjected to reduction, alkylation and digestion with trypsin. Then, tryptic peptide mixtures were analysed in triplicate by nanoUHPLC/High Resolution nanoESI-MS/MS.

Overall, the results obtained show that the number of proteins identified in the eyes treated with the postbiotic is higher than in the placebo treated ones.

No proteins were exclusively observed in the placebo treated eyes. On the contrary, twelve proteins were frequently identified in the eyes treated with the postbiotic, but were not observed in the placebo treated eyes.

These proteins are involved in biological pathways that mainly include immune response, keratinization, and protein regulation.

References

1. Hu B, Guo H, Zhou P, Shi ZL. *Nat Rev Microbiol.* 2021 Mar;19(3):141-154.
2. Pieragostino D, Lanuti P, Cicalini I, Cufaro MC, Ciccocioppo F, Ronci M, Simeone P, Onofrj M, van der Pol E, Fontana A, Marchisio M, Del Boccio P. *J Proteomics*. (2019) Jul 30;204:103403.

From micro- to non-invasive approaches to recognize painting binders on artworks

E.C.L. Rigante[1], C.D. Calvano[2,3], R.A. Picca[1,3], T.R.I. Cataldi[1,3], L. Sabbatini[1,3]

[1]Dipartimento di Chimica, [2]Dipartimento di Farmacia-Scienze del Farmaco, [3]Centro Interdipartimentale SMART, Università degli Studi di Bari Aldo Moro, via Orabona 4, 70126 Bari (Italy)

Keywords: *painting binders, MALDI-ToF, proteomics and lipidomics.*

The analysis of painting layers in artworks is of paramount importance when restoration and conservation strategies are planned[1]. Drying oils and proteinaceous materials have been used as painting media to bind suspended particles of pigments. The present communication will focus on the identification of organic binders covering some stony sculptural elements composing the Nativity scene of Altamura's Cathedral dating back to the XVI century. The restoration work carried out on these sculptures highlighted the existence of several overlapping painting layers most likely applied in different occasions. To get an overview of both lipid and protein fractions, previously developed non-invasive[2] and micro-invasive protocols[3] were applied. While protein binder's characterization was accomplished by a bottom-up approach, which involves protein fraction enzymatic digestion and mass spectrometry (MS) analysis of the ensuing peptide mixture[2] by matrix-assisted laser desorption/ionization – tandem mass spectrometry (MALDI-MS/MS) and reversed-phase liquid chromatography coupled to electrospray ionization (RPLC-ESI) and MS/MS, the lipidic binders were investigated by MALDI-MS carried out on the chloroform phase upon a conventional Bligh and Dyer extraction. Free fatty acids and the mixture of triacylglycerols of the drying oils were examined using 4-chloro-α-cyanocinnamic acid as MALDI matrix. Micro-samples, previously examined by Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR), were used to investigate both proteinaceous and lipidic paint layers. Both the protocols were rapid and micro-invasive and allowed us to gain information from minute amounts (*i.e.*, micrograms) of sample relying on the high selectivity and sensitivity[4] of MALDI-MS and RPLC-ESI-MS. As expected, the presence of drying oils, most likely linseed oil from flax (*Linum usitatissimum*) and peptides of egg proteins in sampled points, was ascertained. The stratigraphy of samples was successfully investigated by ATR-FTIR and the identity of pigments was recognised as in the case of light golden ochre mixed with drying oil.

References

1. Dallongeville, S.; Garnier, N.; Rolando, C.; Tokarski, *C. Chem. Rev.* 116 (2016), , 2–79.
2. Calvano, C. D.; Rigante, E. C. L.; Cataldi, T. R. I.; Sabbatini, L. *Anal. Chem.* 92 (2020), 10257–10261.
3. Van Der Werf, I. D.; Calvano, C. D.; Palmisano, F.; Sabbatini, L. *Anal. Chim. Acta* 718 (2012), 1–10.
4. Calvano, C. D.; van der Werf, I. D.; Palmisano, F.; Sabbatini, L. *Anal. Bioanal. Chem.* 408 (2016), , 6957–6981.

A novel MEPS-UHPLC-MS/MS analytical platform for the analysis of salivary oxylipins in heart failure patients

D. Biagini[1], S. Ghimenti[1], T. Lomonaco[1], C. Vigor[2], C. Oger[2], T. Durand[2], R. Fuoco[1], F. Di Francesco[1]

[1] Department of Chemistry and Industrial Chemistry, University of Pisa, Via Giuseppe Moruzzi 13, Pisa, Italy;
[2] Insitut des Biomolécules Max Mousseron IBMM, UMR 5247 CNRS, Université de Montpellier, ENSCN, France.

Keywords: *oxylipin, micro-extraction by packed sorbent, heart failure*

Oxylipins are powerful bioactive lipid mediators generated from both ω-3 and ω-6 pol-yunsaturated fatty acids (PUFAs) through enzymatic (*e.g.* prostanoids, epoxy and hy-droxy fatty acids) and non-enzymatic (*e.g.* isoprostanes) oxidation reactions [1]. The production of these lipid mediators is considerably increased during inflammation and oxidative stress, which play a key role in the pathogenesis and pathophysiology of a great number of diseases, as Heart Failure (HF) [2]. Heart Failure is a complex clinical syndrome caused by a wide range of cardiovascular disorders, such as structural or functional abnormalities of the heart. Besides the conventional HF markers, targeting oxylipins could represent an emerging way of monitoring disease severity and progression. Mass spectrometry-based profiling of lipid mediators has drawn considerable attention during recent years, notably in the active field of biomarker discovery. Therefore, the demand of analytical techniques for accurate quantification of oxylipins at very low concentration level (ppt range) is intensifying.

This work illustrates the very reliable combination of micro-extraction by packed sorbent technique (MEPS) and ultra-high performance liquid chromatography coupled to electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS/MS) for the de-termination of salivary oxylipins. The proposed analytical method was fully validated and guaranteed excellent analytical performances, *i.e.* precision (RSD ≤ 10%), recovery (90-110 %) and LODs in the range of 10-100 pg mL-1, by substantially reducing the extraction time (20 min), the required volume of both solvents (30-500 µL) and sample (100-500 µL). The straightforward application of the present method for the monitoring of HF patients is widely displayed, furnishing a comprehensive description of lipid mediator rearrangement upon HF acute episodes. The importance of lipid mediators in guiding disease progression and responsiveness to the therapy is further highlighted.

References

1. P. B. Curtis-Prior, ed. *The eicosanoids*. Chichester: John Wiley & Sons (2004).
2. J. M. Galano, J M, *et al.; Prostaglandins & other lipid mediators*, 107 (2013), 95-102.

A metabolomic approach reveals hippuric acid as a possible hallmark of frailty

G. De Simone[1], *Roberta Pastorelli*[1], *Laura Brunelli*[1]

[1]Mario Negri Institute for Pharmacological Research – IRCCS, Milan, Italy - Department of Environmental Health Sciences

Keywords: *Frailty, Metabolomics, Hippuric acid*

Aging is a natural biological event that has some downsides though, as the increase of frailty, the decline in cognitive and physical functions, leading to chronical diseases and lower quality of life. Underlying frailty status are alterations of many biological processes, including chronic inflammation, energy imbalance, oxidative stress and mitochondrial dysfunction. [1]
The aim of the present study is to identify possible plasma biomarkers of frailty in an ongoing longitudinal elderly population-based study in Italy (Invece.Ab). Plasma sample from 130 individuals, aged between 76 and 78 years old and ranked into Fit and Frail groups using the Frailty Index, [2] was profiled using untargeted and targeted mass spectrometry (MS)-based metabolomics approaches.
We identified hippuric acid, the glycine conjugate of benzoic acid derived from microbial degradation of polyphenolic dietary compounds [3], as the only metabolite able to discriminate between Fit and Frail elderly. Subsequently, a liquid chromatography coupled with selected reaction monitoring (SRM) mass spectrometry method was developed to validate this result derived from the untargeted strategy. Hippuric acid concentration significantly drops in Frails relative to Fits. Mediation analysis using Frailty Index, hippuric acid levels and fruit-vegetable intake suggested the role of fruit-vegetable consumption in modulating the hippuric acid relationship with Frailty Index. Furthermore, logistic regression analysis showed that high hippuric acid levels significantly reduced the risk of frailty over four years.
Overall, these findings highlighted the relevance of hippuric acid level in plasma as plausible hallmark of frailty status related to low fruit-vegetable intake and diet-gut-microbe rearrangement.

