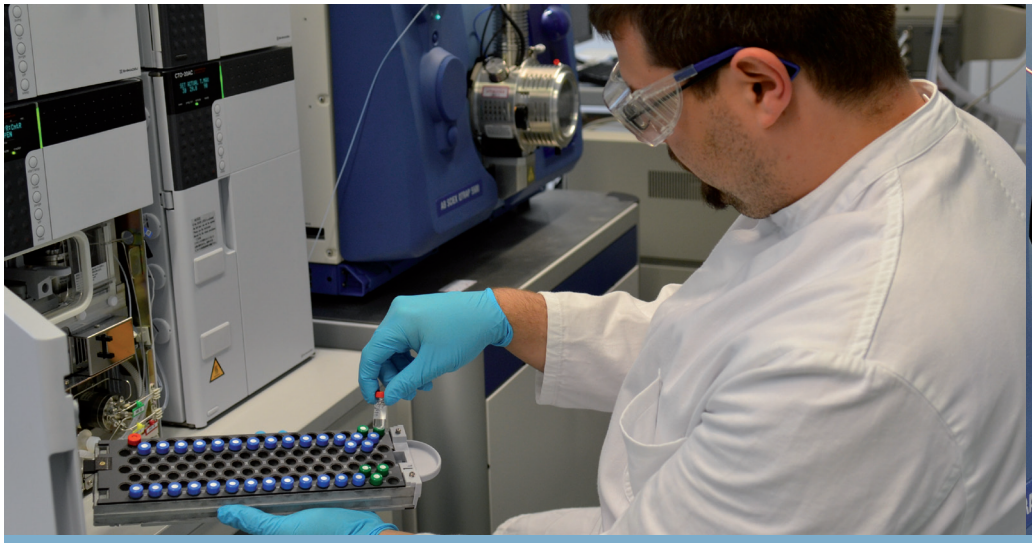
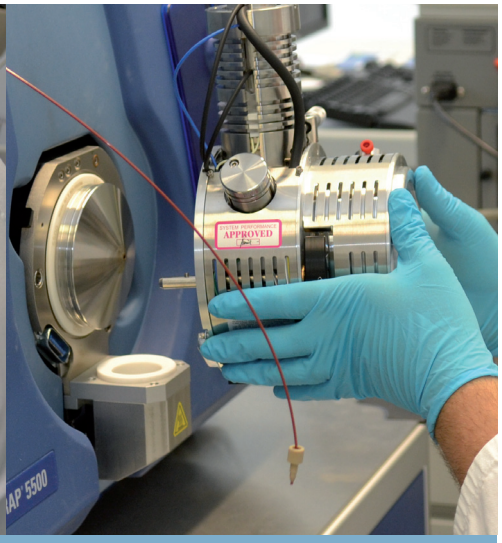
MRI
Max Rubner-Institut





Max Rubner Conference 2011

Food Metabolomics

October 9-11, 2011
Karlsruhe, Germany

MRI
Max Rubner-Institut

Speaker Abstracts

MRI
Max Rubner-Institut

Speaker Abstracts

Poster Abstracts

MRI
Max Rubner-Institut

Poster Abstracts

MRI
Max Rubner-Institut

Poster Abstracts

Max Rubner Conference 2011
October 9-11, 2011

MRI
Max Rubner-Institut

Speaker Abstracts

MRI
Max Rubner-Institut

Metabolomics: Principles and Potentials

Warwick (Rick) Dunn
Centre for Advanced Discovery and Experimental Therapeutics (CADET), School of Biomedicine
University of Manchester
Manchester, UK

Metabolomics is a highly suitable system to investigate the interaction of genotype and environment in biological systems, defined as the phenotype or metabotype [1]. The interaction of multiple molecular and biological processes are integrated in the metabolomes of cells, tissues and biofluids; a systems-level view is observed within a top-down systems biology context. Subtle changes in the genotype or environment can be rapidly and sensitively detected in the metabolome. Metabolomics can provide a high-throughput strategy where hundreds of samples are analysed in a single week and thus enables the diversity of the human phenotype, through the epidemiological study of thousands of people, to be accurately defined and changes related to nutrition, lifestyle (including diet and smoking), ageing (healthy or unhealthy), diseases and drug toxicity/efficacy to be experimentally observed.

The early stages of the emergence of metabolomics were dominated by developments in technology (for example, mass spectrometry instrumental advances) and bioinformatics (for example, HMDB). However, more recently these tools have being applied to the study of microbial, plant, environmental and mammalian systems. In mammalian systems [1] studies are diverse from understanding healthy ageing, molecular mechanisms related to disease initiation/progression [2] and drug toxicity/efficacy, putative and validated biomarkers and risk factors related to disease and drug treatment [3,4] and the role of gut microflora on health and disease.

In this presentation I will discuss why and how metabolomics is being applied in Manchester for both basic research (MCISB) and clinically-focussed (CADET) mammalian studies. The presentation will include (a) a general introduction to metabolomics and experimental strategies, (b) the development of robust methodologies for large-scale metabolic profiling applying mass spectrometry and quality assurance [5], (c) the study of a large healthy human population in an epidemiological-type study to define metabolome changes related to disease risk factors (BMI, smoking, blood pressure) and the 'health' status of populations and (d) the determination of molecular mechanisms of cardiovascular diseases (diabetes, heart disease, preeclampsia) in mammalian tissue and biofluids. I will finish with some foresight on limitations in metabolomics and potential future advances.

[1] Dunn et al. Chem Soc Rev. 2011, 40, 387-426.
[2] Dunn et al. Placenta. 2009, 30, 974-80.
[3] Mamas et al. Arch Toxicol. 2011, 85, 5-17.
[4] Kenny et al. Hypertension. 2010, 56, 741-9.
[5] Dunn et al. Nature Protocols. 2011, 6, 1060-1083.

MRI
Max Rubner-Institut

Metabolite Profiling – a Versatile Tool for the Assessment of Food Quality and Safety

Karl-Heinz Engel
Technische Universität München
Freising-Weihenstephan, Germany

Metabolites are the end products of cellular processes and represent the ultimate reflection of the response of biological systems to genetic and environmental changes. Metabolomics-based approaches to metabolite analysis have been developed in recent years providing tools that complement other unbiased techniques, such as transcriptomics and proteomics. They aspire to provide a comprehensive picture by extracting, detecting, identifying and quantifying a broad spectrum of metabolites present in complex biological systems. The unbiased and non-targeted screening of metabolic profiles in combination with appropriate statistical tools enables the evaluation of major impact factors on food quality and safety.

