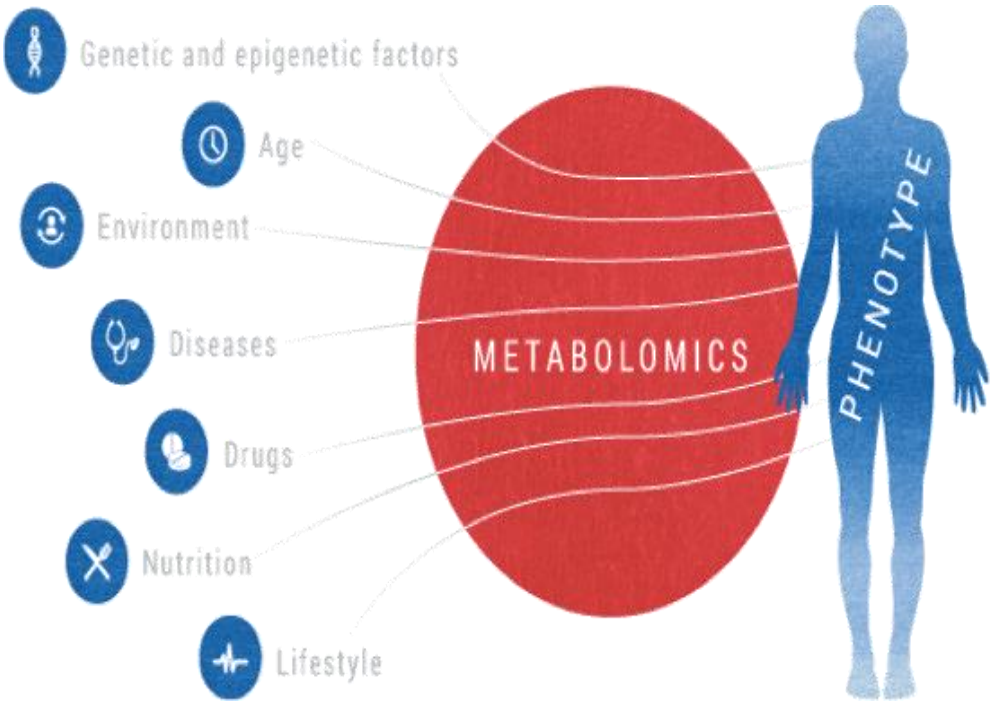

Biomic auth
Center for Bioanalysis & Omics

FoodOmicsGR
National Research Infrastructure for the Comprehensive Characterisation of Foods

Aristotle University Thessaloniki, Greece
Bioanalysis Network, BIOMIC
http://bioanalysis.web.auth.gr/metabolomics/

Thursday 16/5		Life Sciences Moderators: Theodoridis / Wilson / Vorkas / Witting	Speaker	Institution
8:20	9:00	**Registration**		
9:00	9:15	Introduction, Overview and Perspective	G. Theodoridis	Aristotle Univ.
9:15	09:45	Rapid Profiling UPLC-IM-MS Methods for Metabolic Phenotyping: Smaller, Better, Faster	I.D. Wilson	Imperial College
09:45	10:10	Endogenous Metabolic Profiling as a fundament in Personalized Theranostics	T. Lundstedt	Uppsala Univ.
10:10	10:35	A New Tool in Metabolomics: SWATH® Acquisition Analysis for Global Profiling and Quantitation	A. Paccou	Sciex
10:35	11:00	**Break**		
11:00	11:25	Workflow for rapid identification of endogenous metabolites based on spectral library search	U. Schweiger-Hufnagel	Bruker Daltonik
11:25	11:50	Urinary metabolic profiling in Autosomal Dominant Polycystic Kidney Disease (ADPKD)	O. Mayboroda	LUMC, Leiden
11:50	12:15	Standardization of metabolomics: IROA TruQuant IQQ	S. Spector	Merck
12:15	12:40	(Metabol)-omics in Neonates: Our Experience	K. Sarafidis	Aristotle Univ.
12:40	13:00	Degradation of D-2-Hydroxyglutarate in the presence of Isocitrate Dehydrogenase Mutations	R. Berger	Regensbug University
13:00	13:50	**Lunch**		

Thursday 16/5		Life Sciences Moderators: Theodoridis / Wilson / Vorkas / Witting	Speaker	Institution
13:50	14:15	Metabolomics analysis of human atherosclerotic plaques reveals a potential novel pathway of macrophage foam cell apoptosis in advanced atherosclerosis	P. Vorkas	Imperial College
14:15	14:40	TBA	F.Michopoulos	AstraZeneca, UK
14:40	15:05	A map to navigate through the lipidome of the model organism Caenorhabditis elegans	M. Witting	Helmholtz Zentrum München
15:05	15:50	**Break/ Poster Session**		
15:50	16:05	Stable-Isotope Dilution GC–MS Quantification of Malondialdehyde (MDA), Nitric Oxide (NO) and Amino Acids in Infant's Serum Samples with Ureteropelvic Junction Obstruction (UPJO)	O. Begou	Aristotle Univ.
16:05	16:20	Global Lipidomics of Normal Appearing White Matter in Multiple Sclerosis using RP-UPLC-TOF-MSE	P. Pousinis	Sheffield Hallam University
16:20	16:35	Metabolomics in Infants with Ureteropelvic Junction Obstruction	A. Pavlaki	Hippokratio General Hospital of Thessaloniki
16:35	16:50	Quantification and 13C-Tracer Analysis of Total Reduced Glutathione by UHPLC-QTOFMS/MS	X. Sun	Regensburg University
16:50		End of programme		

Friday 17/5		FoodOmics Moderators: Theodoridis / Thomaidis / Spyros / Kalogiannis	Speaker	Institution
9:00	9:30	FoodOmicsGR_RI	G. Theodoridis	Aristotle Univ.
9:30	10:00	Implementation of Metabolomics and Information Technologies in the Food Systems	G. Nychas	Athens Agricult. Univ.
10:00	10:30	HRMS Foodomics for Authenticity and Quality: From basic Research to Industrial Applications	N. Thomaidis	Athens Univ.
10:30	10:55	NMR-based Food Metabolomics and Analytics	A. Spyros	Univ. of Crete
10:55	11:25	**Break/ Poster Session**		
11:25	11:50	Food Quality and Authenticity control by 1H-NMR methods – new developments	C. Napoli	Bruker BioSpin, Italy
11:50	12:15	Similarity and Differential NMR Spectroscopy, An Alternative Way to Find Marker Compounds in Metabolomics	J. Schripsema	State Univ. of Northern Rio de Janeiro
12:15	12:40	Metabolomics-Based GC×GC-TOFMS Technology as the New Avenue to Understanding the Microorganisms Behaviour	S. M. Rocha	University of Aveiro
12:40	13:05	SONAR in combination with Imaging (MSI)	E. Claude	Waters
13:05	13:50	**Lunch**		
13:50	14:15	The era of functional and nutraceutical foods	A. Agapiou	Univ. of Cyprus
14:15	14:30	The effect of sugar bee feeding on the metabolic profile of royal jelly	D. Kanellis	Aristotle Univ.
14:30	14:45	LC-HRMS Technique as a tool to Access the Impact of Conventional and Mild Presentation Technologies on Fruit Syrup Metabolome	K. Hurkova	UCT Prague
14:45	15:00	How to Harmonize Non-Targeted Analytical Approaches? – Investigation of the Instrument Comparability Using the Example of Wine Authentication	M. Ehlers	German Federal Institute for Risk Assessment
15:00	15:30	**Break/ Poster Session**		
15:30	17:00	Round Table FoodOmics		