References

1. Pan Y, Ji T, Li Y & Ma L; *Clin Chim Acta* 510 (2020), 363–372
2. Rockwood K. & Mitnitski A. J*; Gerontol A Biol Sci Med Sci*. 62 (2007), 722–727
3. Lees HJ, Swann JR, Wilson ID, Nicholson JK & Holmes E; *J Proteome Res* 12 (2013), 1527–1546

Development of a method for the quantification of estrone and estradiol in serum by LC-MS/MS

V. Fiorini[1], F. Villanelli[1], G. Bartolucci[2], G. Danza[1]

[1] Department of Experimental and Clinical Biomedical Sciences "Mario Serio"
[2] Department of Neurosciences, Psychology, Drug Research and Child Health
University of Florence

Keywords: *Estrone, Estradiol, Tandem mass spectrometry*

Estrogens are the main female sex hormones and play a key role both in physiological processes and in some pathological conditions. They are present in both sexes, but their serum levels are much higher in fertile women. In pre-menopause the most abundant estrogen is estradiol (E2), while in post-menopause estrone (E1) prevails. In addition to intervening in the development of primary and secondary sexual functions, the beneficial effects of estrogens are reflected on various organs and tissues. However, elevated estrogen levels in women have been associated with breast cancer [1] and ovarian cancer [2], while in men, an imbalance between estrogens and androgens appears to be associated with the development of prostate cancer [3]. Their dosage is therefore necessary but the immunoassays currently used in clinical laboratories result inaccurate because of their cross-reactivity and are not sufficiently sensitive to detect concentrations <10 pg/ml typical of menopausal and male serum samples. For this purpose LC-MS/MS methods have been developed for their quantitative determination and are actually indicated as the best choice for diagnostic purposes . Unfortunately the low levels of serum estrogens and difficulties in the ionization of these molecules force to introduce time and cost expensive sample purification and concentration steps that are unsuitable for a routine method. To overcome this problem we set up a method that involves a simplified sample preparation through a single LLE with hexane /Ethyl acetate 75/25, supported by an on line 2D chromatography consisting of a trapping/purification step on a C18 luna 5□m 20x2mm, , and a separation step on a 3 □m 50x2mm PFP analytical column. The mobile phases consist of NH4F 0.2mM in water, and methanol [4]. The analysis was performed with a mass spectrometer AB Sciex 6500 QTRAP with an ESI source, operating in negative ion mode; E2-d3 and E1-d4 are used as ISTD and two transitions for each analyte are acquired and starting serum volume is 300 □l. Method validation showed an excellent sensitivity (E1: LOD 1.8 pg / mL and LOQ 5.3 pg / mL; E2 LOD 1.2 pg / mL and LOQ is 3.7 pg / mL) and a very good precision and accuracy (Intra-assay% CV is 7% and inter-assay% CV = 2% and accuracy% 95% at 83 pg / mL for E2). Matrix effect% and recovery% calculated at100pg / ml for E2 are 46.2% and 80.9% respectively. Statistical comparison with the Elecsys Estradiol III immunoassay method produced by Roche Diagnostics showed good overall correlation (R2= 0.97) however becoming decisively less evident (R2= 0,61) in the low concentration range 5-25 pg/ml were the immunoassay is less sensitive and precise. The method performances and the easy and fast sample preparation allow its introduction in the routine of a clinical laboratory.

References

1. R.J. Santen, W. Yue, J.-P. Wang; *Steroids*, 99 (2015) 61–66.
2. M. Zahid, C.L. Beseler, J.B. Hall *et al.*; *Int. J. Cancer*, 134 (2014) 2414–2423.
3. A. Black, P.F. Pinsky, R.L. Grubb, *et al.*; *Cancer Epidemiol. Biomarkers Prev*. 23 (2014) 2374–2382
4. T. Fiers, B. Casetta, B. Bernaert *et al*; *J Chromatogr B*, 893-894 (2012) 57-62.

Mass Spectrometry Imaging as a tool to investigate region specific lipid alterations in symptomatic human carotid atherosclerotic plaques

F. Greco[1,2], L. Quercioli[3], A. Pucci[4], S. Rocchiccioli[5], M. Ferrari[3], F.A. Recchia[1,6], L.A. McDonnell[2]

[1] Institute of Life Sciences, Sant'Anna School of Advanced Studies, 56127 Pisa, Italy;
[2] Fondazione Pisana per la Scienza ONLUS, 56017 San Giuliano Terme (PI), Italy;
[3] Department of Vascular Surgery, Azienda Ospedaliero Universitaria Pisana, 56124 Pisa, Italy;
[4] Department of Histopathology, University Hospital, 56124 Pisa, Italy;
[5] Institute of Clinical Physiology, National Research Council, 56124 Pisa, Italy;
[6] Cardiovascular Research Center, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA

Keywords: *MALDI MSI; atherosclerosis; plaque outcome.*

Atherosclerosis is characterized by fatty plaques in large and medium sized arteries [1]. Their rupture can causes thrombi, occlusions of downstream vessels and adverse clinical events [2]. The investigation of atherosclerotic plaques is made difficult by their highly heterogeneous nature. Here we propose a spatially resolved approach based on matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging to investigate lipids in specific regions of atherosclerotic plaques. The method was applied to a small dataset including symptomatic and asymptomatic human carotid atherosclerosis plaques. Tissue sections of symptomatic and asymptomatic human carotid atherosclerotic plaques were analyzed by MALDI mass spectrometry imaging (MALDI MSI) of lipids, and adjacent sections analyzed by histology and immunofluorescence. These multimodal datasets were used to compare the lipid profiles of specific histopathological regions within the plaque. The lipid profiles of macrophage-rich regions and intimal vascular smooth muscle cells exhibited the largest changes associated with plaque outcome. Macrophage-rich regions from symptomatic lesions were found to be enriched in sphingomyelins, and intimal vascular smooth muscle cells of symptomatic plaques were enriched in cholesterol and cholesteryl esters. The proposed method enabled the MALDI MSI analysis of specific regions of the atherosclerotic lesion, confirming MALDI MSI as a promising tool for the investigation of histologically heterogeneous atherosclerotic plaques.

References

1. A.J. Lusis; *Nature*, 407 (2010), 233-241.
2. G.L. Basatemur, H.F. Jørgensen, M.C.H. Clarke, M.R. Bennett and Z. Mallat; *Nat Rev Cardiol*, 16 (2019), 727-744

An innovative UHPLC-MRM method for monitoring the cholesterol synthetic pathway in biological samples

A. Lanno[1], *R. Bagnati*[1], *A. Passoni*[1]

[1]Mario Negri Institute for Pharmacological Research – IRCCS, Milan, Italy
Department of Environmental Health Sciences

Keywords: *UHPLC-MRM, oxysterols, neurodegeneration*

One of the latest evidences in neurodegenerative disease research is the linkage between the alteration of brain cholesterol metabolism and neurodegeneration [1]. The brain cholesterol synthetic pathway is essential to maintain the cholesterol levels, since the blood-brain barrier prevents the uptake of cholesterol from blood circulation. Brain cholesterol is involved in synaptic functions and several studies have shown that cholesterol precursors and metabolites levels are reduced in patients affected by neurodegenerative diseases [2] [3]. The analytical challenge is the development of a method that can measure all cholesterol metabolites and the synthetic intermediates (which have different polarities) in a single analysis. The most appropriate technique to achieve this goal is hyphenated mass spectrometry (MS), in which a gas or a liquid chromatograph are coupled to a mass spectrometer. The aim of this work is the development of a new Ultra High Performance Liquid Chromatography – Mass Spectrometry (UHPLC-MS) method to determinate oxysterols and cholesterol precursors in biological samples. In biological matrices, sterols can be found in two different forms: free or esterified to several fatty acids. For the determination of the total sterol levels it is necessary to hydrolyse samples before the analysis. The method we have developed consist of an alkaline hydrolysis followed by the extraction and concentration of samples. Unlike methods described in the literature [4], we decided to not include a purification step with solid-phase extraction (SPE) or a sterol derivatisation step; despite this, we have obtained a detection sensitivity (10 ng/mL in plasma) comparable to that of other published methods.

The analytical method was developed using a liquid chromatographic system (Nexera 2) coupled to a triple quadrupole mass spectrometer (Shimadzu LCMS-8060), operating in Multiple Reaction Monitoring (MRM) mode. The separation of the analytes was obtained with a reverse phase column, using a segmented gradient coupled with a temperature ramp to help the elution of the less polar cholesterol precursors in less than 35 minutes.

The newly validated method can be used to measure the levels of cholesterol precursors and metabolites in plasma during clinical monitoring of patients, but also for the characterization of animal models of several neurodegenerative disease such as Huntington's or Alzheimer diseases.