Different crops including cereals (maize, rice, barley) and legumes (soybeans, mung beans) were subjected to a metabolite profiling approach based on gas chromatography – mass spectrometry (GC-MS). The objective was to investigate the impact of genetic background (different cultivars), breeding strategy (genetic engineering, mutation breeding), environmental conditions (growing location, season), farming practice (organic farming, conventional farming) and food processing (malting, sprouting) on the respective crop metabolic phenotype. The employed extraction and fractionation methodology allowed a comprehensive coverage of a broad spectrum of low molecular weight metabolites ranging from lipophilic (fatty acid methyl esters, hydrocarbons, free fatty acids, fatty alcohols, sterols, tocopherols) to hydrophilic (sugars, sugar alcohols, organic acids, amino acids, amines) compounds. The resulting GC metabolite profiling data were assessed by means of multivariate and univariate statistical methods.

The presented metabolite profiling was shown to be suitable to the investigation of different plant breeding systems. The impact of induced mutations (low phytic acid rice and soybean mutants generated through irradiation) and the potential effects of genetic engineering (Bt- and Roundup Ready-maize) on the crop metabolite profiles were assessed. Investigations of the crops grown under different environmental conditions enabled the search for consistent differences. This allowed to distinguish between natural variability and changes induced by different treatments. In addition to the investigation of breeding systems, the applied non-targeted metabolite profiling was shown to be a useful tool to follow the metabolic changes during food processing. Metabolite profiling data generated in the course of crop sprouting (mung beans) and malting (barley) can provide valuable data regarding the safety status and the nutritional quality of the processed foods.

MRI
Max Rubner-Institut

Mass Spectrometry Based Metabolomics

Katja Dettmer
University of Regensburg, Institute of Functional Genomics
Regensburg, Germany

Metabolomics is an important building block of systems biology. It strives for the comprehensive and quantitative analysis of metabolites in a biological system. Investigating metabolism, as a key descriptor of phenotype, means analyzing the distribution of metabolites as a function of disease, nutrition, environmental influences, genetic modifications or toxicity. It will help to understand the mechanisms by which phenotypic changes develop. However, the comprehensive evaluation of the metabolome is still a challenging task, which cannot be met by a single analytical platform. The successful implementation of metabolomics requires quantitative analyses with high throughput, resolution, reproducibility, and sensitivity. Due to the complexity of metabolites in biological samples, the assembly of different analytical platforms is necessary to obtain maximum coverage of the metabolome. Mass spectrometry coupled to chromatography is a powerful analytical platform offering high detection sensitivity and resolution as well as the ability for structure elucidation of unknowns.

A central focus of our work in the field of metabolomics is the development of an array of robust methods to qualitatively and quantitatively probe the metabolome. A potpourri of those methods will be presented. This includes both metabolic fingerprinting and metabolic profiling approaches. Using chromatographic separation techniques, such as gas chromatography and liquid chromatography coupled to mass spectrometry, a broad range of metabolites can be covered.

Metabolic fingerprinting is primarily performed based on gas chromatography coupled to mass spectrometry (GC-MS) after derivatization. This approach delivers both metabolic fingerprinting data as well as the quantitative analysis of selected metabolites using both one-dimensional GC-MS as well as comprehensive two-dimensional GC (GCxGC-TOFMS). A major bottleneck for metabolic fingerprinting by GC-MS is the identification of unknowns. Although commercial mass spectral libraries are available, they still fail to identify all signals in complex GC-MS chromatograms. The complex fragmentation caused by electron ionization and the potential absence of the molecular ion renders the structural elucidation of unknown metabolites cumbersome. To tackle this problem atmospheric pressure chemical ionization (APCI) is used to couple GC to a high resolution TOFMS (Bruker MicrOTOF). This soft ionization technique produces quasi molecular ions that in combination with high resolution accurate mass measurement and consideration of the isotope pattern can be used to generate a sum formula as a first step to identify unknown metabolites. Furthermore, LC-MS/MS based methods for metabolic profiling of selected metabolite classes such as amino acids and amino acid metabolites will be presented.

MRI
Max Rubner-Institut

NMR-Based Metabolomics

Burkhard Luy
Institute of Organic Chemistry and Institute for Biological Interfaces II,
Karlsruhe Institute of Technology (KIT)
Karlsruhe, Germany

Nuclear magnetic resonance spectroscopy and mass spectrometry constitute the two major techniques in modern metabolomics studies. While mass spectrometry allows the detection of smallest amounts of metabolites, NMR spectroscopy is inherently quantitative and allows remarkable reproducibility even between laboratories.

In this talk, a brief introduction into NMR spectroscopy will be given with a special focus on its advantages and limitations with respect to metabolomic studies. It will be shown that NMR spectroscopy is very well suited to characterize even small changes in samples for example on storage conditions used. The technique is very well applicable to all kinds of body fluids, but also to the investigation of biological model organisms. Example spectra will demonstrate the achievable quality for such systems.

MRI
Max Rubner-Institut

Data Processing and Data Analysis Tools in Metabolomics

Joachim Selbig
University of Potsdam
Potsdam, Germany

The metabolome is the entirety of small molecules present in an organism and can be regarded as the ultimate expression of its genotype in response to environmental changes. It is regulated among other regulatory mechanism by enzyme activities that are themselves dependent on gene expression levels. Furthermore, it can be assumed that metabolite concentrations and their fluxes are a reflection of the physiological status, as manifested by whole-plant phenotypic properties.

In contrast to gene expression analysis by microarray experiments, metabolite profile analysis starts with much more complicated experimental measurement values. However, the subsequent data reduction and the possibility to measure a larger amount of samples helps in uncovering relationships between molecular data and the phenotype.

To date, more than 100 000 different metabolites of broad biochemical complexity have been discovered in the plant kingdom and typical non-plant eukaryotic organisms are estimated to contain 4000 to 20 000 metabolites [1]. The high number of metabolites, together with their biochemical complexity and a wide dynamic range of abundances, hampers a comprehensive analysis. The technical and analytical challenges in metabolome analysis have been recently reviewed in detail [2].

In order to reliably measure a large fraction of the small molecules in the biological matrix, samples are analyzed using high-resolution techniques such as NMR and MS and are often preceded by a separation method like liquid chromatography, GC or capillary electropho-resis. While NMR is non-invasive and allows compound structure elucidation in a low number of samples of low complexity, MS approaches are widely used in large-scale experiments because of their high throughput capacity, robustness and sensitivity. The method of choice therefore depends on the experimental design as well as on the biological question.