POSTERS

1. **Univariate batch correction for UHPLC-MS metabolomics data.** N. Torbica, C. Nakas, A. B. Leichtle
2. **GC-MS based metabolomics as a tool to evaluate freshness of sea bream (sparus aurata) during storage on ice.** Th. Mikrou, A. Mallouchos
3. **Mass spectral molecular networking as tool to investigate the microbial interactions in leaf cutting ants symbionts.** A. Pereira, A. Bauermeister, D. Spiteller, J. B. Fernandes
4. **Human melanoma: metabolomics in and out of the cells.** M.A. Kosmopoulou, A.F. Giannopoulou, A. Iliou, E. G. Konstantakou, A. D. Velentzas, I. S. Papassideri, D. Benaki, E. Mikros, E. Gikas, D. J. Stravopodis
5. **A metabolome wide association study for colorectal cancer genetic variants in humans**. A.Iliou, I.Karaman, R.Pinto, G. Graça, A.Dehghan, D.Benaki, E.Gikas, E.Mikros, I.Tzoulaki
6. **Characterization of poppy seeds after heat treatment using metabolomic fingerprints.** M. Jiru, J. Stastny, K. Hurkova, V. Kocourek, J. Hajslova
7. **Exploring the formation of Advanced Glycation End Products (AGE) in different reaction conditions.** S. Visentin, C. Medana, C. Butnarasu
8. **Detection of "olive quick decline syndrome" markers using an untargeted metabolomics approach by HPLC-HRMS technique**. M. Zorzi, R. Bartolo, M. D'Addabbo, R. Aigotti, F. Dal Bello, G. Ciccarella, C. Medana
9. **Determination of endogenus metabolites in Muscat of Alexandria grape and must samples using UPLC(HILIC)-MS/MS analysis.** A. Ketsetzi, O. Deda, M. N. Marinaki, H. Gika, G. Theodoridis, A. N. Assimopoulou
10. **Targeted Metabolomics Method Development Across Liquid Chromatography Domains.** D. J. Floros , F. Berthiller , H. Schwartz-Zimmermann
11. **Method development for LC-MS/MS measurement of carboxylic acids in animal matrices.** N. Reiterer, D. J. Floros, F. Berthiller, H. Schwartz-Zimmermann
12. **NMR-based cheese metabolomics, quality control and authenticity**. E. Ralli, A. Spyros
13. **NMR-based metabolomics of the lipid fraction of organic and conventional bovine milk.** C. G. Tsiafoulis, O. Tzamaloukas, M. Malet-Martino, I. P. Gerothanassis
14. **Targeted and untargeted metabolomics of mice urine and fecal samples, in the discovery of alcohol toxicity biomarkers.** O. Deda, Ch. Virgiliou, A. Orfanidis, H.Gika
15. **Effect of alkannin and shikonin on hormone-dependent breast cancer cell line MCF-7: an LC-MS based metabolomics study.** A. Nakas, C. Virgiliou, A. Beyer, J. Wober, H.G. Gika, G. Vollmer, A.N. Assimopoulou
16. **Fatty acid profiling in blood of healthy individuals and patients with hyperlipidemia and association with ABCB1 2677G>T/A, PNPLA3C>G, CAT C>T, Taq1B-CEPT polymorphisms.** T. Mouskeftara, A. Goulas, A. Asimopoulou, N. Raikos, G. Theodoridis, H. Gika
17. **Building a local UPLC-QTOF-MS Mass Spectral Library using the MSMLS Kit.** D. Diamantidou, P. Katechis, E. Lazaridou, H. Gika, S. Dove, M. Fields, S. Spector, J. Wrigley, F. DeJong, G. Theodoridis
18. **Metabolomics analysis for the *in vitro* toxicity assessment of cocaine in HepG2.** A. Krokos, C. Virgiliou , H. Gika, N. Raikos, E. Aggelidou, A. Kritis, G. Theodoridis
19. **MQACC: A community-led effort to strengthen quality assurance and quality control practices in metabolomics research and reporting.** G. Theodoridis, F. Abdi, A. Bandukwala, A. Barsch, Dan Bearden, Chris Beecher, R. Beger, B. Bethan , J. Bowden, D. Broadhurst, C. Clish, S. Dasari, L. Derr, S. Dhungana, W. Dunn, T. Ebbels, A. Evans, S. Fischer, R. Flores, T. Flynn, C. Grieser, T. Hartung, M. Haznadar, D. Herrington, R. Higashi, Ping-Ching Hsu, T. Huan, J. Jans, C. Jones, M. Kachman, J. Kirwan, A. Kleensang, M. Lewis, K. Lippa, P. Maruvada, S. Meyer , M. E. Monge, J. Mosley, I. Ntai, C. O'Donovan, G. Papanicolaou, R. Pinto, M. Playdon, D. Raftery, S. Ross, M. Schmidt, T. Schock, A. Souza, J. Sun, F. Tayyari, F.Torta, B. Ubhi, V. Velagapudi, M. Verma, M. Viant, D. Vuckovic, T. Walk, I. Wilson, Li-Rong Yu, K. Zanetti

20. NUCLEAR MAGNETIC RESONANCE AND RAMAN PROFILING OF MUSCAT OF ALEXANDRIA GRAPES MUST DURING FERMENTATION

Maria N. Marinaki, Alexandros Nakas, Nebojša Rodić, Elli Kampasakali, Dimitrios Christofilos, Andreana N. Assimopoulou

Conference proceedings

5th Workshop on Analytical Metabolomics

Thessaloniki, 16-17 May 2019

Oral Presentations

RAPID PROFILING UPLC-IM-MS METHODS FOR METABOLIC PHENOTYPING: SMALLER, BETTER, FASTER

Ian D Wilson, Imperial College, London

For the discovery of biomarkers via MS and LC/MS-based metabolic phenotyping (metabotyping) studies employing untargeted metabonomic/metabolomic methods there is often a need to compromise between high-throughput, where metabolite coverage is sacrificed to some extent for speed, and more comprehensive metabolic profiling, where a longer analysis time is required. So, rapid LC-MS-based profiling methods will inevitably suffer from greater ion suppression than longer runs, leading to analytes being missed. The more lengthy LC-MS analyses provide "deeper" profiling but at the expense of increased instrument time and consumables costs. The introduction of ultra (high) performance LC provided a significant improvement in efficiency and enabled many more metabolites to be detected per unit time compared to conventional HPLC. Here the use of a strategy using UHPLC with rapid solvent gradients in combination with ion mobility spectrometry (IMS) will be described and illustrated with applications in both RP- and HILIC modes of LC to demonstrate the potential of UHPLC/IMS/MS for this type of analysis to deliver high throughput metabolome analysis, illustrated with examples of applications to biological fluids.

DEGRADATION OF D-2-HYDROXYGLUTARATE IN THE PRESENCE OF ISOCITRATE DEHYDROGENASE MUTATIONS

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Gain-of-function mutation in isocitrate dehydrogenase (IDH) 1 or 2 have been founds in certain cancers, such as AML or glioma, resulting in increased D-2-HG levels [1,2]. Normal function of IDH1/2 is oxidative decarboxylation of isocitrate forming α-ketoglutarate. However, the mutation confers a neomorphic activity to the enzyme – the production of D-2-HG out of α-ketoglutarate. D-2-HG is regarded as an oncometabolite promoting tumor initiation and progression [2]. The production is of high capacity, while its degradation is catalyzed by the high-affinity/low-capacity enzyme D-2-hydroxyglutarate dehydrogenase (D2HDH). So far, it has not been proven experimentally that the accumulation of D-2-HG in IDH mutant cells is the result of its insufficient degradation by D2HDH.

We have established a sensitive assay to determine D2HDH activity in cell homogenates by direct quantification of 2-HG concentrations using LC-MS/MS [3]. In detail, cells are lysed in an appropriate assay buffer by sonification. The buffer was optimized e.g. regarding pH and redox equivalents. Cellular lysate is then incubated with enantiomerically pure D-2-HG at 37°C and aliquots are taken over time to determine degradation rate. These aliquots are extracted for LC-MS/MS analysis which is performed on a TripleQuad-instrument operated in MRM (multiple reaction monitoring) mode. Quantification is achieved by adding stable isotope labeled 2-HG-D3.
Using this DH-assay and non-linear fitting we determined the K_m-value of D2HGDH in MCF-7 cells. Furthermore, D2HDH activity was also compared within several cell lines. Those included HT1080 (which harbours an IDH1R132C mutation and therefore produces D-HG in high amounts) and a HCT116 cell panel with three different mutations in IDH1/2. Furthermore D2HGDH protein abundance was analyzed by Western Blot to check for correlation with enzyme activity. Our data clearly indicate, that the maximum D-2-HG degradation rate by D2HDH is reached in vivo, as v_{max} is low in comparison to production of D-2-HG by mutant IDH1/2. The latter seems to be limited only by substrate availability. Further, incubation of IDH wild type cells for up to 48 hours with 5 mM D-2-HG did not result in a significant increase in either D2HDH protein abundance or enzyme activity.