References

1. A. M. Petrov, M. R. Kasimov, A. L. Zefirov; *Acta naturae*, 8 (2016), pp 58-73.
2. M. Markianos, M. Panas, N. Kalfakis, D. Vassilopoulos; *Molecular Genetics and Metabolism*, 93 (2008), 341-346.
3. V. Leoni, C. Mariotti, L. Nanetti *et al.*; *Neuroscience Letters*, 494 (2011), pp 245-249.
4. W. J. Griffith, J. Abdel-Khalik, P. J. Crick, E. Yutuc, Y. Wang; *Journal of Steroid Biochemistry & Molecular Biology*, 162 (2016), pp 4-26.

Determination of salivary short chain fatty acids and hydroxy acids in heart failure patients by in-situ derivatization and Hisorb-probe sorptive extraction coupled to thermal desorption and gas chromatography-tandem mass spectrometry

A. Lenzi[1], *D. Biagini*[1], *S. Ghimenti*[1], *F. Vivaldi*[1], *A. Bonini*[1], *P. Salvo*[2], *R. Fuoco*[1], *F. Di Francesco*[1], *T. Lomonaco*[1]

[1] Department of Chemistry and Industrial Chemistry, University of Pisa, Via G. Moruzzi 13, Pisa, Italy
[2] Institute of Clinical Physiology, CNR, Via G. Moruzzi 3, Pisa, Italy

Keywords: *Short chain fatty acids; HiSorb; Heart failure;*

Short chain fatty acids (SCFAs) are the predominant products of dietary fiber fermentation operated by gut microbiota. Their variation in human can emphasize the predisposition to metabolic diseases. Studies have also highlighted their implication in the regulation of blood pressure, and in the increased risk of Heart Failure (HF) [1]. On the other hand, compounds unrelated to microbiota activity, such as 3-hydroxybutyric acid and lactic acid, are used as energy sources during acute energies crisis. In the last few years, a link between oral and gut microbiomes was underlined. Differences in the oral metabolome, as for example in the SCFAs family of compounds, are known to be correlated to the health of salivary microbiome, which is also related to the development of a low-grade inflammation in the host, and consequently to potential increasing risks of cardiovascular diseases [2].

The aim of this work was to develop and validate an analytical procedure based on an innovative single step in-situ derivatization with pentafluorobenzyl bromide (PFB-Br) and HiSorb-probe sorptive extraction for the determination of a panel of low-molecular weight salivary metabolites (SCFAs, 3-hydroxybutyric acid, and lactic acid). Reaction's derivatives released from HiSorb probe by thermal desorption were analyzed by gas chromatography-tandem mass spectrometry. A Central Composite Face-Centered experimental design was used for the optimization of the molar ratio between PFB-Br and target analytes, the derivatization temperature and time which resulted respectively 100, 60 °C and 180 min. A sample volume of 20 µL of saliva guaranteed limits of detection between 0.1-100 µM. Intra- and inter-day precision and recovery were in the range of 10-15% and 70-98%, respectively, thus highlighting the reliability of the method.

The validated method was employed as a proof-of-concept method to monitor and compare SCFAs and hydroxy acids collected from saliva of 13 HF patients during hospitalization, with the aim to preliminary evaluate the role of these compounds as potential salivary indicators of the course/progression of the disease.

References

1. L.B. Richards, M. Li, B.C.A.M. van Esch, J. Garssen, G. Folkerts; *PharmaNutrition*, 4 (2016), pp 68-111.
2. M. Pietiäinen, J.M. Liljestrand, E. Kopra, P.J. Pussinen, *Eur. J. Oral Sci.*, (2018), pp 26-36.

A new LC-MS/MS method for the quantification of 1-Hydroxypyreneglucuronide in urine samples

C. Maccari[1], *R. Andreoli*[1], *M. Corradi*[1,]

[1] Laboratorio di tossicologia industriale e metodiche analitiche avanzate, Dipartimento di Medicina e Chirurgia, Università di Parma

Keywords: *1-Hydroxypyreneglucuronide, LC-MS/MS, urinary biomarker*

Polycyclic aromatic hydrocarbons (PAHs) are a complex mixture of carcinogenic and non-carcinogenic substances present in environmental air pollution and in various industrial processes, usually a very low levels of concentrations.
In the case of exposure to a complex mixture of compounds, it is essential to identify a tracer, that is representative of all the PAHs and of the exposure levels. It is also very important that this biomarker is sufficiently sensitive to characterize the exposure of both the general population and workers, and to characterize different confounding factors such as smoking habits, food, lifestyles. Pyrene is the most representative PAHs and 1-hydroxypyreneglucuronide (1-OHPG) is the mainly urinary metabolite. In occupational medicine, the biomarker used to characterize PAHs exposure is 1-hydroxypyrene after enzymatically hydrolysis of the urine samples.
The aim of the Project is to develop a method that allows to dose 1-OHPG in the urinary matrix with the least possible pre-analysis treatment, without enzymatic hydrolysis or SPE purification, in order to reduce the possibility of artifacts, the loss of compound and optimization of time. To achieve this goal, liquid chromatography associated with mass spectrometry is certainly very helpful [1].
Moreover, urinary sample is a complex matrix full of confounding factors, so it is necessary to characterize matrix effect and separate 1-OHPG from all matrix components.
During the development of the method, both the UPLC and MS/MS parameters were optimized to obtain the maximum sensitivity, evaluating different operating flows, columns, mobile phases, acid or basic environments and different ionization conditions.
Interferers from the matrix were observed mainly in negative ionization mode and as a function of urinary pH; for this reason, after evaluating both the positive and negative ionization and different pH conditions, it was decided to resort to monitoring the adduct with ammonium in positive ion mode [2].
The use of a labelled internal standard (1-OHPG-d_9) in the analysis increased the specificity of the method and confirmed the adduct's stability.

References

1. K. Kakimoto, A. Toriba, T. Ohno, M. Ueno, T. Kameda, N. Tang, K. Hayakawa, *Journal of Chromatography B*, 961 (2002);107-112
2. J. Srneraglia, S. I Baldrey, D. Watson, Huntingdon Life Sciences, Alconbury, Huntingdon, Cambridgeshire,

Phosphatidic acid methyl esters as markers of phospholipid profile alterations induced by phospholipase D during lipid extraction from microgreen crops: a hydrophilic interaction liquid chromatography – mass spectrometry study

A. Castellaneta[1], I. Losito[1,2], V. Losacco[1], B. Leoni[3], P. Santamaria[3], C.D. Calvano[2,4], T.R.I. Cataldi[1,2]

[1]Dipartimento di Chimica, [2]Centro Interdipartimentale SMART, [3]Dipartimento di Scienze Agro-Ambientali e Territoriali, [4]Dipartimento di Farmacia e Scienze del Farmaco, Università degli Studi di Bari "Aldo Moro", via Orabona 4, 70126 Bari, Italy.

Keywords: Lipid extraction, phospholipase D, phosphatidic acid methyl esters.

The application of mass spectrometry (MS), often coupled to liquid chromatography (LC), greatly contributed to unveil the manifold roles that glycerophospholipids (GPLs) play in plant cells. GPLs are not just structural elements of bio-membranes; they also appear as dynamic informational elements in the complex network of molecular signalling [1]. Nevertheless, special care should be paid when GPLs are extracted from plant tissues. Indeed, the uncontrolled activation of endogenous phospholipases may introduce both qualitative and quantitative artifacts. Recently, we have recognized a relevant activity of phospholipase D (PLD) during lipid extraction based on the Bligh and Dyer (BD) [2] protocol performed on five different oleaginous microgreen crops, namely soy, flax, chia, rapeseed and sunflower. PLD is a transphosphatidylase, able to catalyze an hydrolysis reaction if water is the acceptor, transforming the substrate GPL in the corresponding phosphatidic acid (PA) [3]. However, since methanol is widely available as an alternate acceptor in BD extraction conditions, a transphosphatidylation reaction occurs and phosphatidic acid methyl esters (MPAs) are generated. Since MPAs are perfectly isobaric with PAs having an odd number of side chain C atoms, they cannot be distinguished in the *m/z* domain. Nevertheless, they can be easily separated by the use of hydrophylic interaction liquid chromatography (HILIC), since the retention mechanism on the HILIC stationary phase is mainly attributable to the GPL polar head structure [4]. We identified both PAs and MPAs as artifacts since the corresponding HILIC bands disappeared when microgreen tissues were suspended in isopropanol rather than in water prior to grinding. Indeed, isopropanol is known to strongly inhibit the PLD activity [5]. Thus, MPAs can be considered a marker of artificial lipidome modifications triggered by PLD. Moreover, we employed HILIC coupled to tandem and sequential mass spectrometry (HILIC-ESI-MS^n, with n = 1-3) to fully assess the regiochemistry of substitution of the glycerol moiety in MPAs. In addition, we recognized both phosphatidylcholines (PCs) and phosphatidylglycerols (PGs) as the main PLD substrates for MPAs formation.