We will focus in this presentation on specific aspects of metabolite profile analysis: the evaluation of the interactions between metabolites, the uncovering of the connection between metabolism and the phenotype (e.g. as measured by the biomass or morphological properties) and the establishment of relationships between gene expression and metabolite profiles. The latter is to date the most difficult task because the number of observations is often much smaller than the number of investigated genes. For the same reason, the uncovering of relationships between gene expression and physiological properties is difficult [3].

[1] Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L (2004) Metabolite profiling: From diagnostics to systems biology. Nat Rev Mol Cell Biol 5: 763–769.
[2] Goodacre R, Vaidyanathan S, Dunn WB, Harrigan GG, Kell DB (2004) Metabolomics by numbers: Acquiring and understanding global metabolite data. Trends Biotechnol 22: 245–252.
[3] Steinfath M, Groth D, Lisec J, Selbig J (2008) Metabolite profile analysis: From raw data to regression and classification. Physiologia Plantarum 132: 150-161.

Max Rubner-Institut

Databases in Metabolomics

David S. Wishart
Depts. of Biological Sciences and Computing Science, University of Alberta
Edmonton, AB, Canada

Thanks to recent technological advances, biology has become a data-driven science. Routine biological experiments now generate terabytes of information. Unfortunately, only a fraction of this information can be presented in the ~1 million life science articles that are published each year. As a result, many life scientists are turning to the internet to store, retrieve, consolidate and exchange data. This has give birth to the “era of biological databases”. As a younger cousin to genomics and proteomics, metabolomics is just beginning to enter into this era. Nevertheless, if metabolomics is going to succeed as a scientific discipline it must develop a solid database infrastructure. In this presentation I will briefly describe a number of metabolomic databases that we, and others, have developed. In particular I will review chemical databases, pathway databases, spectral databases and integrated metabolomic databases. I will also highlight some of the critical features that need to be included in modern, integrated metabolomic databases, especially those pertaining to food and nutrition. As most metabolomic databases are maintained only through the dedicated efforts and generosity of individual labs, one of the key efforts that metabolomics must undertake in the near future is the development of a common, community funded metabolomic data repository. I will conclude this presentation by discussing some of the benefits and challenges associated with such an effort.

MRI
Max Rubner-Institut

Metabolomics of Genetically Modified and Non-transgenic Crops

Derek Stewart
The James Hutton Institute
Dundee, Scotland

Food has, over the last several years, been brought swiftly to the top of the world governmental agendas. This is the result of multiple driving forces: global finance and food insecurity, a population predicted, by the United Nations, to hit 8.92 billion by 2050 and the now de rigueur requirement for food to be generated sustainably in a changing environment. The consumers are also getting more educated and their expectations of food have increased significantly compared with 20 years ago. The end result of this is that we need to produce more food at an equivalent or higher quality with lower inputs. These aims are achievable using conventional breeding, but with a time line of 10-20 years, and this has meant that state-of-the-art genetic and analytical technologies are coming to the fore. The concept of metabolomics, underpinned by mainstream (GC-MS, LC-MS, NMR) and specialist (e.g. MALDI-ToF-MS) analytical technologies addressing broad chemical (class) targets and dynamic ranges, offers significant potential to add significant value to crop and food science and deliver on future food demands. Metabolomics has now found a home in the food analytical toolbox with raw material (crop) quality and safety the major quality areas. However, as we will show, it is translating beyond this into food storage, shelf-life and post harvest processing.

Food research is undergoing a renaissance with the requirement for a greater understanding of how our food is produced, its origins and the changes associated with the multiple and, often highly specialised, post harvest processes. At all stages of these post-harvest processes, and indeed during the crop developmental and ripening stages, the inherent (bio)chemistries change, often with a modification in one component directly affecting another. This has major consequences throughout the food chain not least with respect to food safety and one where metabolomics has made many advances.

If, as alluded, to above we are to satisfy the global demand for more and better crop derived food we must adopt the molecular genetic approaches of marker-assisted breeding through to genetic modification (GM). Although a sensitive topic, the application of GM technologies to crop modification and enhancement offers huge potential in delivering the world's food expectations. The safety concerns regarding the potential for unintended effects, metabolic compensation etc, have been the subject of many national and international projects such as SFEFOODS, NOFORISK etc within which metabolomics has been at the forefront of a new paradigm in risk assessment and examples of this will be highlighted.

Metabolomics is also being utilised to address global concerns around the impact of climate change in many crops systems. The innate untargeted nature of metabolomics is ideally suited to this as climate change is a multifactorial problem which impacts on many crop processes such as temperature and environment sensing, responses to drought/flooding etc. Examples of how metabolomics is being applied to these problems in crops will highlighted using ongoing transnational projects, such as Climafruit (www.climafruit.com).

MRI
Max Rubner-Institut

Metabolomics Approach to Residue and Contaminant Analysis

Arjen Lommen, Jeroen Rijk, Ainhoa Ruiz-Aracama,Toine Bovee, Michel Nielen
RIKILT, Institute of Food Safety, Wageningen UR
Wageningen, The Netherlands

Advances in separation (p.e. UPLC, GCxGC) and full-scan MS technology (p.e. TOF, FTICR and Orbitrap MS) are increasing sensitivity and selectivity, but also increasing the volume of raw data. This in turn is increasing the total full costs, since more man hours are needed for data analysis. In order to deal with the increasing data stream a higher degree of automation is needed. The field of metabolomics is driving an increasing automation in data handling and is starting to provide us with exciting opportunities in targeted and untargeted analysis as well as databasing. We present a cost-efficient processing strategy – termed instrumental screening - which deals with the large amounts of data we encounter in residue and contaminant analysis.

New software tools using multiple cores have been developed which are now starting to be used for pre-processing and querying data sets. Since raw data are reduced by a factor 100 to 1000 in size prior to querying, this approach shows great promise for databasing complete profiles. Furthermore targeted querying of small files has the inherent benefit of being much faster than using raw data. Therefore, a strong collaboration with the MADMAX (Management and analysis database for multiple ~omics experiments) project is in progress to combine the strong points of this database and the developed analytical data-querying.