BIOMEDICAL APPLICATION IN INFANT'S SERUM SAMPLES WITH URETEROPELVIC JUNCTION OBSTRUCTION (UPJO) OF STABLE-ISOTOPE DILUTION GC–MS METHOD FOR THE QUANTIFICATION OF MALONDIALDEHYDE (MDA), NITRIC OXIDE (NO) AND AMINO ACIDS IN ELECTRON-CAPTURE NEGATIVE-ION CHEMICAL IONIZATION (ECNICI).

Olga Begou[A,B], Alex Bollenbach[C], Kathrin Drabert[C], Antigoni Pavlaki[D], Helen Gika[B,E], Nikoleta Printza[D], Georgios Theodoridis[A,B], Dimitrios Tsikas[C,*]

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Ureteropelvic junction obstruction (UPJO) constitutes the predominant cause of obstructive hydronephrosis/neuropathy in infants and children. However, fundamental

questions regarding its management remain unanswered. Metabolomics-based analysis aims at the determination and quantification of significant /key-end metabolites in order to elucidate potential biochemical changes in response to pathophysiological stimuli or genetic modifications. Aim of this study was to clarify whether the metabolic profile of children with UPJO differs significantly from healthy matched controls. Serum samples were collected from 22 patients preoperatively, 21 patients with mild stenosis treated conservatively, and 19 healthy controls. Two targeted metabolomics-based analyses by Gas Chromatography coupled to mass spectrometry were performed for the quantification of malondialdehyde (MDA), Nitrite and Nitrate as pentafluorobenzyl derivatives and of amino acids and their derivatives as *N*-pentafluoropropionic amides of methyl esters and all their externally added labeled analogues in serum. Selected-ion monitoring for each analyte in electron-capture negative-ion chemical ionization (ECNICI) was also performed. Univariate statistical analysis was applied in order to elucidate potential biomarkers of the aforementioned decease, with homoarginine (hArg) (pValue 0.03), asymmetric dimethylarginine (ADMA) (pValue 0.04) and malondialdehyde (MDA) (pValue 0.02) being statistically differentiated among the studied groups.

METABOLOMICS IN INFANTS WITH URETEROPELVIC JUNCTION OBSTRUCTION

Antigoni Pavlaki[A*], Olga Begou[B,C], Olga Deda[C,D], Evangelia Farmaki[A], John Dotis[A], Helen Gika[D], Anna Taparkou[A], Nikolaos Raikos[D], Fotios Papachristou[A], George Theodoridis[B], Nikoleta Printza[A].

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Despite the fact that ureteropelvic junction obstruction (UPJO) is the most common obstructive nephropathy, fundamental questions regarding its management remain unanswered. Aim of the present study was to clarify whether the metabolic profile of children with UPJO differs significantly and if through metabolic analysis new biomarkers can emerge that will allow early diagnosis of renal dysfunction in these children. Serum samples were collected from 19 patients preoperatively, 19 patients with mild stenosis treated conservatively, and 17 healthy controls. Targeted metabolomic analysis by Hydrophilic Interaction Liquid Chromatography coupled to mass spectrometry was performed. All perinatal and clinical characteristics were recorded. Multivariate and univariate statistical analysis enabled the discrimination of patients who required surgery from those followed by systematical monitoring as well as from healthy controls. A set of metabolites, including Homocysteine, Creatinine,

Choline and Pyroglutamate showed high performance as biomarkers of UPJO in serum.

QUANTIFICATION AND 13C-TRACER ANALYSIS OF TOTAL REDUCED GLUTATHIONE BY UHPLC-QTOFMS/MS

Xueni Sun‡, Paul Heinrich‡, Raffaela Berger, Peter J Oefner, Katja Dettmer

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Glutathione is an essential intra- and extracellular antioxidant. The level of glutathione in the body is highly related to different disease states and is a useful indicator of disease risk and oxidative stress status. So far, various methods are available for the determination of GSH in biomedical specimens. However, to date, studies have focused mostly on the quantification of GSH under different biological conditions, but not on the distribution of GSH isotopologues in 13C-tracer experiments. Concentration changes of a metabolite in the body can only provide information on alterations in the producing or consuming reactions (ana-/catabolic reactions), but for the investigation of the origin and fate of metabolites stable isotope tracing experiments are needed.

In this study, we have developed a sensitive, selective, and comprehensive LC-MS/MS method for the absolute quantification and ^{13}C-tracer analysis of total glutathione using dithiothreitol (DTT) for the reduction of glutathione disulfide. The limit of detection (LOD) was 0.01 µM, while the lower limit of quantification (LLOQ) was 0.78 µM, with the linear (R=0.9997) range extending up to 100 µM. Intra-run and inter-run coefficients of variation are 2.49 % and 2.04 %, respectively, attesting high repeatability. Mean (±SD) recoveries of three different concentrations (low, medium, high) of GSH spiked into aliquots of HCT116 cells prior to cell extraction were 108.9 % (±2.1), 100.8 % (±8.3), and 99.9 %(±7.1), respectively. Finally, using a 12-Da wide Q1 window in MRM mode on UHPLC-QTOFMS, we were able to detect all isotopic labeling states of GSH extracted from HCT116 cells fed with either ^{13}C-labeled glucose or glutamine.

NMR-BASED FOOD METABOLOMICS AND ANALYTICS

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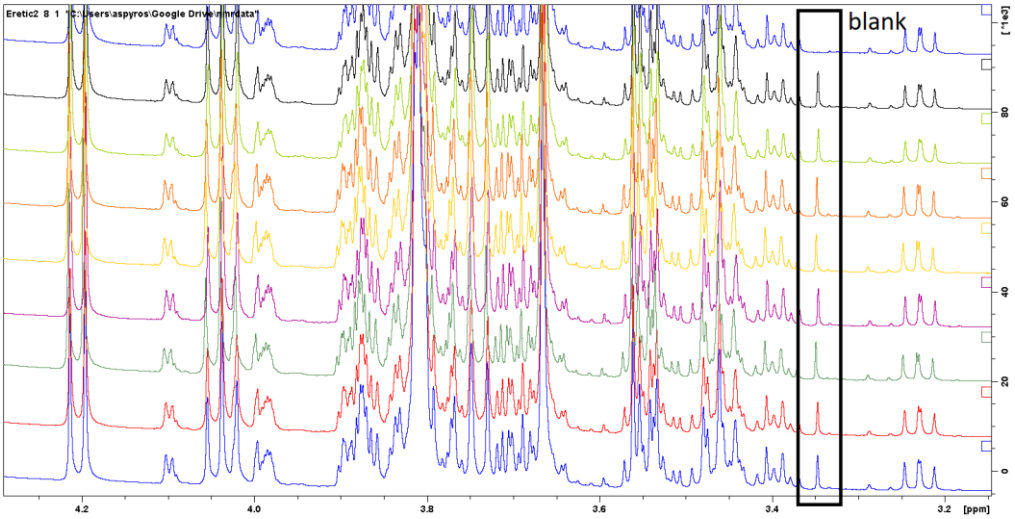
In recent years, high resolution NMR spectroscopy has been an important analytical tool for the development of fast and effective analytical methods of food analysis, and contributed immensely to the rise of the -omics era in food metabolomics. Applications of NMR in food analysis include the quality control of foods, authentication of the geographical origin, such as PDO and PGI labeling, and the detection of controlled chemicals, adulterants and unauthorized practices.

In the present report we will present results from ongoing projects in our laboratory dealing with a wide range of NMR applications in food analytics. These applications will include the compositional analysis of goji berry fruits, the quality control of refreshment drinks, authentication of geographic origin and quality control of traditional Cretan cheeses.



SIMILARITY AND DIFFERENTIAL NMR SPECTROSCOPY, AN ALTERNATIVE WAY TO FIND MARKER COMPOUNDS IN METABOLOMICS

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In NMR based metabolomics the routine procedure to analyze the spectra consists of the following steps: 1. Processing the NMR spectra, 2. Performing binning of the spectra, 3. Performing multivariate data analysis (PCA or PLS-DA), 4. Deduce marker compounds through the coefficient plots. This routine procedure has a number of problems. First of all data about minor components of the extracts are lost in the binning process. Furthermore the procedure is not completely transparent in relation to reproducibility and quality of the original spectra.
In a recent publication[1] an alternative and complementary method was presented, in which the similarity of spectra is calculated and differences between spectra or sets of spectra are investigated with differential spectroscopy.
In this presentation the procedures are compared, using examples from the analysis of a series of different vegetable oils. The samples were analysed by both ^{1}H and ^{13}C NMR.