References

1. T. Munnik, R.F. Irvine, A. Musgrave; *Biochimica et Biophisica Acta*, 1389 (1998), pp 222-272.
2. *E.G.* Bligh, W.J. Dyer; *Canadian Journal of Biochemistry and Physilogy*, 37 (1959), pp 911-917.
3. S.F. Yang, S. Freer, A.A. Benson; *Journal of Biological Chemistry*, 242 (1967), pp 477-484.
4. I. Losito, E. Conte, T.R.I Cataldi, N. Cioffi, F.M. Megli, F. Palmisano, *Lipids* 50 (2015), pp 71-84.
5. I.A. de la Roche, C.J. Andrews, M. Kates; *Plant Physiology*, 51 (1973), pp 468-473.

Unambiguous positional assignment of double bonds in unsaturated fatty acyl chains of arsenosugar phospholipids (As-PL) in wakame seaweed by LC-ESI multistage mass spectrometry

D. Coniglio[1], C.D. Calvano[2,3], G. Ventura[1], I. Losito[1,2], T.R.I. Cataldi[1,2]

[1]Dipartimento di Chimica, [2]Centro Interdipartimentale SMART and [3]Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari Aldo Moro, via Orabona 4, 70126 Bari (Italy)

Keywords: *seaweeds, lipids, LC-ESI-MS*

Algae have been receiving much attention in general due to their high-quality lipid content, which makes algae production and application by the food industry very appealing [1]. Wakame seaweed (*Undaria pinnatifida*) is an edible marine macroalga largely consumed for centuries as foodstuffs being tasty and rich in benefits [2]. However, little attention has been paid to soluble arsenolipids and polar arsenophospholipids (As-PL) [3] for their possible involvement in membrane biochemistry and potential human health issues related to As toxicity. The detailed characterization of unsaturated fatty acyl chains of As-PL [3] occurring in wakame seaweed chiefly focussing on the identification of the double bond (DB) position will be introduced.
Lipid fraction was firstly extracted from rehydrated specimens obtained upon Bligh-Dyer extraction [4]. Then, a derivatization reaction was carried out using a chloroform suspension (0.12 M) of *meta*-chloroperoxybenzoic acid (*m*-CPBA) to generate epoxide species of unsaturated fatty acyl chains [5]. Upon ca. 15 minutes, the reacted sample was analysed by liquid chromatography using a silica-based hydrophilic interaction liquid chromatography (HILIC) column coupled with electrospray ionization (ESI) and multistage mass spectrometry (MS^n, n=2, 3). Deprotonated epoxidized species, $[epoM\text{-}H]^-$, were examined by a linear ion trap (LIT) instrument (VelosPro) with collision-induced dissociation (CID). Since little to no reliable evidence of the DB positions was provided by tandem mass spectra (MS/MS) of derivatized reaction products, the investigation was accomplished by CID-MS/MS/MS of gas-phase isolated fatty acids $[epoFA\text{-}H]^-$, which arose from the fragmentation pattern of As-PL. Pairs of product ions featuring the key structural information on the acyl chain double bond positions were successfully identified [5]. The complete assignment of each As-PL was obtained through a combined approach of chemical reactions, HILIC separation and multistage MS. In this communication the most important experimental results will be presented and discussed.

References

1. S. Ścieszka and E. Klewicka, *Critical Reviews in Food Science and Nutrition*, 59 (2019), 3538-3547
2. G.B. Martínez–Hernández, N. Castillejo, M. del M. Carrión–Monteagudo, F. Artés, and F. Artés-Hernández, *Food Science and Technology International*, 24 (2018), 172–182.
3. D. Coniglio, C.D. Calvano, G. Ventura, I. Losito, and T.R.I. Cataldi, *Journal of the American Society for Mass Spectrometry*, 31 (2020) 1260-1270.
4. *E.G.* Bligh and W.J. Dyer, *Canadian Journal of Biochemistry and Physiology*, 37 (1959), pp 911-917.
5. Y. Feng, B. Chen, Q. Yu, and L. Li, *Analytical Chem*istry, 91 (2019), pp 1791−1799.

Detection of products of the reaction between 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) and common antioxidants by high-resolution mass spectrometry

L. Angeli[1], S. Imperiale[1], Y. Ding[1], M. Scampicchio[1], K. Morozova[1]

[1] Faculty of Science and Technology, Free University of Bozen-Bolzano,
Piazza Università 1, 39100 Bolzano, Italy

***Keywords**: high-resolution mass spectrometry, reaction products, side reaction*

Food antioxidants play an important role in improving public health since they help to fight oxidative stress in cells. In vitro assays are usually applied to evaluate such activity in food matrices. The 2,2-diphenyl-1-picrylhydrazil (DPPH•) is a stable radical used to assess the antioxidant activity of food products and extracts. A kinetic model of second order follows the absorption decay of DPPH• at its maximum of 515 nm during the reaction with antioxidants [1]. Such reaction can follow a simple model or a more complex model employing a secondary reaction, related to the development of reaction products.
High-resolution mass spectrometry (LC-MS) was used to analyze the reaction products of common antioxidants with the DPPH radical. A reaction mixture with an excess of DPPH• was injected in the LC system after 1 h in the dark [2]. The MS operated in negative ionization mode with a capillary voltage of 4.5 kV at a temperature of 350 °C. Full-MS analysis was carried out at a resolution of 70,000 and the scan range was set at 135-1500 *m/z* and the AGC Target at 2e5. For data-dependent analysis (dd-MS2) the instrument operated at a resolution of 17,500 with AGC Target set at 1e5. Some antioxidants, such as gallic, ferulic and caffeic acid, showed complex reaction products that are justified by the presence of a side reaction in the kinetic model. For other antioxidants, such as ascorbic acid, Trolox and sinapic acid the oxidized form or a dimer were found. Indeed, they did not show any side reaction in the kinetic model.
In summary, this study describes a promising kinetic approach coupled to mass spectrometry to monitor the antioxidant activity of food antioxidants and to analyse the mechanism of radical reactions.,

References

1. M. C. Foti et al; *Journal of Organic Chemistry*, 69 (2004), 2309-2314.
2. S. B.R. Berton *et al.*; *Industrial Crops & Products*, 154 (2020), 112701.

Accelerated dehydration of D-fructose performed in microdroplets by a commercial ESI Z-spray source

C. Salvitti[1], *A. Troiani*[1], *F. Pepi*[1]

[1] Dipartimento di Chimica e Tecnologie del Farmaco, "Sapienza" Università di Roma, P.le Aldo Moro 5, 00185 Roma, Italy

Keywords: *ambient ionization, microdroplets, D-fructose dehydration*

Besides its use as an analytical tool, mass spectrometry (MS) has long been employed in reaction monitoring to intercept elusive intermediates and highlight the mechanistic details of a chemical transformation. The introduction of electrospray ionization (ESI) by Fenn *et al.* [1] enables one to directly generate from a diluted aliquot of a reaction mixture a plume of charged droplets showing a diameter less than 1,0 μm. Once desolvated, the microdroplets release isolated ionic species that provide an accurate picture of the reaction progress in solution. Interestingly, the desolvation time of the charged droplets can be easily increased in the air by increasing the distance between the ESI source and the MS inlet. Such a dramatic change of the reaction conditions can indeed accelerate the reaction rate up to 10^5 times compared to the same process occurring in bulk [2]. As a consequence, the ionized reagents, typically detected by MS at short distances, are promptly replaced by the reaction intermediates or even by the ionized products.

Since several milestone reactions of organic chemistry have recently benefited from acceleration in confined volumes [3], we studied the dehydration reaction of D-fructose in microdroplets under ambient conditions. Furan derivatives, such as 5-hydromethylfuraldehyde (5-HMF), are indeed produced by the acid-catalysed dehydration of hexoses, thus obtaining key-building block molecules from "green" resources [4]. To this end, we used a commercial ESI Z-spray source of a Q-TOF Ultima mass spectrometer already employed in our laboratory to successfully modify electrode surfaces by ambient ion soft landing experiments [5]. High conversion ratios of D-fructose into 5-HMF were obtained by using $KHSO_4$ metal-free and green catalyst in millimolar concentrations. Nonetheless, the reaction outcome was found to be highly sensitive to the catalyst and solvents employed, as well as to the ionization and desolvation parameters of the ESI source. Further attempts to scale up the reaction for potential industrial application are actually in progress in our laboratory.