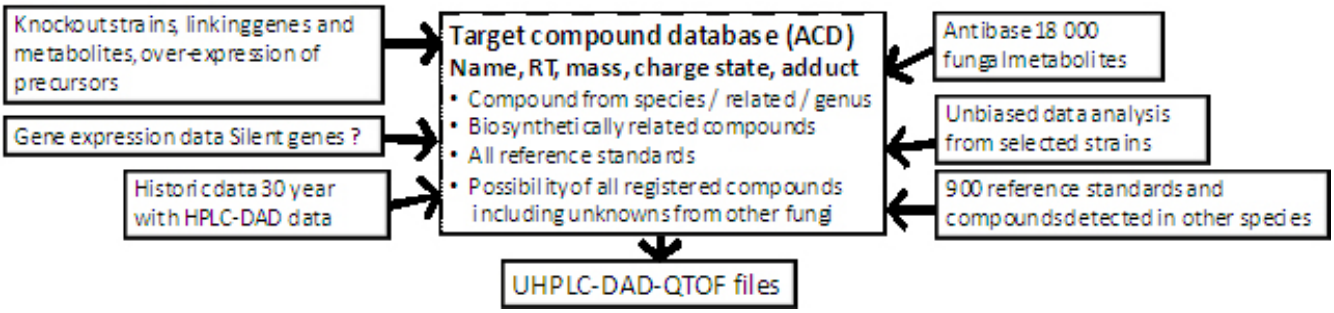
Pre-processed datasets can be aligned and then further analyzed with univariate/multivariate statistics. Selections of signals can be used for further identification. We will show 3 examples of this. The first example is finding and identifying metabolites in urines of calves treated with the natural prohormone DHEA. The second is studying the mode of action of endocrine disruptors in the human H295R adrenocarcinoma cell line, which expresses the complete steroid biosynthesis pathways. The last example is finding and identifying paracetamol metabolites in urine of humans treated with dosages of paracetamol well below maximum daily allowed dosage.

UHPLC-DAD-qQTOF Profiling, Identification and Dereplication of Fungal Metabolites using a Multi-Target Approach

Kristian F. Nielsen, Anita Iversen, Dorte K. Holm, Mikael R. Andersen, Jens C. Frisvad, Thomas O. Larsen
Department of Systems Biology, Technical University of Denmark
Lyngby, Denmark

Fungal secondary metabolomics aims at identification of all metabolites in a given sample. This can be important in drug discovery, food safety, and for establishment of connections between gene-sequences and products. However detection of peaks and especially the significant ones under particular biological conditions is only the first step. Next step is linking known structures to peaks, which is an extremely challenging part, as reference standards are only available for a minor part of the described compounds in the literature.

At CMB we have worked with chemotaxonomy since the early 1980'ties first using TLC, latter HPLC-DAD, Direct-ESI-MS, and HPLC-DAD-TOFMS, and most lately the new UHPLC-qTOF instrument (Bruker maXis). Our latest strategy is to combine our in-house compound-data with databases such as Antibase (38 000 microbial secondary metabolites).



This presentation will illustrate how we search all known compounds (typically 600-1100) in the data files using the Bruker TargetAnalysis software. Examples will be given from our recent work on Aspergillus, Alternaria and Fusarium where we tentatively identify the compounds by combining accurate MS , MS/MS, UV/VIS, logD, taxonomy, ion-exchange properties, unless they are not identified as one of the 900 fungal secondary metabolites we have as reference standards. Information about all compounds including unknowns (registered as mono-isotopic mass, UV/VIS, retention time, and fungal species) are being collected in an database (ACD format). This allows sorting of compounds, including finding related compounds by cross referencing between all detected peaks in all running projects. Expression data as well as gene knockout strains are further being used link compounds and genes as well as indicate the fraction of unexpressed genes, subsequently requiring improved cultivation media and conditions.

MRI
Max Rubner-Institut

Mass Spectrometry Based Metabolomics of Plant Resistance Against Pathogen Attack

Ajjamada Kushalappa
McGill University
Quebec, Canada

Target biochemical analyses, of plant-pathogen interactions, have revealed several mechanisms by which plants resist biotic stresses. However, a comprehensive biochemical, metabolomics and proteomics, technology offers visualization of an array of biochemicals, which can be correlated to biotic stress, and linked to metabolic pathways and genes to better understand the mechanisms of plant-pathogen interactions. Metabolomics and proteomics of plant biotic stress is the comparison of biochemical profiles of biological systems, at their homeostasis or perturbed environment status, to extract system information, from which the system knowledge is generated. Mass spectrometry technology has been applied to detect not only thousands of biochemicals but also the monomers and oligomers of structural components of plants. This comprehensive information can be used to improve resistance in plants to biotic stress. Here the mass spectrometry based metabolomics approach to better understand mechanisms of resistance in plants against pathogen stress will be presented.

Plants, in nature and as well under commercial production, are subjected to abiotic and biotic stresses. The plant-pathogen system relationship is a parasitic relationship where the pathogen derives food from the host. Such relationships could be biotrophic, where the pathogen establishes infection in living host to live and to multiply, or necrotrophic, where the pathogen kills the plant cells before colonizing and feeding on them. A necrotrophic system, Triticeae-Fusarium graminearum, has been used here to describe various steps involved in metabolomics.

The application of comprehensive metabolomics technology to better understand, phenotype and improve resistance in plants against biotic stress can be grouped into ten major heuristic steps: 1) Plant and pathogen systems for comparison; 2) Factors influencing plant-pathogen interaction; 3) Assessment of resistance against stress; 4) Sample collection; 5) Metabolite extraction; 6) Metabolite analysis; 7) Mass spectral output analyses; 8) Data visualization and information extraction; 9) New knowledge generation; and 10) Application of knowledge and technology transfer. These steps will be presented using Triticeae-Fusarium graminearum as a plant-pathogen system.

Barley spikelets were inoculated with Fusarium graminearum, ground in liquid nitrogen, metabolites were extracted with aqueous methanol and analyzed based on a hybrid mass spectrometer. Several resistance-related metabolites were identified, but these were mainly constitutive (Bollina et al. 2010. Mol. Plant Path. 11:769-782; Kumaraswamy et al. 2011. European J. Plant Path. 130:29-43). However, instead of spray inoculation of pathogen the drop inoculation reduced the experimental error and yielded several resistance-related induced metabolites (Kumaraswamy et al. 2011. J. Chem Ecol. DOI: 10.1007/s10886-011-9989-1; Bollina et all. 2011. Plant Mol. Biol. - accepted). These metabolites belonged to phenylpropanoid-flavonoid, terpenoid and fatty acid pathways. These compounds varied in thier antimicrobial properties. An isolate producing trichothecenes induced a signal molecule jasmonic acid but not a mutant lacking in trichothecene production (Kumaraswamy et al. 2011. Plant Path. - accepted). The resistant related metabolites reduced the synthesis of trichothecene (deoxynivaleonol production almost completerly at LD50 concentrations much lower than for biomass reduction (Bollina et al. 2011. International J. Myocology - accepted). Several of these resistance-related metabolites occuring in more than one resistant genotype were selected as biomarkers for potential screening of breedinglines.