SONAR DESI IMAGING: A NOVEL METHOD TO IMAGE PRECURSORS AND FRAGMENTS FOR MOLECULAR IDENTIFICATION IN A SINGLE EXPERIMENT

Emmanuelle Claude[1]

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Mass spectrometry imaging (MSI) allows the correlation of spatial and chemical information directly from biological tissues. Desorption Electrospray Ionization (DESI) is an ambient ionization technique that has gained popularity over the past few years due to the ease of sample preparation, the ESI-like spectra, and also the non-destructive nature of the DESI technique.

Typically MSI experiments are untargeted and are performed using the full scan MS mode of data acquisition. After mining the MSI data and identifying potential

biomarkers, the next step is their identification which is usually performed using a limited number of manually entered MS/MS experiments.

Recently a new Data Independent Acquisition (DIA) method called SONAR^{TM} has been introduced, utilizing a scanning quadrupole mass filter m/z window in a Q-ToF geometry. In this method, a resolving quadrupole mass filter m/z window is scanned repetitively with precursor and MS/MS data acquired at rapid spectral acquisition rates. The method produces a highly specific and unbiased two-dimensional dataset that can be viewed and processed using a variety of informatics tools.

Here, we describe the SONAR^{TM} mode of acquisition implemented on a bench top quadrupole - ToF Xevo G2-XS mass spectrometer that has been embedded into a DESI imaging workflow for lipid imaging and identification directly from rat brain tissue sections.

THE ERA OF FUNCTIONAL AND NUTRACEUTICAL FOODS

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The last few years, there has been an increased interest towards the biological food market. Consumers' demands seem to change, driven by the modern health trends (e.g. natural products, gluten- and caffeine-free products, products of PDO and PGI, demand for natural hydrocolloids, etc.), following the basic aspects of the Mediterranean diet. Carob fruit seems to fulfill the above modern trends, as carob is considered a multi-factory tree, serving both the need for high nutritional value, as well as the many current pharmaceutical needs. Although it has been neglected the last decades and became mainly a food for animals, its return to the global market is feasible and appears very promising, serving many health and nutritional claims (i.e. as cocoa supplement, E 410, low fat content, rich in metals and dietary fibers, abundance source of D-pinitol, natural chocolate-rich in sugars, high polyphenols content, unique sensory characteristics, long shelf-life, etc.). Cultivated initially in the eastern Mediterranean area, soon due to humans' distribution, it travelled all over the world and hosted in climates resembling that of Middle East, as in Australia, Africa and the USA. The overall research scientific efforts towards highlighting the unique composition and properties of carobs will be highlighted, aiming to regenerate the global interest towards the carob fruit. The use of hyphenated mass spectrometric analytical techniques, along with chemometrics for further data processing will be

shown and discussed next to sensory studies, in an effort to distinguish the seeds from the fruit and the cultivated country.

LC-HRMS TECHNIQUE AS A TOOL TO ASSESS THE IMPACT OF CONVENTIONAL AND MILD PRESERVATION TECHNOLOGIES ON FRUIT SYRUP METABOLOME

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Sea buckthorn (*Hippophae rhamnoides*) is a cold resistant shrub, which produces yellow-red berries with a tough skin covering pulp and small hard seed. In recent years, these fruits have gained a global attention for their nutritional benefits, which can be related to bioactive compounds, such as polyphenols, carotenoids, tocopherols and vitamin C. Several studies have associated sea buckthorn with beneficial effects on cardiovascular system, protective and curative effects on skin, immunomodulatory, antioxidant and radioprotector activity. These valuable effects have made sea buckthorn a desirable product for medicinal, food and cosmetic purposes.

Over the years, consumers demand for fresh and naturally tasting products with a longer shelf life has increased. For this reason, new mild preservation technologies such as Pulsed Electric Field (PEF), Ohmic Heating (OH) or High Pressure Processing (HPP) were developed in order to achieve a gentle, non-thermal pasteurisation of foods. In comparison with conventional heating processes, mild technologies allow final product to retain flavour, texture and nutritional value. These techniques are suitable mainly for liquid foods such as fruit juices, purees, oils etc.

In our study, two varieties of sea buckthorn (Botanica and Leicora) were used to prepare fruit syrup. This product was than treated by PEF, OH, HPP and conventional heating and stored for 8 weeks in the refrigerator, in order to find differences caused by preservation technology or storage time. Metabolomic fingerprints of methanolic extracts of fruit syrups were obtained using ultra-high performance liquid chromatography coupled to tandem high resolution mass spectrometry (U-HPLC-HRMS/MS). Subsequently, chemometric evaluation was performed to assess the differences between the samples. The PCA (Principal Component Analysis) revealed significant differences between components in analyzed samples. Clear clustering, which was more pronounced in negative ionization mode, was found between different types of treatment. Among the compounds influenced by the treatment the

most were flavonoids, phospholipids and free fatty acids. The impact of storage time was not as significant as the impact of preservation technologies.

HOW TO HARMONIZE NON-TARGETED ANALYTICAL APPROACHES? – INVESTIGATION OF THE INSTRUMENT COMPARABILITY USING THE EXAMPLE OF WINE AUTHENTICATION

Mona Ehlers[a*], Carsten Fauhl-Hassek[a], Janet Riedl[a], Armin Hermann[b], Leos Uttl[c], Jana Hajslova[c], Jens Brockmeyer[d], Julia Raeke[a]

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Metabolomics has become a helpful tool in many research areas, e.g. food authentication. Non-targeted analytical techniques with subsequent multivariate data analysis are used to answer complex authenticity questions like the differentiation of botanical/ geographical origin or the detection of unknown adulterants [1]. Although a lot of progress has been made in the past years to develop such methods, a major question is still unanswered: How to harmonize non-targeted analytical approaches? The comparability of the results, e.g. between instruments or laboratories, is an important prerequisite for the implementation of non-targeted methods in official control and for the creation of joint databases [2].

In this study, using the example of grape variety differentiation in wine, several comparability aspects are investigated. 201 monovarietal commercial wines were analyzed using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). For both platforms, two identical spectrometers in two different labs were used to measure the same sample set in order to investigate the instrument comparability.

In this talk, first results of the ^{1}H-NMR analysis will be presented. Spectra obtained with the two instruments show differences, e.g. in the signal intensity. The applicability of several approaches to improve the comparability, e.g. correction with an instrument-specific factor, is discussed. Also, classification results for grape variety differentiation of both instruments are shown and compared.

POSTERS

UNIVARIATE BATCH CORRECTION FOR UHPLC-MS METABOLOMICS DATA

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Introduction In the field of metabolomics, ultra high pressure liquid chromatography coupled to mass spectrometry (UHPLC-MS) has proven to be a powerful method for acquisition of high dimensional metabolic data. Nevertheless, data analysis of non targeted UHPLC-MS data is heavily obstructed by batch effects. Subtle variations in sample quality and measurement conditions result in a large bias between batchwise organized sample material, yielding statistically incomparable results. Previous investigations show that the anticipation of batch effects enable their handling, and methods have been proposed to achieve removal of batch effects.
Objectives We introduce a package for R statistical software designed to remove batch effects from non targeted UHPLC-MS data and evaluate the effectivity of the batch effect removal. The pipeline is intended to be easy to use and modular, such that extensions may be introduced at any time.
Methods The core functionality of the pipeline lies in batch correction through analysis of covariance (ANCOVA) of the data features, which has been shown as promising method for removal of batch effects. The procedure consists of application of univariate regression models on the data features in order to account for and remove batch effects. The primary correction relies on the presence of a documented injection sequence and a sufficient amount (i. e. 10 - 15 per batch) regularly measured quality control samples (QCs) to fit the regression models. The regression models are also applicable on study samples given a randomized injection sequence. Missing data is replaced by very small numbers larger than zero, such that no obstruction through missing data occurs. Additionally we implement locally weighted scatterplot smoothing (LOESS) as a complementary pre-processing step. We investigate the effectivity of the batch effect removal by applying the pipeline on clinical study data with two sample batches of identical samples and computing the metrics proposed in the paper introducing the method.
Results Batch correction by ANCOVA performs well when using study samples for regression. The usage of QCs for regression shows inferior correction compared to correction with study samples. Applying LOESS as a pre- or post-correction step for removal of noise produces rather questionable results requiring further clarification.
Conclusion The effectiveness of ANCOVA for batch correction is solidified by our investigations. Yielding better results when using study samples implies that the QCs require special care such that they notably improve batch effect removal. As no actual imputation but rather replacement of missing data has been performed, further improvement of batch correction may be achieved with the introduction of more sensible imputation methods. We therefore mainly conclude that the prepartions for the occurrence of batch effects are invaluable for reliable removal of batch effects from on targeted LC-MS data.