References

1. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, C. M. Whitehouse; *Science*, 246 (1989), pp 64-71.
2. X. Yan, R. M. Bain, R. G. Cooks, *Angewandte Chemie Int. Ed.*, 55 (2016), pp 12960-12972.
3. Z. Wei, Y. Li, R. G. Cooks, X. Yan, *Annual Review of Physical Chemistry*, 71 (2020), pp 31-51.
4. A. Shrotri, H. Kobayashi, A. Fukuoka, *Advances in Catalysis*, 60 (2017), pp 59-126.
5. C. Salvitti, A. Troiani, F. Mazzei, C. D'Agostino, R. Zumpano, C. Baldacchini, A. R. Bizzarri, A. Tata, F. Pepi, *International Journal of Mass Spectrometry*, submitted (2021).

MS-based molecular networking: a modern strategy for the fast detection of new natural products

S. Scarpato[1], R. Teta[1], A. Mangoni[1]

[1]Department of Pharmacy, University of Naples Federico II, via D.Montesano 49, 80131, Naples, Italy

Keywords: *marine sponges; dereplication; molecular networking*

In natural product research, extracts of marine micro- and macro-organisms are the most prolific source of bioactive compounds with diverse and unique structures. The current issue in natural product drug discovery is the rapid dereplication of known compounds from complex mixtures and the consequent identification of new structural analogues. Dereplication can be best approached using liquid chromatography coupled with tandem mass spectrometry (LC-HRMS/MS). However, this technique provides huge amounts of data, which prevents any efficient data analysis based on visual inspection. Molecular networking is an innovative computational approach based on liquid chromatography/tandem mass spectrometry (LC−MS/MS) data, giving as result a visual representation of the structural similarity between molecules, as inferred by the relatedness of their LC−MS/MS data [1]. The metabolites are visually represented in a diagram (the network) where similar metabolites (the nodes) are connected by lines (the edges). Furthermore, each LC-MS/MS spectrum is also compared with MS/MS spectral archives of known natural products. The powerful complementation of molecular networking with traditional dereplication strategies was proved in the study of extracts from the marine sponge *Stylissa caribica*, in search of new bioactive secondary metabolites. Although the Bahamian sponge *Stylissa caribica* has been thoroughly investigated, having shown to contain a wide variety of cyclic peptides and different brominated alkaloids, the molecular networking approach led to the fast detection of a new cyclic heptapeptide, stylissamide L [2]. The structure elucidation of stylissamide L including the cis/trans geometry of the three proline residues was determined by extensive NMR studies. Moreover, the L configuration of the seven amino residues was confirmed using advanced Marfey's methodology.

References

1. Wang, M. *et al.*, *Nat. Biotechnol*., 2016, *34*, 828–837 (2016).
2. S. Scarpato, R. Teta, G. Della Sala, J.R. Pawlik, V. Costantino, A. Mangoni, *Mar Drugs.*, 2020, 27;18(9): E443.

Characterization of *Rumex abyssinicus*, a Traditional Rwandan Medicine

C. Spaggiari [1,2], L. Righetti[1],G. Annunziato[1], R. Bruni[1], G. Costantino[1]

[1] Department of Food and Drug, University of Parma, Parma, Italy
[2] INES-Ruhengeri, Institute of Applied Sciences, Musanze, Rwanda

Keywords: *Traditional Healers Knowledge, Medicinal Plants, Untargeted Metabolomics*

Due to the favorable climate condition characterized by dry and rainy seasons, Rwanda is a prominent fertile ground for cultivation and growth of different medicinal plants.
Medicinal plants are integral parts of life in many communities in Rwanda, about 80% of rural population still rely on traditional herbal medicines due to the limited availability and affordability of pharmaceuticals. Natural products had played an important role throughout the world in treatment and prevention of human diseases. Indeed, many food supplements and *cosmaceuticals* are still produced from plants. The aim of our study is to provide a scientific rationale for *Rumex abyssinicus*, which is one of the commonest medicinal plant used by traditional healers of Rwanda. By applying high-resolutions mass spectrometry based-metabolomics we discriminate the metabolite pattern of the different rumex organs (stems, leaves and flowers). Chemometric evaluation revealed an optimal sample clustering according to the rumex organs and the presence of several significant markers able to discriminate the groups. Among the metabolites resulted statistically significant *xanthones* and *chromenones* derivatives might be the responsible of the broad- spectrum antimicrobial activity of flowers.
The result demonstrates that untargeted metabolomics, in conjunction with *in vitro* antimicrobial assays have the potential to validate and promote traditional Rwandan medicine.

References

1. T. Eklov, P. Mårtensson, I. Lundstrom; *Analytica Chimica Acta*, 381 (1991), pp 221-232.
2. F. Takens, D. Rand and L.S. Young editors, *Dynamical Systems and Turbulence*, Warwick 1980, pp 36-38.

Determination of carbazole alkaloids in *Murraya Koenigii* by means of LC-MS/MS analysis with a predictive multi experiment approach

E. Viteritti[1], *F. Fanti* [1], *E. Oliva*[1], *S. Palmieri*[1], *F. Eugelio*[1], *A. Pepe*[1], *M. Sergi*[1], *D. Compagnone*[1]

[1]University of Teramo, Faculty of Bioscience and Technology for Food, Agriculture and Environment, Teramo, Italy.

Keywords: *Carbazole alkaloids; HPLC-MS/MS; MRM-IDA-EPI*

Murraya Koenigii is recognized as the most significant Indian medicinal plant due to its huge spectrum of therapeutic properties. In fact, different parts of *M. koenigii*, such as its leaves, root, bark, and fruit, are known to promote various biological activities including anti-inflammatory and antifungal. The properties of *M. koenigii* leaves have been attributed to several chemical constituents such as Carbazole alkaloids and other important metabolites [1]. Carbazole alkaloids are a class of tricyclic hetero-aromatic alkaloids, with two benzene rings fused onto a pyrrole ring structure, but it is still difficult to achieve a full classification of these compounds because of their heterogeneous structures. For this reason, it was necessary the development of new strategies for the identification of carbazole alkaloids [2]. Recently, they were found in higher plants and other natural sources such as microorganisms; their structures were determined by analysis of high resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) spectroscopic method. The analysis is usually performed in untargeted mode through quadrupole time-of-flight (Q-ToF) [1]. In this work, a sensitive method involving the use of liquid chromatography (LC) coupled to a hybrid triple quadrupole-linear ion trap mass spectrometer was developed, with the aim of the simultaneous quantification and determination of three target analytes (Mahanimbine, Koenimbine and Koenigicine) and a putative identification for other compounds belonging to the huge family of carbazole alkaloids. It was developed an efficient extraction procedure followed by a suitable clean-up step, in order to obtain reliable recoveries (resulted for all the analytes from 60% to 85%). The analyses was performed by HPLC-MS/MS using predictive multi experiment approach coupling multiple reaction monitoring (MRM), information-dependent acquisition (IDA) and enhanced product ion scan (EPI). Competitive Fragmentation Modeling-ID (CFM-ID) was used to predict MS/MS spectra from the chemical structures of the compounds. The obtained MRM transitions were used as survey experiment; the IDA method then was used to trigger the EPI scans by analyzing MRM signals. Dependent MS/MS spectra acquired in EPI mode for target and unknown compounds were confirmed by matching them with MS/MS spectra found in literature. The obtained results showed that the MRM-IDA-EPI method can simultaneously provide quantitative information for the target analytes and a putative identification for other compounds belonging to different classes of carbazole compounds which are not included in the target list.

References

1. Nalli, Y., Khajuria, V., Gupta, S., *et al.*; *Organic and Biomolecular Chemistry*, 14 (2016), 3322–3332.
2. Greger, H; *Phytochemistry Reviews*, 16 (2017), 1095–1153

Poster Presentations

Development and validation of an optimized UHPLC-MS/MS method for high-throughput determination of lipophilic marine toxins in fresh and processed shellfish

T. D'Amore [1], *V. Vita*[1], *A. Di Taranto*[1]

[1] Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Via Manfredonia 20, 71121 Foggia, Italy

Keywords: *lipophilic marine toxins; UHPLC-MS/MS; SPE*

Under the name of lipophilic marine toxins there are included more than 1000 toxic secondary metabolites produced by phytoplankton, with the common chemical property of lipophilicity. These phycotoxins have natural tropism for lipid-enriched and hepatopancreatic gland tissues of shellfish, mainly mussels, oysters, clams, cockles and scallops, representing a relevant health and economic concern in food system [1].