MRI
Max Rubner-Institut

Metabolomics to Characterize and Study the Quality of Fleshy Fruits

Annick Moing
INRA Bordeaux, UMR 1332 Fruit Biology and Pathology
Metabolome Facility of Bordeaux Functional Genomics Center
Villenave d'Ornon, France

Variations in fruit flesh development and composition, along fruit growth and maturation, have a major impact on the organoleptic and nutritional quality of ripe fruit. Metabolomics approaches renew our strategies to characterize and study the compositional changes of fruit. Several cases will be described illustrating targeted or untargeted metabolic profiling of tomato (Solanum lycopersicum), melon (Cucumis melo L.) or other fleshy fruits for diagnostic or functional genomics.

For tomato,1H-NMR profiling was used in order to characterize the organoleptic quality of fruit at commercial maturity along an entire season. Compositional changes were followed for successive harvests in a greenhouse. Multivariate analyses revealed discriminant metabolites for season or variety and showed that nutrient solution recycling had little effect on fruit composition. A similar approach was used within the European project ISAFRUIT on several Prunus species to provide data on the biochemical phenotype of ripe fruit and reveal mQTL. In order to get new insights concerning the mechanisms controlling the fleshy trait in fruit, we undertook a comparative study of two expanding fruit tissues in tomato by using detailed cytological analyses, transcriptome and a combination of metabolic profiling approaches. Multivariate analyses showed that pericarp and locular tissues considerably differed in their metabolite composition. In addition, the integration of transcriptome and metabolome data revealed that changes in tissue composition were related to transcriptional changes, not only of genes involved in central metabolism but also of genes implicated in diverse signalling pathways.

For melon, we studied the metabolic and mineral element changes in fruit, using an unprecedented range of analytical platforms within the European project META-PHOR: targeted LC-DAD and ICP-MS, untargeted proton NMR, GC-MS and LC-MS. Metabolite and element profiling of several commercial varieties allowed characterizing the effects of year, culture conditions and genotypes on fruit flesh quality and highlighted discriminant compounds. We also dissected the spatial and temporal variability of metabolic and mineral element profiles in the flesh of one variety in order to improve our knowledge of fruit metabolism and physiology. We took advantage of the metabolite and element variability, to study co-regulated compounds using clustering or correlation networks. These analyses revealed hubs in the networks, and highlighted the crosstalk between primary and secondary metabolites or between metabolites and mineral elements. Proton NMR and GC-MS spectra and data, as well as metadata of the tomato and melon studies, were deposited into MeRy-B database (http://bit.ly/meryb) and are open to the community.

The present examples demonstrate the potential of combined metabolomics approaches for diagnostic studies of fruit quality as well as new physiology or genomics studies corroborating previously known metabolic links and revealing unexpected relationships. Such approaches, integrated with enzymatic measurements will contribute to characterize and model the effect of environmental factors on carbon metabolism of tomato fruit during its development within the Eranet EraSysBio+ FRuit Integrative Modelling project.

Deborde et al. (2009) Metabolomics 5: 183–198.
Ferry-Dumazet et al. (2011) BMC Plant Biology 11:104.
Moing et al. (2011) New Phytologist 190: 683-696.
Mounet et al. (2009) Plant Physiology 149: 1505-1528.

Max Rubner-Institut

Influence of Postharvest Conditions on the Metabolite Profile of Deciduous Tree Fruits

James Mattheis and David Rudell
USDA, ARS Tree Fruit Research Laboratory
Wenatchee, WA USA

Market life of apples and pears is extended after harvest by holding fruit in cold storage often in controlled atmosphere rooms where O2 and CO2 concentrations are managed. While temperature management directly impacts metabolic rate resulting in slowed respiration and other metabolic processes, atmosphere management contributes to further reductions in ethylene production and action, processes critical for promoting ripening of these climacteric fruits. Chemical inhibition of ethylene action via 1-methylcyclopropene rapidly alters climacteric fruit metabolism in a manner similar to that induced over a longer time period by controlled atmospheres. All of these postharvest technologies alter primary and secondary metabolism resulting in changed profiles of many compounds including alcohols, aldehydes, amino acids, carbohydrates, esters, membrane components, pigments and others. Of particular importance to both cosmetic and edible aspects of fruit quality, controlled atmospheres and 1-MCP influence development of market limiting physiological disorders of both peel and cortex tissues. The potential for exploiting identification of metabolic patterns related to disorder development is the objective of ongoing research to enable disorder prediction prior to symptom development and to differentiate disorders with similar appearance to assist in forensic analysis.

Max Rubner-Institut

Metabolomics as a Tool for Food Processing

Ric de Vos[1,2,3], Roland Mumm[1,2], Jules Beekwilder[1,2], Flavio Borém[1,5], Esra Capanoglu[1,6], Patricia Lopez-Sanchez[3,4], Lucy Bialek[3,4], John van Duynhoven[3,4], Robert Hall[1,2,3]

[1] Plant Research International and [2] Centre for Biosystems Genomics, Wageningen-UR, Wageningen, The Netherlands;

[3] Netherlands Metabolomics Centre, Leiden, The Netherlands;

[4] Unilever Research Centre, Vlaardingen, The Netherlands;

[5] Federal University of Lavras, Lavras, Brazil; 6Food Engineering Department, Istanbul Technical University, Turkey

Plants and products derived thereof are known to contain a huge array of metabolites, including the large and economically important group of so-called secondary metabolites such as terpenoids, alkaloids, saponins, flavonoids, glucosinolates, polyamines and all kinds of derivatives thereof. As most of the compounds present in crude plant or food extracts are still unknown, so-called untargeted metabolomics approaches, in which all metabolites detected in extracts are taken into account, enable the most comprehensive comparison of plant-derived samples for differences and similarities in metabolite composition. These untargeted comparative metabolomics approaches, followed by identification of differential metabolites, are nowadays frequently applied in plant research e.g. in relation to disease resistance, genetic variation, fruit ripening, and quality of plant products.

Within this presentation, we will provide some examples of using untargeted LCMS and GCMS based metabolomics approaches as a tool for food processing. For instance, we investigated the alterations in metabolite profiles taking place during the industrial processing of fresh tomato fruits towards canned tomato paste. This untargeted approach, in addition to targeted analyses of compounds of prime interest. identified those tomato secondary metabolites that were mostly influenced by each separate processing step and elucidated the most important steps influencing the overall metabolite profile and level of health-related phytochemical compounds in tomato paste. Also, different orders and temperatures of food treatments are being compared for their effects on the overall metabolite composition of raw carrot, broccoli and tomato vegetables. These studies help us to understand and control the mechanisms underlying differential metabolite compositions and to define the best strategy for achieving optimal nutritional profiles of vegetable products. With regard to coffee, we are applying large-scale metabolomics to investigate the effects of post-harvest treatments on the metabolite composition of green coffee beans and their relation to compounds that determine cup quality.