GC-MS BASED METABOLOMICS AS A TOOL TO EVALUATE FRESHNESS OF SEA BREAM (SPARUS AURATA) DURING STORAGE ON ICE

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A complete GC-MS based fingerprinting protocol is described for the assessment of freshness of gilthead sea bream (Sparus aurata). Ice-storage experiments were conducted in two fish batches from different harvesting periods (spring, summer). After tissue sampling, the polar metabolite fraction was extracted according to a modified Bligh-Dyer method, followed by lyophilization, silylation and analysis of the derivatives with gas chromatography-mass spectrometry. After data preprocessing, various multivariate analysis methods were applied using the MetaboAnalyst web platform. In addition, sensory assessment was carried out in accordance with Regulation (EC) 2406/96. Shelf life was determined at 17 days according to sensory attributes. According to the results of the Principal Component Analysis, a clear separation of samples was achieved based on storage time. In addition, it was revealed that the first Principal Component (PC1) describes the time of storage on ice and consequently, the freshness of the samples. Using the Partial Least Squares-Discriminant Analysis method, a satisfactory prediction model of the four freshness categories (E: excellent, A, B, C: spoiled) has been developed. The most significant metabolites (VIP score > 1) were divided into two groups: i) metabolites whose concentration showed an increasing trend during storage and ii) metabolites whose concentration decreased during the storage of the sea bream, which can be further exploited as biomarkers of spoilage or freshness, respectively.

MASS SPECTRAL MOLECULAR NETWORKING AS TOOL TO INVESTIGATE THE MICROBIAL INTERACTIONS IN LEAF CUTTING ANTS SYMBIONTS

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Understading the microbial communication in different environments has been goal of study in chemical ecology, once microorganisms co-exist in close associations. Leaf cutting ants are part of a complex symbiosis: the mutualistic fungus with their garden fungus, Leucoagaricus gongylophorus that constitutes their main food source. Since the garden fungus is threatened by pathogenic fungi from the genus Escovopsis, and the ants need to defend their nest and to do so they joined alliance with symbiotic actinomycetes that produce antibiotics compounds that are active against the parasites. There are other microbes in the microcosmos of leaf cutting ants: the black yeast from the genus Phialophora that its ecological role is little studied so far. This work shows how to improve the data management from different interactions of leaf cutting ants` symbionts through the use of MS/MS molecular networking and metabolomics analysis. Ten different networks were created to compare the metabolic induction in di_erent solid media cocultures, with five symbionts

microbes of leaf cutting ants. Different secondary metabolites was identified in the libraries GNPS such as abyssomicin C, shearinines, disul_ram and others. MS network approach can be used together to metabolomics to detect some m/z ratio that only produced in the cocultures and it can deeppen future studies. In conclusion, a combination of molecular networks and metabolomic approaches can advance the MS/MS data analysis of secondary metabolites in microbial interactions and it can be possible to understand how it works the communication in complex environments as the leaf cutting ants` garden.

HUMAN MELANOMA: METABOLOMICS IN AND OUT OF THE CELLS

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The recent advances in mapping metabolomic landscapes have unearthed the important roles of plasticity and reprogramming of cellular metabolism in tumorigenesis, thus paving the way to novel drug-treatment strategies and therapies. Metabolic activities, which are correlated with malignant hallmarks, such as cell survival under stress conditions and the ability to successfully encounter high-energy demands, may significantly differ between primary tumor- and metastatic-cell populations of the same type of cancer, leading to distinct metabolic networks and metabolomes. In this study, we present a comparative NMR- and UHPLC-HRMS/MS-mediated untargeted metabolic profiling of melanoma to landscape the metabolic alterations tumor cells are subjected during metastasis.

Two different cell lines, the WM115 and WM266-4 ones (10 individual samples from each cell line), were examined. Both cell lines have derived from the same patient, with WM115 cells having originated from a primary, pre-metastatic, tumor and WM266-4 cells clonally expanded from individual lymph-node metastases. Application of orthogonal partial least-squares discriminant analysis (OPLS-DA), following receiver operating characteristic (ROC) curve of NMR data, revealed significantly differentiated metabolite profiles in each cell-malignancy grade, with WM115 cells being mainly characterized by upregulated levels of choline products, inosine, guanosine and the notable 7-hydroxy-6-methyl-8-ribityl lumazine. Interestingly, WM266-4 cells showed notably increased contents of hypoxanthine, myo-inositol, glutamate and organic acids, as well as AMP, ADP, ATP and UDP(s), strongly indicating the critical roles of purine, pyrimidine and amino acid metabolism, during metastasis. Further analysis of the supernatants (“fluxomics”) obtained from cells cultured with 1x PBS for 24 h (starvation conditions), allowed the identification of extracellular metabolites, where guanosine and inosine were enriched in WM115, while myo-inositol and hypoxanthine were significantly upregulated in WM266-4 cells, thus corroborating the importance of these metabolites in melanoma progression, invasion and metastasis, both at intra- and extra-cellular settings.

A METABOLOME WIDE ASSOCIATION STUDY FOR COLORECTAL CANCER GENETIC VARIANTS IN HUMANS

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Colorectal cancer (CRC) is the second most common malignancy in Western populations. Genome-wide association studies (GWAS) have identified over 98 common genetic variants unequivocally associated with the risk of CRC1. Molecular mechanisms implicating a small number of these variants in CRC are well-defined; however for the vast majority of them these mechanisms are less well understood or completely unknown. Better understanding of the metabolic pathways through which these genetic variants affect CRC risk could lead to new insights on cancer pathogenesis and/or novel therapeutic or preventive strategies. To build on that knowledge we performed metabolome-wide association analyses with each genetic variant known to be associated with CRC using metabolic profiling data from the Airwave Health Monitoring Study.

GWAS data available for 1,974 participants from the Airwave Health Monitoring Study as well as 1D 1H-NMR spectra for urine and blood were used. For each genetic variant, Spearman and Pearson partial correlation analyses were conducted against the NMR spectral data while adjustment for confounders and FDR correction for multiple hypothesis testing were applied. Moreover, a multivariate approach was used to construct correlation networks of SNPs and metabolic traits using the sparse partial least squares regression (sPLS) method. The latter allows the integration of genomics and metabolomics data as well as simultaneous variable selection in the two datasets. Both annotated metabolites and their ratios were examined.

Overall, 69 and 54 metabolites have been identified in urine and serum respectively. We found that variants in 14 out of 98 reported loci for CRC were associated with metabolites at FDR 5%. These metabolites mainly originated from lipoprotein subclasses, lipids from several lipoprotein particles, fatty acid residues, amino acids and sucrose. This information could produce new knowledge on the causal pathways linking genes to CRC and novel preventive targets. These results highlight the importance of lipid, fatty acid and sugar metabolism in CRC.

CHARACTERIZATION OF POPPY SEEDS AFTER HEAT TREATMENT USING METABOLOMIC FINGERPRINTS

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In recent years many cases of adulteration of poppy seeds with significantly cheaper opium poppy seeds (waste product) have been reported. Opium poppy varieties are characteristic with a high content of opium alkaloids (hundred of mg/kg) on the seed surface and couldn´t be used as a food. Potential counterfeiters have found a way how to decontaminate seeds by a process called thermostabilization (exposition to hot steam). Primary purpose of this process is inactivation of lipolytic enzymes, but the steam reduces the content of opium alkaloids from the seed surface which can be than sold as food varieties. This process can also reduce the stability of poppy seeds to oxidation.

This study firstly reports the effect of thermostabilization on the changes of poppy seeds quality during storage (38 days) in oxygen and nitrogen atmosphere. The both, basic fat quality parameters (acid value, peroxide value), as well as changes in metabolome and lipidomic profiles (U-HPLC-HRMS/MS; infrared spectroscopy), were monitored. The obtained data were chemometrically processed (PCA, PLS-DA, PLSR) and characteristic markers of thermostabilized and untreated poppy seeds were found and identified. For untreated seeds, a significant increase in free fatty acids was observed after milling. As a marker of thermostabilization, lipid oxidation products were found, so reduced oxidation stability of treated poppy seeds was confirmed.