Due to their toxicological effects and geographical distribution, the European Commission set maximum permitted levels for relevant compounds, divided into four subgroups, and assigned to them the toxic equivalency factor, as recommended by the European Food safety Authority - EFSA. Their determination is accomplished with the reference method based on liquid chromatography-tandem mass spectrometry, according to the Regulation EU 15/2011 [2,3].

In this study, a modified ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method was developed for identification and quantification of all EU-regulated lipophilic toxins. Through the optimization of extraction procedure and SPE-C18 purification, matrix interferences were minimized. Improved chromatographic conditions and upgraded ammonia-based gradient ensured the best separation of all analytes and, in particular, of the two structural isomers, okadaic acid - OA and Dinophysistoxin-2 - DTX_2 ($C_{44}H_{68}O_{13}$). Moreover, different MS parameters, along with identification and confirmation criteria, were tested and finally established. The validation study confirmed that all parameters were satisfactory. Recovery values ranged from 73 to 101%, limit of quantification was 8 µg kg^{-1}, and precision, expressed as coefficient of variation, was lower than 12%. A mussel-tissue certified reference material for multiple marine toxins, CRM-FDMT1, was used for further assessment.

In recent years, a marked increase in algae proliferation events and intoxication cases was reported; concurrently, new studies of important toxic effects of these contaminants were carried out. These indications suggest that implementation of analytical methods and extraction procedures, especially for official and routine purposes, still represents an important task for public health and food control laboratories [4].

References

1. P. Visciano, M. Schirone, M. Berti, A. Milandri, R. Tofalo, G. Suzzi; *Frontiers in Microbiology,* 7:1051 (2016), pp 1-10
2. European Food Safety Authority; *The EFSA Journal*, 1306 (2009), pp 3-23.
3. Commission Regulation EU 15/2011; *Official Journal of the European Union,* L 6 (2011).
4. S. Morabito, S. Silvestro, C. Faggio; *Natural Product Research,* 32:6 (2018), pp 621-631.

Understanding of grapevine communication mediated by volatile organic compounds against downy mildew using a metabolomics approach

S. Avesani[1,2,3], V. Lazazzara[3], M. Oberhuber[2], P. Robatscher[2], M. Perazzolli[1,3]

1 Center Agriculture Food Environment (C3A), University of Trento, Via E. Mach 1, 38098 San Michele all'Adige, Italy
2 Laboratory for Flavours and Metabolites, Laimburg Research Centre, Laimburg 6, Pfatten (Vadena), 39040 Auer (Ora), Italy
3 Department of Sustainable Agro-ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38098 San Michele all'Adige, Italy

Keywords: *volatile organic compounds, downy mildew, metabolomics, grapevine*

Plants can produce a wide variety of volatile organic compounds (VOCs), which play a crucial role in the interaction with other organisms and the regulation of plant responses against stresses. Different modes of action against phytopathogens have been attributed to VOCs, such as induction of plant resistance and direct inhibition of pathogen growth. In particular, the amount of some VOCs was higher in resistant than in susceptible grapevine genotypes upon *Plasmopara viticola* inoculation, indicating their possible involvement in resistance mechanisms against this pathogen. This work aims at identifying the metabolic response of VOC-treated grapevine leaves and the potential activation VOC-mediated resistance mechanisms. Susceptible grapevine leaf disks were treated with pure VOCs produced by resistant genotypes, or with water as control. Functional analyses confirmed that two VOCs (one C_{13}-norisoprenoid and one pyrone) reduced the disease severity on downy mildew on susceptible leaf disks and leaf disks were collected at two time points (one and six days after inoculation) from VOC-treated and control samples. transcriptional analyses revealed an increased expression level of defence-related gene, such as hypersensitivity-related, osmotin, chitinase and pathogenesis-related genes. Metabolomic analyses will be applied to clarify the mechanisms of action of VOCs and the response of VOC-treated leaves using ultra-high pressure liquid chromatography-high resolution-quadrupole-time of flight-mass spectrometry (UHPLC-Q-TOF-MS) analysis.

Fatty acid profile and quantification in *Danio rerio* reared on new eco-sustainable insect-based diets

F. Girolametti[1], S. Illuminati[1], A. Annibaldi[1], I. Olivotto[1], M. Zarantoniello[1], F. Tulli[2], G. Cardinaletti[2], C. Truzzi[1]

[1]Department of Life and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy
[2]Department of Agricultural, Food, Environmental and Animal Science, Università di Udine, 33100, Udine, Italy

Keywords: *Aquaculture, Insect-based diets, Fatty acid profile*

Aquaculture is the fastest growing food production sector worldwide [1] and, based on the circular economy concept, it should become more responsible and sustainable, promoting the use of environmentally friendly ingredients in aquafeeds. Insects are considered as an alternative and sustainable ingredient for feed production [2]. In this study, *Hermetia illucens* (HI) (fed on coffee roasted by-product added with 10% microalgae *Schyzochytrium* sp., rich in polyunsaturated fatty acids) was processed into meal and used to replace fish meal (FM) in five experimental diets to be tested in a feeding trial using zebrafish (*Danio rerio*) as experimental model. The test diets were formulated by increasing the inclusion levels of full-fat HI pre-pupae meal (0, 25, 50, 75 and 100% respect to fish meal). The fatty acid (FA) profile of fish larvae (20 days), juveniles (2 months, deprived of the viscera) and adults (6 months, deprived of the viscera) specimens was studied to evaluate the impact of dietary FM replacement with full-fat HI prepupae meal. In particular, the content of Saturated (SFA), Monounsaturated (MUFA), and Polyunsaturated (PUFA) fatty acids were evaluated, together with omega-3 (n3) and omega-6 (n6) fatty acids content. The determination of FAs composition was performed optimizing an analytical methodology based on a fast microwave-assisted extraction of lipids from the lyophilized sample, a base-catalysed trans-esterification of lipid extract to obtain Fatty Acid Methyl Esters, which were identified and quantified by gas chromatography-mass spectrometry [3]. The mass fraction of fatty acids in mg g^{-1} tissue dry weight (dw) was measured using the response factor method against nonadecanoic acid methyl ester used as internal standard. Results obtained from the HI-based diets showed significantly higher content of SFA and n6, and significantly lower content of MUFA, PUFA, and n3 with respect to the control diet. Quantified FAs compute for larvae, juvenile, adult male and adult female for about 135, 235, 267 and 265 mg g^{-1} dw, respectively. As regard FA classes, PUFA (in particular 18:2n6, 20:5n3 and 22:6n3) are the most represented in all life stages, except in specimens fed with the diet containing the highest HI meal content. In fish, the increasing amount of HI prepupae meal in the diet resulted in: i) a statistically significant increase of SFA and n6, and a statistically significant decrease of PUFA and n3 in all life stages; ii) a statistically significant decrease of MUFA in larvae; iii) a statistically significant increase of MUFA and n9 in adult specimens. From the results, we can assume that adults have a different ability to synthesize fatty acids compared to the other earlier life stages. Moreover, adult female showed a higher content of PUFA with respect to adult male, which could be justified by the needs of physiological reproductive processes.

References

1. FAO. State World Fish. Aquac. 2018-Meet.
2. T. Bandara; *J. Entomol. Zool. Stud*, 6 (2018), pp 3087-3094.
3. C. Truzzi, S. Illuminati, A. Annibaldi, M. Antonucci, G. Scarponi; *Chemosphere*, 173 (2017), pp 116-123.

Identification of metabolites involved in the cold stress tolerance promoted by psychrotolerant bacteria in plants.

G. Licciardello[1,2,3], B. Vicelli[2], V. Lazazzara[1], R. Schuhmacher[3], M. Perazzolli[1,2]

[1] Department of Sustainable Agro-ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38098 San Michele all'Adige, Italy; [2] Center Agriculture Food Environment (C3A), University of Trento, Via E. Mach 1, 38098 San Michele all'Adige, Italy; [3] Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad-Lorenz-Straße 20, 3430 Tulln, Austria.