These examples indicate that metabolomics, in particular techniques involving comprehensive untargeted profiling approaches combined to dedicated statistics, can be a powerful tool to identify plant metabolites that are most affected upon food processing, to unravel the mechanisms underlying the processing-induced metabolite changes, and to define and control key steps in food processing that determine product quality.

Max Rubner-Institut

NMR-based Quality Screening of Food: from Research Application to High Throughput Screening

Léa Heintz, Birk Schütz, Fang Fang, Eberhard Humpfer, Claire Cannet, Monika Mörtter, Hartmut Schaefer and Manfred Spraul
Bruker BioSpin
Rheinstetten, Germany

Based on the metabolomics approach, ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) screening has rapidly expanded in recent years in the area of food quality control.

^{1}H-NMR is a global, reliable and reproducible method allowing the acquisition of spectral fingerprints. ^{1}H-NMR screening is therefore an efficient method for both targeted and non-targeted analysis.

Classical targeted quantification of multiple compounds as well as non-targeted statistical analysis can be applied to the spectra. The statistical data analysis consists of sample classification, sample verification and indirect quantification of parameters that are not detectable in ^{1}H-NMR spectra.
This allows not only to assess the authenticity of food samples but also to detect unknown frauds.

The workflow and the requirements for the development of such a method will be discussed through examples given on different food material like fruit juice, wine and edible oil.

Sample preparation has to be optimized in order to generate absolutely robust samples, while modifying the mixtures as less as possible.

NMR measurements have to be set up for optimum usage of the dynamic range.
Data analysis needs to answer questions of sample classification, verification and quantification.

Finally, the conversion of a research application into an expert, fully automated system, able to take decisions and answer proper questions, will be discussed through the example of fruit juice screening.

MRI
Max Rubner-Institut

Organic vs Conventional Farming of Wheat: Influence on Metabolite Profiles

Georg Langenkämper[1], Anja Bonte[1,2], Heiko Neuweger[2], Isabell Hildermann[3], Paul Mäder[3], Karsten Niehaus[2]
[1] Department of Safety and Quality of Cereals, Max Rubner-Institut, Detmold, Germany
[2] Faculty of Biology, Proteome and Metabolome Research, Bielefeld University, Bielefeld, Germany
[3] Research Institute of Organic Agriculture (FiBL), Frick, Switzerland

The increasing popularity of organic farming and organic food leads to a great economic interest in finding discriminating analytical methods to ensure the authenticity of organic labeled products. Using a metabolite profiling approach, we set out to identify biomarkers capable of distinguishing organic and conventional wheat.

Wheat grown under the well controlled conditions of the long term DOK-field trial of the Research Institute of Organic Agriculture (FiBL) and the research station Agroscope Reckenholz-Tänikon (ART), Switzerland was chosen for analysis. In the DOK-field trial organic and conventional cultivation is performed in four plot replications at the same location. We analysed 11 different wheat varieties of the harvest year 2007 to assess the influence of a diverse genetic pool on the spread of analytical results. Additionally, samples of the wheat variety "Runal" were taken over three harvest years in order to account for influence of seasonal variations. Metabolite profiles were generated with GC-MS from derivatised methanol extracts of finely ground whole wheat grains. Employing these techniques on the variety "Runal", we were able to identify 48 metabolites and additionally to detect 245 not identified metabolites (TAGs). In this pool of biomolecules, three metabolites showed significant differences in normalised peak areas in all three harvest years of "Runal". Across all 11 varieties of the 2007 harvest year, 5 metabolites and 11 TAGs with significant differences in peak areas between the cultivation forms were detected, using Student's t-tests. PCA performed on data for the individual varieties revealed a clustering according to the cultivation forms. However, PCA of metabolites and TAGs of combined data of all 11 varieties did not result in a clustering.

Based on individual varieties, metabolite profiling has shown promising results with respect to discriminate organic and conventional wheat. Results viewed across all 11 varieties indicated a higher influence of the variety and seasonal effects than the cultivation form on metabolite concentration. Further work will prove, if significant differences of concentrations in individual metabolites and TAGs can be used to discriminate between cultivations forms across multiple wheat varieties. This work is conducted as part of the research project "Advancement and recommendation for the use of selected methods applicable to differentiate organic and conventional products" (Project No 08OE023 and 08OE044), funded by the German Federal Ministry of Food, Agriculture and Consumer Protection within the „Bundesprogramm Ökologischer Landbau und andere Formen nachhaltiger Landwirtschaft".

MRI
Max Rubner-Institut

Metabolite Profiling and the Phenol-Explorer Database as Tools to Characterize Phytochemical Intake and Exposure

A Scalbert[1], J Rothwell[2], C Manach[2], M Urpi-Sarda[3], M Boto-Ordóñez[4], R Llorach-Asunción[4], C Andres-Lacueva[4], J Perez-Jimenez[5], C Knox[6], R Zamora-Ros[7], L Fezeu[8], M Touvier[8], N Arnault[8], S Hercberg[8], P Galan[8], N Slimani[1], I Romieu[1]

[1] **International Agency for Research on Cancer (IARC), Nutrition and Metabolism Section, France.**
[2] **INRA, Unite de Nutrition Humaine, Saint-Genès-Champanelle, France.**
[3] **Department of Internal Medicine, Hospital Clinic, Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), University of Barcelona, Spain**
[4] **Nutrition and Food Science Department, XaRTA, INSA, School of Pharmacy, University of Barcelona, Spain**
[5] **Institute for Advanced Chemistry of Catalonia, CSIC (IQAC–CSIC), Barcelona, Spain**
[6] **In Siliflo Inc, Edmonton, Canada**
[7] **Unit of Nutrition, Environment and Cancer, Catalan Institute of Oncology (ICO-IDIBELL), L'Hospitalet de Llobregat, Spain**
[8] **INSERM U557, INRA U1125, CNAM, Universite de Paris 13, Bobigny, France**

The key role played by phytochemicals in the prevention of chronic diseases is more widely recognized today. However the exact nature of the most protective polyphenols remains elusive. In particular, more than 500 different polyphenols have been described in various foods, but most studies have been focused on a single or a very limited number of compounds. Bioinformatics and analytical techniques available today have evolved considerably in the last few years and now allow to take into account the complexity of the polyphenol family. In particular, it has now become possible with metabolomics to analyse hundreds of metabolites at a time in human urine or plasma and to study associations with diseases in metabolome-wide association studies. New tools have been developed to characterize in populations exposure to the polyphenol metabolome. Phenol-Explorer, the most complete database on polyphenol content in foods, allows to build food composition tables for polyphenols and to calculate intake of all known polyphenols in various cohorts. The recent addition to the Phenol-Explorer database of a module on all polyphenol metabolites described in humans and experimental animals together with pharmacokinetic data also allows to evaluate their respective value as biomarkers of intake. Recent results and perspectives offered by these new tools to evaluate the effects of polyphenols and other phytochemicals on disease risk will be presented.