EXPLORING THE FORMATION OF ADVANCED GLYCATION END PRODUCTS (AGE) IN DIFFERENT REACTION CONDITIONS

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The rapid increase in metabolic diseases, which occurred in the last three decades in both industrialized and developing countries, has been related to the rise in sugar-added foods and sweetened beverages consumption. An emerging topic in the pathogenesis of metabolic diseases related to modern nutrition is the role of Advanced Glycation End products (AGEs). AGEs can be ingested with high temperature processed foods, but also endogenously formed as a consequence of a high dietary

sugar intake Evidence also in humans suggest that the impact of dietary AGEs on different signalling pathways can contribute to the onset of organ damage in liver, lung, intestine, cardiac muscle and the brain, affecting not only metabolic control, but global health (1) . (AGEs) are generated in the late stages of Maillard reaction in foods and biological systems. These products are mostly formed by the reactions of reducing sugar or degradation products of carbohydrates, lipids, and ascorbic acid. In recent years, the Maillard reaction has gained considerable importance in areas as diverse as human pathology and flavour chemistry (2). This reaction, also called non-enzymatic browning or glycation, is of outstanding importance for the formation of colour, aroma and flavour precursors in foods. The majority of literature considers the Maillard reaction as a series of subsequent and parallel reactions-the early, advanced and final Maillard reaction steps. The molecules involved in these reactions are carbonyl and amino compounds, which include reducing carbohydrates and the free amino groups of amino acids, peptides or proteins. Maillard reaction products (MRPs), especially early stage MRPs and melanoidins, are currently gaining a lot of attention due to their reported health-promoting properties and their potential to be used as functional food ingredients. It is often not clear which specific biological function is assigned to which MRP, due to the large amount of MRPs formed during the reaction and difficulties in their purification and identification.

In this work we investigated by searching for AGE products that are formed in different reaction conditions (targeted metabolomics) and by evaluating the interaction of AGE products with mucin as a model of intestinal barrier for adsorption.

DETECTION OF "OLIVE QUICK DECLINE SYNDROME" MARKERS USING AN UNTARGETED METABOLOMICS APPROACH BY HPLC-HRMS TECHNIQUE

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The "Olive Quick Decline Syndrome" (OQDS) is a destructive disorder that since 2013 decimate olive trees in Apulia (Salento peninsula, southern Italy). The major incitant of this disease is *Xylella fastidiosa*, a quarantine no spore-forming pathogenic bacteria of American origin. The most seriously OQDS affected plants are heavily pruned by the growers to facilitate a future growth that, unfortunately, is scarce and quickly dries up.
From 2013 until nowadays, this gram-negative bacterium continues to proliferate and to expand, and an unprecedented turmoil has occurred for Apulia economy. A concrete resolution is hard to find also because OQDS is a multifactorial disease resulting in an unknown and very complex pathosystem. It is then essential to have

the tools to recognize the infected trees not only by detecting the bacterium itself, but also other characteristic compounds involved in the infection, such as those released by the plant for defence.
Therefore, the primary purpose of this work is to develop an efficient HPLC-HRMS analytical method based on untargeted metabolomics to identify metabolites that are differentially expressed in olive trees and involved in disease development or defence responses to OQDS. Firstly, the sample preparation protocol developed by University of Salento plans to finalize the best analytical method to extract all the metabolites of interest from healthy (HP) and infected (OP) olive trees samples. Subsequently, a liquid chromatography separation coupled to high-resolution mass spectrometry (LTQ Orbitrap Thermo Scientific) was set up by University of Torino for the identification of the metabolites. Finally, online software like XCMS were exploited for putative identification of the main metabolites involved in the host-pathogens interaction and for clustering pools of HP and OP samples. The poster shows the results obtained so far.

DETERMINATION OF ENDOGENUS METABOLITES IN MUSCAT OF ALEXANDRIA GRAPE AND MUST SAMPLES USING UPLC(HILIC)-MS/MS ANALYSIS.

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Muscat of Alexandria, a *V. vinifera* species' variety, with great commercial and nutritional value, that produces except from grapes, well-known aromaric wines and raisins. A widespread variety, in all the wine-growing regions of Greece and mainly in Lemnos, where protected designation of origin (PDO) wines are produced. Metabolomics-based analysis is a very useful tool, revealing valuable characteristics and nutritional components, as well as the origin-based differences, in a great range of foods and natural products. The present project aims to obtain the metabolic profile of grapes and must from single-variety vines of Lemnos, which produce the PDO Moschato Lemnos wine and to investigate its variation between different vineyards in Lemnos, at different stages of grape maturation and during the alcoholic fermentation of the must. A total of twenty-seven grape samples, harvested at three time points during the natural maturation of the grapes, from three different vineyards in Lemnos

and ten must samples, one for each day of the alcoholic fermentation, were analyzed using a targeted HILIC-MS/MS method of one hundred and one small polar compounds. Fifty-two and thirty-five metabolites were detected in must and in grape samples, respectively. Data handling, further statistical evaluation and biochemical interpretation of the obtained metabolic profiles demonstrated a reduction in organic acids, with a parallel increase in sugars, during the ripening process of wine grapes, while a decrease in fermented sugars and an increase in organic acids, in alcoholic fermentation. Overall, intense biosynthesis of organic nitrogenous compounds was observed for both specimens. Metabolic pathways of Kreb's cycle, of glycolysis, of biosynthesis-catabolism of nitrogen compounds (amino acids, amines, nucleosides) were found to be affected during grape ripening. Grape maturation was proven to be a more statistically significant factor to the influence it exerts on the metabolic profile compared to the environmental conditions (microclimate, cultivation conditions) prevailing in the vineyard. Regarding the alcoholic fermentation, statistically important affection of the phosphate pentose cycle and biosynthesis-catabolism of nitrogen compounds was observed.

TARGETED METABOLOMICS METHOD DEVELOPMENT ACROSS LIQUID CHROMATOGRAPHY DOMAINS

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The purpose of this study is the development of targeted metabolomics methods for biomarker discovery in cattle and swine gut health. Livestock biomatrices like rumen fluid, serum, urine, and feces vary wildly in composition, yet have been poorly characterized compared to human samples. In order to comprehensively search such a metabolomic space for quantitative biomarkers of health and disease, multiple chromatographic domains are likely to be required even within the boundary of liquid chromatography coupled mass spectrometry (LCMS). To that end, analytical reference standards that are part of the metabolic pathways of mammalian hosts and gut fermenters have been collected. These range from polar analytes like sugars, amines, and short chain fatty acids to highly apolar fatty acids, as well as intermediate compounds like prostaglandins, acyl amines, and sterols like bile acids. Here we present the systematic approach being used to build an in-house library of tandem mass spectrometric (MS/MS) transitions and their use to survey the retention of these metabolites on C18 and F5 (Pentafluorophenylpropyl) stationary phases.

METHOD DEVELOPMENT FOR LC-MS/MS MEASUREMENT OF CARBOXYLIC ACIDS IN ANIMAL MATRICES

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Determination of carboxylic acids like various fatty acids, keto- or hydroxy acids, and dicarboxylic acids is crucial in animal metabolomics. As especially short-chain carboxylic acids (SCCAs) are hardly retained under reversed phase liquid chromatography conditions, derivatization is a possibility to make SCFAs accessible to LC-separation. Recently, a method for derivatizing short chain fatty acids with aniline and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride as cross-linker has been published.

The first aims of our work are to establish a high performance liquid chromatography tandem mass spectrometric (HPLC-MS/MS) method for analysis of derivatized carboxylic acids and to determine which types of carboxylic acids can be derivatized by the published protocol. A further aim is to investigate whether derivatization also works in the presence of animal matrices like rumen fluid and to optimize the reagent concentrations required for complete derivatization of the target analytes in animal matrix. Further steps will be inclusion of internal standards produced by derivatization of the target analytes with ^{13}C-labelled aniline, validation of the method and application to biological samples of animal origin.

This poster presentation will give an overview of the challenges encountered and the results obtained on the way to establishment of a method for determination of carboxylic acids by HPLC-MS/MS after derivatization with aniline.