Keywords: *plant-growth promoting bacteria, psychrotolerant bacteria, cold stress*

Cold stress causes serious negative impacts on growth and yield of economically valuable crops. While some plants are adapted to cold environments, crops originating from sub-tropical regions, such as tomato, are more sensitive to cold stress. The efficacy of plant-associated microorganisms to protect plants against cold stress was reported, but scarce information is available on the molecular mechanisms underlying this process. The aim of this project is to understand the physiological mechanisms activated by psychrotolerant bacteria on tomato plants and to identify plant and/or bacterial metabolites and genes responsible for cold stress tolerance. Antarctic plants are a possible understudied source of psychrotolerant endophytic bacteria to be exploited in the plant protection against cold stress and four isolates were selected on a bacterial collection for their ability to promote tomato plant growth at low temperatures. The four bacterial isolates are currently being tested for their ability to stimulate plant physiological responses related to cold stress adaptation. In particular, the content of proline and malondialdehyde (MDA) was previously associated to cold stress in tomato plants and it will be analysed in bacterized and non-bacterized plants exposed to cold stress (7 days at 0°C) and recovery condition (0, 2 and 4 days at 25°C) using two different biochemical assays. Metabolomic and transcriptomic changes will be then analysed in bacterized and non-bacterized plants exposed to cold stress. In particular, amino acids, sugars and sugar alcohols previously associated with cold tolerance will be assessed in bacterized and non-bacterized plants using gas chromatography mass spectrometry (GC-MS) and hydrophobic interaction liquid chromatography (HILIC)-MS. The outcome of this project will provide a deeper knowledge in the mechanisms of cold adaptation enhanced by endophytic bacteria in plants.

LC-MS^n with targeted and semi-untargeted approach for the analysis of phenolic compounds in plant matrices

E. Oliva [1], E. Viteritti[1], F. Fanti[1], F. Eugelio[1], A. Pepe[1], S. Palmieri[1], M. Sergi[1], D. Compagnone[1]

[1]University of Teramo, Faculty of Bioscience and Technology for Food, Agriculture and Environment, Teramo, Italy.

Keywords: *Precursor Ion Scan, Neutral Loss, MS^3*

Polyphenol compounds (PCs) represent an important class of bioactive compounds present in plant matrices, in which they perform different biological functions, mainly related with their antioxidant capacity; it is closely linked to their structure, glycosylation or presence of other substituents. They are generated by two basic metabolic pathways: the shikimate pathway and the acetate pathway [1]. Furthermore, in plants, they perform various and important biological functions; such as defence from ultraviolet rays or from attack by pathogens [2]. Several analytical methods have been reported for the determination of PCs in plants; among the best known and most used is liquid chromatography (LC) coupled to various detectors, such as diode array detectors (DAD), UV-vis and mass spectrometry (MS). The latter is certainly the most effective for these purposes, due to high sensitivity and selectivity; in addition, high resolution instruments (HRMS) such as Q-TOF or Orbitrap MS are also used for the level of information they can provide. With LC-MS it is possible to work both through the use of standards, in targeted mode, generally combining the LC technique with low resolution MS (LRMS), as a triple quadrupole (QqQ) or linear ion trap (LIT) as detector, and by working with LC with HRMS, in untargeted mode.In this work two different acquisition approaches were used for the quantification or tentative identification of PCs in plant matrices by HPLC-MS/MS. A targeted approach, based on MRM acquisition mode, was used for the identification and quantification of a list of target analytes by comparison with standards; a semi-targeted approach was also performed by using the precursor ion scan and neutral loss for the tentative identification of further compounds not included in the target list.The analysis of phenolic compounds in three different plant matrices (curry leaves, cannabis and blueberry) was performed. The extraction and clean-up steps were set up according to the characteristics of the matrix allowing to minimize the interfering compounds present in such complex matrices, as proved by the low matrix effect below (<16%) and recoveries between 45% and 98% for all analytes in the different matrices.This approach provided a sensitive and robust quantitative analysis on the target compounds with LOQs between 0.0002 and 0.05 ng mg-1, while different moieties were considered as neutral losses or as precursor ions, which led to the putative identification of different glycosylated forms of flavonoids; a further fragmentation study was carried out by MS3, in order to discriminate compounds with similar aglycones, such as luteolin and kaempferol.

References

1. J. Zhang, X. Sun, *Phytochemistry*, 181 (2021), 112588.
2. A.B. Santhakumar, M. Battino, J.M. Alvarez-Suarez, *Food Chem*, 113 (2018), pp 49–65.

Characterization of SARS-CoV-2 proteins and human proteome in COVID-19 patients' saliva and plasma: an untargeted mass spectrometry approach

L. Pagani[1], C. Chinello[1], A. Mahajneh[1], F. Clerici[1], G. Panarello[1], A. Favalli[2], P. Gruarin[2], R. Grifantini[2], A. Bandera[3,4,5], R. Ungaro[3], A. Muscatello[3], F. Blasi[3,4], A. Gori[3,4,5], F. Magni[1]

[1] Department of Medicine and Surgery, University of Milano-Bicocca, Clinical Proteomics and Metabolomics Unit, Vedano al Lambro, Italy; [2]Istituto Nazionale di Genetica Molecolare (INGM), Milan, Italy; [3]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [4]Department of Pathophysiology and Transplantation, University of Milano, Milan, Italy; [5]Centre for Multidisciplinary Research in Health Science (MACH), University of Milano, Milan, Italy

Keywords: *COVID-19, mass spectrometry, proteomics*

In March 2020 Coronavirus Disease 2019 (COVID-19), caused by SARS-CoV-2, was declared pandemic by WHO [1]. Mass spectrometry-based proteomics could represent an useful tool to obtain biological and clinical information about this new disease, especially if an untargeted approach, which doesn't need a prior knowledge, is used [2]. The aim of our study is to develop an untargeted nLC-ESI MS/MS approach to study SARS-CoV-2 proteins in saliva and plasma samples of COVID-19 patients with different outcomes and to analyse their salivary and plasmatic proteome [3]. At first, four SARS-CoV-2 recombinant proteins (S1, S2, RBD of Spike Glycoprotein, and Nucleoprotein) were purified, de-glycosylated, digested with trypsin and analysed. Then, saliva and plasma from healthy donors were collected and spiked with viral proteins (S1, S2, RBD, N): different viral inactivation, de-glycosylation and enzymatic digestion approaches were studied. The method which gave better results was chosen to process saliva and plasma samples of COVID-19 patients. All samples were injected and analysed with nLC-UHRTOF, while protein identification was performed through Mascot and PEAKS studio X+ platforms. Injecting different quantities of SARS-CoV-2 recombinant proteins, we established the limit of detection (LOD) of the method, which is of 10 pg for both Spike glycoprotein (S) and Nucleoprotein (N). We also analysed saliva and plasma samples spiked with a decreasing quantity of viral proteins (S1, S2, RBD, N) to evaluate the matrix effect and our sensibility in biological samples: from these results it has emerged a limit of characterization for N and S proteins of 90pg, allowing a coverage of 20% and 5%, respectively, in saliva and of 33pg and 330pg, respectively, with a coverage of ~5% in plasma. In the end, from the comparison of salivary and plasmatic proteome of patients with different outcomes (mild or severe), it is possible to highlight 77 and 18 proteins which are differentially expressed between the two different groups in saliva and plasma, respectively. With this untargeted proteomic approach is possible to explore both SARS-CoV-2 proteins and the whole proteome of saliva and plasma samples of COVID-19 patients, allowing an overall view of possible proteomic pathways involved in the development of the disease.

References

1. Pourrajab F, Zare-Khormizi MR, Sheikhha MH, Infect. Drug Resist., vol. 13, *pp.* 2385–2405, 2020
2. Messner CB, Demichev V, Wendisch D, *et al.*, Cell Syst., vol. 11, no. 1, *pp.* 11-24.e4, 2020
3. https://www.who.int/csr/don/31-december-2020-sars-cov2-variants/en/ (accessed 31 December, 2020)

ACKNOWLEDGMENTS: Il progetto/intervento è stato cofinanziato a valere sulle risorse POR FESR 2014-2020: LINEA 2 "RICERCA INDUSTRIALE E SVILUPPO SPERIMENTALE": CO-iMMUNITY - Caratterizzazione della risposta immunitaria protettiva in pazienti affetti da covid-19 e realizzazione di un saggio immuno-diagnostico.