Max Rubner-Institut

Metabolomics as a Tool to Characterise Food Intake

John Draper
Aberystwyth University
Aberystwyth, UK

Western diets are generally complex and conventional methods of measuring habitual dietary exposure such as Food Frequency Questionnaires (FFQs) depend upon food intake estimates and are subject to errors, which can confound interpretation of subsequent data. Descriptors of individual FFQ food components vary in degree of distinctiveness and consumption patterns generally typical of each food component display great variability, including effects of seasonality. Against this background we have been exploring the use of metabolomics to help validate FFQ dietary component descriptors without prior knowledge of biochemical markers potentially indicative of habitual exposure to specific foods.

Initially we demonstrated that non-targeted metabolite fingerprinting using Flow Infusion ESI-MS (FIE-MS) in conjunction with machine learning data analysis can be used to explore relationships between the chemical content of overnight or fasting urine and reported levels of citrus exposure in 24 humans consuming a freely-chosen diet. Fourier-Transform Ion Cyclotron Resonance MS (FT-MS) and tandem MS, followed by signal annotation using MZed-DB suggested that correlated explanatory signals indicative of high citrus consumption were ionisation adducts of proline betaine (stachydrine) and hydroxyproline betaine.

In an expansion of this preliminary study we describe a high throughput, data-driven approach to explore the food consumption habits (>130 standard food components) of a larger cohort of free-living humans. Using FFQ information and FIE-MS analysis of 24hr or 'spot' urines (fasting or overnight/first void) we identify food components that are well discriminated between groups of individuals reporting either high or low habitual consumption. Ultra-high accurate mass analysis and tandem MS has revealed potential biomarkers for a range of foods of high public heath significance (including oily fish, red and white meats and specific fruit/vegetables). The likely role and impact of the use of biomarkers on future dietary exposure monitoring in human epidemiological studies will be discussed.

MRI
Max Rubner-Institut

Use of Metabolite Profiling for the Detection of Biomarkers in Cohort Studies

Dagmar Drogan
German Institute of Human Nutrition Potsdam-Rehbrücke
Nuthetal, Germany

Because of recent advances in the development of robust, high-throughput analytical techniques, metabolomics is increasingly being used in human studies to investigate metabolic alterations associated with pathological processes. The potential to simultaneously measure large numbers of metabolites within a given biological sample has generated high expectations in finding markers of exposure as well as novel and reliable biomarkers of disease risk and diagnosis.

Important areas of metabolomics research have been cancer, cardiovascular diseases and diabetes mellitus. However, many human studies conducted so far had methodological limitations, the most important ones being the small number of study participants, the cross-sectional design, and lack of control for confounders. The metabolic profile of a biological specimen is influenced by numerous factors such as diet, lifestyle and drug use. Apparently, environmental factors increase the biological variability and consequently decrease the statistical power to detect subtle metabolic alterations. Even more problematic, several of these environmental factors are likely be affected by the disease itself. In the setting of case-control studies, metabolic changes in diseased individuals may therefore not only reflect causes but also consequences of the disease, thereby potentially biasing the biological conclusion.

The above limitations can be partly overcome by well-designed prospective cohort studies with appropriate sample size and detailed documentation of potential confounders. Since biological samples are collected before disease onset, prospective cohort studies are conceptually suitable to study early metabolic alterations and the natural history of disease. Several prospective cohort studies that are available today have collected biological samples and extensive phenotypic data. The presentation will focus on methodological considerations and first successful efforts to integrate metabolomics into large cohort studies. Furthermore, ongoing metabolomics activities in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study will be described, in which more than 25,000 individuals have been followed up for incident diseases since baseline examination in 1994 to 1997.

Max Rubner Conference 2011
October 9-11, 2011

MRI
Max Rubner-Institut

Poster Abstracts

Max Rubner-Institut

Poster 1

LC-MSMS Based Metabolomics Reveals for the First Time That Bioactive Benzoxazinoids in Rye Bread are Absorbed and Metabolized in Pigs

Khem B. Adhikari[1], Bente B. Laursen[1], Helle N. Lærke[2], Inge S. Fomsgaard[1]
[1] Department of Agroecology, Faculty of Science and Technology, Aarhus University, Slagelse, Denmark
[2] Department of Animal Health and Bioscience, Faculty of Science and Technology, Aarhus University, Tjele, Denmark

Benzoxazinoids are a group of naturally occurring plant secondary metabolites with remarkable allelopathic, pharmacological and health promoting attributes. In addition to roots and leaves, these bioactive compounds are also present in the whole cereal grains and bread products which could contribute to the health promoting properties of whole grain diets. To our knowledge, study related to the bioavailability and transformation of dietary benzoxazinoids in animals and/or humans has not been published so far. The objective of the present study was to evaluate the uptake, distribution and metabolism of dietary benzoxazinoids in pigs.

A total of 12 pigs were fed high-fibre wheat or rye bread-based diets (n = 6 per treatment). After 8 weeks on the experimental diets, faeces and urine were collected quantitatively for 7 days. Subsequently, the pigs were euthanized and blood from 4 different parts of the circulation system and bile were sampled three hours postprandial. Until now, 10 benzoxazinoids and 4 derivatives have been quantified by an AB SCIEX 3200 liquid chromatography-ion trap triple quadrupole mass spectrometer (LCMSMS) using electrospray ionization and multiple reaction monitoring (MRM) mode.

Rye bread contained highest concentration of 2-β-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one (DIBOA-glc; 102 nmol/g DM) followed by 2-benzoxazolinone (BOA) > 2-β-D-glucopyranosyloxy-1,4-benzoxazin-3-one (HBOA-glc) > 2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-glc) > 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) > 2-β-D-glucopyranosyloxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA-glc), while wheat bread had only DIBOA-glc and HBOA-glc in negligible amounts. Benzoxazinoids and their metabolites were detected in blood plasma and urine at various proportions. DIBOA-glc in the diet was apparently reduced into HBOA-glc, the most dominant benzoxazinoid in the blood (829 nmol/l in rye fed pigs). Benzoxazinoid compounds were excreted through the urine mainly as HBOA-glc (18577 nmol/l in rye fed pigs). Moreover, considerable proportion of BOA, 2-hydroxy-1,4-benzoxazin-3-one (HBOA) and HMBOA-glc also excreted in urine and thus seemed to be renal metabolism products. Interestingly, 2-amino-3H-phenoxazin-3-one (APO) was also detected in the urine. This study is the first of its kind to elucidate the evidence of absorption and metabolism of dietary benzoxazinoids in animals. The outcome of the present study can be a milestone for the interpretation and intervention studies on the health effects of benzoxazinoids in animals and humans. Results of the complete study will be published in the near future.