NMR-BASED CHEESE METABOLOMICS, QUALITY CONTROL AND AUTHENTICITY

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High resolution NMR spectroscopy is an important analytical methodology for polar metabolite and lipid profiling of cheese. This method has been used successfully on cheese samples to determine changes in the metabolic profile during maturation,1 determine the origin of buffalo milk and mozzarella PDO and PGI cheeses,2 and study the production chain of Asiago d'Allevo cheese.

In this report we present results of an ongoing project involving the detailed metabolite profiling and compositional mapping of Cretan cheeses. Complementing previous work on PDO Cretan graviera and salty white cheeses, this study presents the determination of the metabolite/lipid profile of the local white cheeses xynomyzithra, xygalo, and pichtogalo. Polar metabolites and lipid profiling were quantified using high resolution 1H NMR spectra obtained from water and chloroform extracts respectively. Additionally, the metabolite data were used to develop multivariate statistical analysis models that provide information regarding chemical composition differences between different categories of cheeses. Such models may be used to monitor quality control, discriminate the cheese samples by their metabolic profiles with respect to cheese type, geographical origin or cheese-making facility, and authenticate PDO and PGI labeling of cheeses.

NMR-BASED METABOLOMICS OF THE LIPID FRACTION OF ORGANIC AND CONVENTIONAL BOVINE MILK

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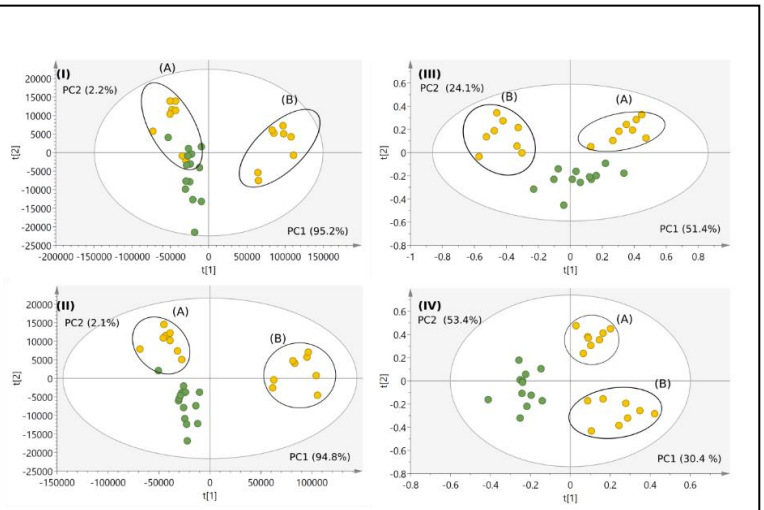
Abstract: Origin and quality identification in dairy products is an important issue and also an extremely challenging and complex experimental procedure. The objective of the present work(1) was to compare the metabolite profile of the lipid fraction of organic and conventional bovine milk using NMR metabolomics analysis. ^{1}H NMR and 1D TOCSY NMR methods of analysis were performed on extracted lipid fraction of



PCA and PLS-DA analyses Score plots on the UNor and NorCont data of the lipid fraction of organic and conventional processed (P) milk samples. (I) PCA score plot of UNor procedure, (II) PLS-DA score plot of UNor procedure, (III) PCA score plot of the NorCont procedure, (IV) PLS-DA score plot of the NorCont procedure. Yellow circles for conventional and green circles for organic samples; (A), conventional milk samples consisted of 1.5% fat content and (B), conventional milk samples consisted of 3.0 % fat content.

lyophilized milk. For this purpose, 14 organic and 16 conventional retail milk samples were collected monthly, and 64 bulk-tank (58 conventional and 6 organics) milk samples were collected over a 14-month longitudinal study in Cyprus. Data were treated with multivariate methods (PCA, PLS-DA) and a normalization of the data series is proposed that allows each analyte to be expressed as % content in the lipid fraction of each sample. Moreover, minor components were identified and quantified and modification of the currently used equations for their content determination is proposed.

Our results show the discrimination between organic and conventional milk produced in Cyprus with differences mainly being assigned to specific fatty acids. A significant increased % content of conjugated (9-*cis*,11-*trans*)18:2 linoleic acid (CLA), α-linolenic acid, linoleic acid, allylic protons and total unsaturated fatty acids (UFA) and decreased % content for caproleic acid were observed in the organic samples compared to the conventional ones. The present work confirms that lipid profile is affected by contrasting management system (organic vs. conventional), and supports the potential of NMR-based metabolomics for the rapid analysis and authentication of the milk from its lipid profile.

TARGETED AND UNTARGETED METABOLOMICS OF MICE URINE AND FECAL SAMPLES, IN THE DISCOVERY OF ALCOHOL TOXICITY BIOMARKERS.

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Metabolomics-based analysis has proven to be a reliable tool in biomarker discovery and in the elucidation of biochemical mechanisms. Alcohol health-related problems can occur at different levels of severity, from mild to life-threatening, such as cirrhosis of the liver, increased cardiovascular disease, malignant neoplasm, hepatic steatosis. The aim of the project is to study the metabolic profile of mice urine and fecal samples in order to gain new insight in the biochemical mechanism of alcohol toxicity in animal models.

For this purpose, a long-term animal experiment was conducted with 38 C57BL/6 mice (8-10 weeks old) of both genders separated into control and ethanol (5% v/v) *ad libitum* feeding groups, with *Lieber-DeCarli* diet for 8 weeks. Urine and fecal samples were collected in two time points (at 10^{th} and 20^{th} day after the beginning of the alcohol administration), while tissues were collected at post mortem. The animal experiment was conducted in agreement with the current national and European legislation (N. 2015/1992, ΠΔ 56/2013, European guideline 2010/63). Both targeted

and untargeted methods were applied for a comprehensive metabolic profiling of fecal samples while only targeted analysis was applied for urine samples due to low sample volume availability. An *in house* HILIC UHPLC-MS/MS method was applied for the detection of 101 polar metabolites, including sugars, amino acids, organic acids, amines, etc, in a single run of 40 min. Additionally UHPLC- QTOF-MS analysis was performed to study differences in lipids and non-polar metabolites in fecal samples. Data were processed using TargetLynx (Waters) and XCMS. Both multivariate and univariate statistical analysis were accomplished using SIMCA-P 13.0, MS Excel and GraphPad Prism version 7.00 for Windows and R programming language. Despite some limitations such as heterogeneity of fecal samples and low volume of urine samples, we could reveal a multitude of disturbances in the polar, non-polar and semi-polar metabolic content of the samples due to the alcohol intervention by both targeted and untargeted approaches.

EFFECT OF ALKANNIN AND SHIKONIN ON HORMONE-DEPENDENT BREAST CANCER CELL LINE MCF-7: AN LC-MS BASED METABOLOMICS STUDY

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Alkannin, shikonin and their esters are naturally occurring hydroxynaphthoquinones, biosynthesized mainly in the roots of plants belonging to the Boraginaceae family. These substances have well established antimicrobial, antioxidative, regenerative and wound healing properties, and nowadays there is a lot of evidence on their cytotoxic and antitumor activity. However, the molecular pathways involved in their action are not yet clearly defined.

Metabolomics aims to provide a global snapshot of all small-molecule metabolites in cells and biological fluids, thus is a promising tool for studying the effect and response of cells under treatment. In this study, we investigated the inhibitory effect of the natural products alkannin and shikonin on human breast cancer cell line MCF-

7, and applied both targeted (UHPLC HILIC-MS/MS) and untargeted (RP UHPLC-HRMS) metabolomics approach to gain further insight.

Alkannin and Shikonin cytotoxic activity on MCF-7 cells was examined at different concentrations (0.05 – 5 μM) and time points. After the cell viability assay, aliquots from both the extracellular and intracellular fluids of some groups were taken and submitted to HILIC-MS/MS and some to UHPLC-HRMS analysis.

UHPLC HILIC-MS/MS was performed on an ACQUITY Amide column with A: acetonitrile/water 95:5 v/v, and B: water: acetonitrile 70:30 v/v, with both in A and B 10 mM ammonium formate. MRM acquisition was applied for the detection of more than 100 hydrophilic metabolites playing key role in cells' metabolic activities. RP UHPLC-HRMS analysis was performed on an ACQUITY HSS C18 column by the application of a linear gradient elution with A: H2O, 0.1% HCOOH, and B: MeOH, 0.1% HCOOH. The detection was performed by an LTQ Orbitrap XL mass spectrometer. Electrospray ionization was applied in both positive and negative mode.