Drug plasma stability of PG-P and Carbonic Anhydrase hybrids inhibitors by LC-MS/MS and application of LEDA algorithm for their characterization

M. Pallecchi[1], *M. Menicatti*[1], *G. Bartolucci*[1]

[1] Università degli Studi di Firenze, NEUROFARBA, via Ugo Schiff, 6 (FI)

Keywords: *Drug Plasma Stability, LEDA algorithm, Matrix Effect*

Recently it has been shown that P-glycoprotein (P-gp), that was able to extrudes a variety of antineoplastic drugs from resistant cancer cells, and Carbonic Anhydrase XII (CA XII), that catalyzes the conversion of carbon dioxide to bicarbonate and proton, are overexpressed in some neoplastic cells and are both responsible of the Multidrug Resistance (MDR)[1]. New hybrids having inhibitory properties toward both targets were then synthesized. Their structure contains two ester groups so it was important evaluating their stability as a preliminary in vitro tests therefore in this study a series of drug stability experiments was carried out in human plasma (H-pl) and phosphate-buffer solutions (PBS) to assess the possible susceptibility of the ester bonds to plasma enzymes or the spontaneous hydrolysis respectively. For this purpose, a liquid chromatography coupled with a mass spectrometer system, operating in tandem mass spectrometry (LC-MS/MS), was used. For the method a Phenomenex C18 20 mm length, 2 mm internal diameter and 3 mm particle size was used. The solvents used were 10 mM formic acid and 5 mM ammonium formate in water: acetonitrile 90:10(solvent A) and 10 mM formic acid and 5 mM ammonium formate in acetonitrile:water 90:10 (solvent B) according to the elution gradient as follows: initial at 90% solvent A, which was then decreased to 10% in 4.0 min, kept for 2.0 min, returned to initial conditions in 0.1 min and maintained for 2.0 min for reconditioning, to a total run time of 8.0 min. Drug stability experiments relied on incubation of each analyte, in PBS and H-pl, for different times (0, 30, 60 and 120 minutes) and analyzed with the method described before. Also, an evaluation of Matrix Effect following Matuszewski protocol was employed[2]. Furthermore this new hybrids are isomer pairs, so they require long chromatographic analysis for separate them. Our approach, instead, use short columns, fast analysis and LEDA algorithm, a mathematical tool that allows the characterization of coeluting isomers relying on their different MS/MS spectra, increasing the productivity and avoiding any operator error due to contamination[3].

References

1. Kopecka J *et al.* P-glycoprotein-mediated chemoresistance is reversed by carbonic anhydrase XII inhibitors. Oncotarget. 2016; 7: 85861-85875.
2. 2. Matuszewski BK *et al.* Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem 2003;75:3019–30.
3. M. Menicatti *et al.* Resolution of co-eluting isomers of anti-inflammatory drugs conjugated to carbonic anhydrase inhibitors from plasma in liquid chromatography by energy-resolved tandem mass spectrometry J Enzyme Inhib Med Chem. 2018, 33, 671.

Cannabinoids profile in C. sativa samples by means to LC-EPI/IDA/MRM analysis: a new approach for cultivar differentiation

S. Palmieri[1], *E. Oliva*[1], *F. Fanti*[1], *E. Viteritti*[1], *A. Pepe*[1], *M. Sergi*[1], *D. Compagnone*[1]

[1] Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Via R. Balzarini 1, 64100 Teramo, Italy

Keywords: *C. sativa L, EPI, LC/MS/MS*

A new multi-target screening (MTS) procedure for 30 cannabinoids in inflorescences of different cultivar of *C. sativa* L. (hemp) has been developed using multiple reaction monitoring (MRM) as coupled with enhanced product ion (EPI) scan in an information-dependent acquisition (IDA) experiment, in one single LC/MS/MS analysis. In literature there are a lot of works regard the identification of new cannabinoids by the untargeted analysis by high-resolution mass spectrometry [1,2,3], but here a different approach based on triple quadrupole configuration is proposed.

Dry inflorescences of different samples of hemp were extracted by ultrasounds, diluted and directly injected for MS/MS analysis. An MRM-EPI-IDA analytical method was used for the analysis of the different samples. The results, processed by multivariate statistical analysis, showed that each cultivar has a different cannabinoidic profile based on not only the common cannabinoids studied as cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), but also for the presence of the less common cannabinoid compounds. Furthermore, the analytical approach demonstrates as MRM-EPI-IDA is capable of semi-targeting recently discovered cannabinoid compounds. The identification is performed through the comparison of mass spectrum analysis of each cannabinoid with literature and the development of MS/MS library based on EPI spectra in positive or negative mode. This approach allows to detect and identify the cannabinoids in one single LC/MS/MS run.

References

1. L. Giupponi, V. Leoni, R. Pavlovic, A. Giorgi, *Molecules*, *25*(6) (2020), 1381.
2. A. Cerrato, C. Citti, G. Cannazza, A. L. Capriotti, C. Cavaliere, G. Grassi, A. Laganà, *Talanta*, *230* (2021), 122313.
3. C. Citti, P. Linciano, S. Panseri, F. Vezzalini, F. Forni, M. A. Vandelli, G. Cannazza, *Frontiers in plant science*, 10 (2019), 120.

AP-MALDI-MSI: simultaneous imaging of a large number of endogenous metabolites in brain tissues

A.M. Siciliano[1], *L. Brunelli*[2], *G. De Simone*[2], *R. Pastorelli*[2], *E. Davoli*[1]

[1] Mass Spectrometry Research Center for Health and Environment, Environmental Health Sciences Department, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy
[2] Protein and Metabolite Biomarkers Unit, Mass Spectrometry Research Center for Health and Environment, Environmental Health Sciences Department, IRCCS - Istituto di Ricerche Farmacologiche Mario Negri IRCCS-Milan, Italy

Keywords: *MALDI-IMS, small metabolites, brain*

The biochemical processes characterization by spatial metabolites distribution in the tissue can be considered as a mirror of health or disease. Spatial metabolomics is a field of omics research focused on the detection and interpretation of metabolites, lipids, drugs, and other small molecules in the spatial context of cells, tissues, organs, and organisms. [1] The spatial map of metabolites can shed light on the biological processes in unexplored tissues. The matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) has developed as a promising tool to investigate the spatial distribution of biomolecules in intact tissue specimens. [2] Since the development of MALDI mass spectrometry, this procedure has been specifically used for analysing proteins or high molecular weight compounds because of the interference of matrix signals in the regions of the low mass range. [3] However, the detection of small molecules and related pathologies are the challenge of recent years. The mapping of metabolites in the brain lead the way to the possibility to visualize endogenous changes in the case of pathologies with unknown mechanisms and to evaluate their modification in response to drug administration. For this reason, the development of strategy to image the small molecules in the brain is the first step to achieve this aim.

Here, we illustrate a high-resolution Atmospheric Pressure-MALDI-mass-spectrometry imaging (AP-MALDI MSI, MassTech inc., MD, USA) approach that allowed us to image small metabolites in the mouse brain through the combination of different matrices with different ionization power. AP-MALDI mass spectrometry imaging was performed on mouse brain tissue to visualize the spatial distribution in a different brain section of 50 metabolites belonging the main cellular metabolic pathways. All spectra were acquired in a 50-500 *m/z* range using Q-Exactive mass spectrometer. MS/MS experiments conducted on brain slices and used to confirm metabolite identity in the Human Metabolome Database. Matrix background was subtracted from the outcome to exclude matrix interference signals.

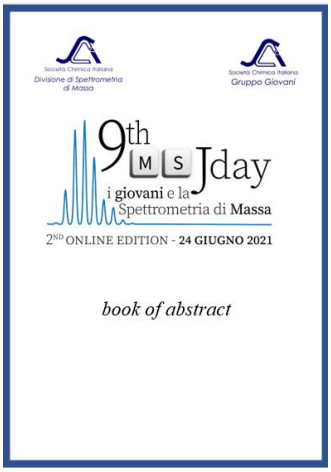
These preliminary results suggest that the combination of different matrices could be the right way to obtain a complete visualization of the metabolites distribution in a cerebral area in health and disease condition.

References

1. T. Alexandrov, Annual Review of Biomedical Data Science, 2020, *pp.* 61-87.
2. B. Chatterji and A. Pich., Expert Review of Proteomics, 2013, pp 381-8.
3. S.R. Shanta, T.Y. Kim, J.H. Hong, J.H. Lee, C.Y. Shin, K.H. Kim, Y.H. Kim, S.K. Kim, K.P. Kim, Analyst, 2012, pp 5757-62.

Proceedings of the

9th MS Jday
i giovani e la Spettrometria di Massa
2ND ONLINE EDITION - 24 GIUGNO 2021



All the scientific contributions of the symposium are collected in this *international volume* with ISBN code.

You can cite your work as follow:
N. Surname, N. Surname, … and N. Surname; <*abstract title*>, in Proceedings of the 9th MS J day 2021, Ed. F. Fanti, R. Pascale, V. Lazazzara, G. Ventura, F. Vincenti, ISBN: 978-88-94952-06-3, <*page number*>, 2021, Rome.

Edited by *F. Fanti, R. Pascale, V. Lazazzara, G. Ventura, F. Vincenti*

9 788894 952063