Interpretation of metabolic profiling data in both techniques was carried out by multivariate data analysis using SIMCA 13. PLS-DA and OPLS-DA models in the intracellular samples analyzed by UHPLC-HRMS revealed clear separation when shikonin vs control group and alkannin vs control group discrimination analysis was applied, both in positive and negative ionization mode. Components with major contribution to these models were determined and their respective m/z defined. By HILIC-MS/MS analysis, changes in cell metabolites (mainly in amino acids) were noticeable. Trypsin affected detection of metabolites and thus scraper was applied for cell viability test.

FATTY ACID PROFILING IN BLOOD OF HEALTHY INDIVIDUALS AND PATIENTS WITH HYPERLIPIDEMIA AND ASSOCIATION WITH ABCB1 2677G>T/A, PNPLA3C>G, CAT C>T, Taq1B-CEPT POLYMORPHISMS

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PNPLA3, CAT, CETP and ABCB1 are enzymes and transfer proteins involved in the metabolism of lipids, and common polymorphisms in their respective genes (rs738409, rs1001179, rs708272 and rs2032582 respectively) have been shown to affect blood lipid homeostasis, among other things. The purpose of this study was to develop and validate a rapid and reliable method for the quantitative determination of fatty acids in blood, with gas chromatography and flame ionization detector, and then examine their association with the afore mentioned polymorphisms.

Twenty FA (C14: 0, C15: 0, C15: 1, C16: 0, C16: 1, C17: 0, C17: 1, C18: , C18: 2cis, C20: 0, C20: 1n9, C20: 2 C20: 3n6, C20: 4n6, C20: 5, C23: 0, C24: 0 C24: 1 and C22: 6) were determined in blood after their esterification with CH3OH/HCl. The total fraction of lipids was extracted by the Folch method. The analysis was performed by gas chromatography with flame ionization detector (GC-FID) in a total run time of 26 min. The method was validated using spiked blood samples with standard solutions of fatty acid methyl estersand as IS the methyl ester of nonadecanoic acid. The parameters of accuracy, precision, linearity, limit of detection, limit of quantification and stability of the analytes were evaluated and were found within the acceptable limits.

The developed method was successfully applied to the analysis of blood clinical samples in a population of healthy (control) and patients with hyperlipidemia carriers of the major genotypes of rs2032582, rs738409, rs1001179, rs708272 (n=109). The differentiation in fatty acid profile was studied between the two groups following stratification according to all genotypes and within the same group between the different genotypes for each separate polymorphism. PLS-DA models were constructed for the discrimination of the groups (p value of CV Analysis was <0.05) and identification of potential biomarkers (VIP values were calculated too). ROCs were constructed for the evaluation of the models (AUC values were calculated as well), while box plots and heatmaps summarizing the potential correlations were obtained. Based on these it was found that differences in the concentrations of C14: 0, C16: 0, C16: 1, C18: 1, C18: 2 between healthy and patients were in almost all cases significant.

BUILDING A LOCAL UPLC-QTOF-MS MASS SPECTRAL LIBRARY USING THE MSMLS KIT

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MSMLS (Mass Spectrometry Metabolite Library) is a collection of high purity (>95%) biochemical compounds that span a broad range of primary metabolism. In this work, a local Metabolite Library has been developed for liquid chromatography-mass spectrometry metabolomics applications. For this purpose, 7 polypropylene plates in 96 well format were analyzed with injections of simple mixtures in an ultra-performance liquid chromatography system combined with a quadrupole time-of-flight mass spectrometer. The chromatographic separation was achieved on a Supelco Ascentis® Express AQ-C18 (100 × 2.1mm, 2.7 μm) HPLC column by gradient elution. Data was recorded in both positive and negative electrospray ionization in separate experiments; MS data were acquired from 70 to 1000 Da using two MS^E functions simultaneously (low and high energy ramp). By taking into account both chromatographic and MS attributes of the analyte peaks (i.e. retention time, parent and daughter ions), useful analytical information for every given compound was provided. The MLSDiscovery software tool was used to build the standard library, supporting the extraction, manipulation, and storage of the data generated when using MLS plates.

METABOLOMICS ANALYSIS FOR THE *IN VITRO* TOXICITY ASSESSMENT OF COCAINE IN HEPG2

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The toxicity of cocaine and its metabolites has been a subject of study for many years since cocaine is one of the most common drugs of abuse. The majority of studies focus on the determination of cocaine and its metabolites in biological matrixes in order to determine cocaine abuse.

Metabolomics-based analysis targets small molecules which take place in the metabolic processes. Metabolomics are mostly used in the study of diseases and focus in the identification of potential biomarkers. However there are several studies on the investigation of a drug intervention. These experiments usually are conducted *in vitro* or *in vivo* in order to focus on the effect of the drug.

In the current project, the toxicity of cocaine on human liver cancer cell line (HepG2) is assessed. Cocaine toxicity (LC_{50}) on HepG2 cells was experimentally calculated using an XTT assay at different concentrations from 0.1 mM to 10 mM. Afterwards metabolic profiling was performed in HepG2 cells to study the cytotoxic activity of cocaine at the estimated LC_{50} at three different time points (3h, 24h and 48h after). Cell medium was collected and frozen at -24^{o}C, then the wells were washed three times with PBS and the metabolism was stopped with the addition of 2 mL of ACN: MeOH: H_2O, 50:30:20 (v/v). The mixture was collected using a cell scraper and after freezing for 20 min at -24^{o}C. The collected samples of the extracellular and intracellular material were further analyzed with a validated HILIC LC-MS-MS method for targeted metabolomics analysis.

The chromatographic analysis was performed in ACQUITY Amide column with A: ACN: H_2O 95:5 v/v 10 mM ammonium formate, and B: H_2O: ACN 70:30 v/v 10 mM

ammonium formate. The triple quadrupole performed MRM acquisition for the detection of more than 100 hydrophilic metabolites of different metabolic pathways.

The obtained data were further processed and PLS-DA and OPLS-DA models were constructed for the discrimination of the groups and the identification of potential biomarkers in the extracellular and intracellular samples; clear separation was found between cocaine affected and the control samples. Following studies will include also untargeted metabolomics analysis using GC-MS and reversed phase LC-Q-TOF analysis in positive and negative mode.

NUCLEAR MAGNETIC RESONANCE AND RAMAN PROFILING OF MUSCAT OF ALEXANDRIA GRAPES MUST DURING FERMENTATION

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The verification of wine identity and authenticity is of urgent importance in the current context of a growing market globalization. As a result, wine authentication is an indispensable, as well as essential aspect in today's consumer protection. Regarding its chemical analysis the matrix wine is challenging, whereas its valuable characteristics are based on different factors, such as the geographical origin associated with the growing conditions, vintage and grape variety, as well as fermentation process parameters. Accordingly, the range of analytical methods to enable a comprehensive characterization of these products is highly diversified. Both Raman and Nuclear Magnetic Resonance (NMR) spectroscopies are valuable analytical techniques providing insights into chemical processes, including catalytic reactions. Since both techniques are chemically sensitive, they yield not only structural, but also quantitative information. In this work, for the first time, the combination of the two techniques has been employed to monitor the alcoholic fermentation processes in terms of targeted metabolite analysis.

In particular, grape must from the Muscat of Alexandria variety (Lemnos, Greece) sampled at different days of alcoholic fermentation were analysed by an ^{1}HNMR profiling method and Raman spectroscopy. Two individual series of samples were prepared for each technique.

The ^{1}HNMR spectra were recorded at 25 ^{o}C on an Agilent 600MHz instrument utilizing a PRESAT pulse sequence with a presaturation pulse to suppress the water signal. For the Raman measurements a LabRAM HR (HORIBA) spectrometer was employed with a laser line at 785 nm and a power of ~15 mW on the sample.

In the Raman spectra, glucose, fructose and ethanol content can be easily monitored. On the other hand, apart from these, ^{1}HNMR can provide information on a multitude of metabolites, indicatively, choline, malic, lactic, succinic, tartaric and other acids. The time evolution of the ethanol, glucose and fructose content of grapes must during fermentation was recorded and exhibited very good agreement between the two techniques. Initially, glucose is rapidly metabolized compared to fructose, the consumption of which accelerates after the fifth day of fermentation when the glucose content is already significantly reduced. Both sugars are almost fully metabolized by the tenth day of fermentation, in line with the measured increase of ethanol content.

KEYWORDS: wine, must, grapes, profiling, NMR, Raman spectroscopy

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