Max Rubner-Institut

Poster 2

Metabolomic Profiling in Food of Animal Origin

S. Andrée[1], D. Martin[2], U. Ostermeyer[2], H. Rehbein[2], F. Schwägele[1]
Max Rubner-Institut
[1] Kulmbach, [2] Kiel, Germany

The aim of the presented project was to establish a profile of metabolites as a basis for the evaluation of food quality. Therefore samples of rainbow trout, carp, milk and pork were tested.

Diadenosine polyphosphates (ApnA) are a group of nucleotide derivatives that are constituents of nearly every cell type in pro- and eukaryotes. In mammalian systems ApnA have been implicated in regulation of vasodilation, platelet aggregation, synaptic neurotransmission and cell cycle control.

For separation of ApnA an ion-pair HPLC method with ESI-high resolution TOF was developed. Furthermore the sample extracts were used for a MS/MS screening for quality relevant metabolites. For mass based search for metabolites the Metabolite and Tandem MS Database of the Scripps Center For Metabolomics was used.

The detection of ApnA (n= 3-5) succeeded in food of animal origin at a physiological relevant concentration level. In some of the meat and fish samples traces of ApnA have been detected. The metabolite screening resulted amongst others in the identification of certain nucleotides. These screening results are in accordance with the results of the ribonucleoside analysis.

Amino acids are not only components of all proteins but also precursor compounds relevant to food flavour, taste and colouring. The content of free amino acids (FAA) in food is also interesting because of nutritional aspects. Taurine, occurring in the free form mainly in the animal kingdom, is important for many physiological processes.
The HPLC determination of FAA occurred using a reversed-phase column and a fluorescence detector after precolumn derivatization with o-phthaldialdehyde.

The total amounts of FAA and of taurine in milk samples were very low and differed clearly from the values found in fish and pork muscle tissues. Fish contain more FAA than terrestrial animals. Taurine was the most prevalent compound in all tested carp and pork samples. In all samples glycine belonged to the 4 most frequent free amino acids.

Ribonucleosides are monomeric metabolites of ribonucleic acids. The characterization of unmodified and modified ribonucleosides is of interest to obtain a better understanding of these compounds as potential quality-related metabolites.

The ribonucleosides were determined using an automated dual-column HPLC analyzer.
In the milk samples, the unmodified ribonucleosides Cyd, Urd, Ino, Ado and Guo and also the modified ribonucleosides m1Ado and t6Ado were detected. Urd had the highest concentration, followed by Cyd. In the evening milkings, slightly higher concentrations were found than in the respective morning milkings. In the trout and carp samples no modified ribonucleosides were found. The levels of Cyd, Urd, Ado and Guo were <2mg/100 g, whereas Ino in much higher concentrations occurred. Also in the pork samples, only unmodified ribonucleosides were detected, and Ino again with the highest contents was determined. In fish and meat clearly higher contents of free available ribonucleosides are present as in raw cow‘s milk.

Poster 3

An Untargeted Metabolomics Approach to Wine Micro-Oxygenation

Panagiotis Arapitsas[1]; Matthias Scholz[1]; Domenico Masuero[1]; Alessandra Biondi Bartolini[2]; Stefano Di Blasi[2]; Daniele Perenzoni[1]; Urska Vrhovsek[1]; and Fulvio Mattivi[1]
[1] Food Quality and Nutrition Department, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
[2] Consorzio Tuscania, Via Sangallo 43 Località Sambuca Tavarnelle Val di Pesa (FI), Firenze, Italy

Micro-oxygenation is a common winemaking practice for red wine, that consist in a continuous addition of small amounts of oxygen into the wine in order to improve its color, aroma, texture and conservation time. Object of our project was to study wine micro-oxygenation through LC-MS untargeted metabolomic profiling.

Eight different theses, differentiated by the dose of oxygen (four levels) and iron (two levels) were applied in a Sangiovese wine, before and after the malolactic fermentation. All other conditions were strictly standardized and monitored, while every thesis was carried out in triplicate. The metabolomics untargeted analysis was performed by a Synapt UPLC-MS QTof system, both in negative and positive electrospray ionization, and every sample was injected twice. Raw data were converted to CDF format and processed by XCMS, for feature extraction, grouping and alignment. The candidate list for separating the various levels of metal and oxygen was identified by using a Support Vector Machine algorithm. Analysis and advanced data analysis has been performed by Matlab.

Based in the standard data set of our laboratory and information from the literature regarding the metabolite profile of wines it was possible to annotate more than 250 compounds. The use of supervised and unsupervised multivariate methods point out both known candidate biomarkers and metabolites never considered before as possible biomarkers of wine micro-oxygenation. Between the known candidate biomarkers were identified various pigments and tannins. Additional and novel information obtained by correlation of oxygen and metal doses with various primary and secondary metabolites. These outcomes could be useful for developing analytical tools able to help researchers and winemakers for a more appropriate use of micro-oxygenation.

Poster 4

Characterization of Black and Green Tea Using ESI-Q-TOF-MS and Data Evaluation by Principle Component Analysis

Aiko Barsch, Gabriela Zurek, Wiebke Lohmann
Bruker Daltonik GmbH
Bremen, Germany

Black and green tea account for more than 95% of the total tea consumption around the world. In order to improve the quality and taste, characterization of food and beverages as well as quality control is a topic of interest in academia and a large market in industry. In our study, we used high resolution electrospray time-of-flight mass spectrometry to study different tea's using statistical methods.

Several black tea's, among them one decaffeinated tea, and one green tea have been analyzed. Infusions were prepared with 100ml hot water for 5 minutes and analyzed using a reversed phase gradient separation on a UHPLC system interfaced to a micrOTOF-Q II high resolution ESI-TOF-MS. Full scan data were acquired in ESI positive mode (scan range m/z 75-1000). A mathematical algorithm was applied to detect all compounds in the analyses. This "Find Molecular Features" algorithm extracts all relevant information and differentiates between real signals and background noise. The processed data were submitted to principal component analysis (PCA) in order to differentiate the samples and to identify differences between the tea types. As expected, the decaffeinated black tea was distinct from the other teas by the absence of caffeine. Excluding caffeine from the PCA calculation, other differences between the teas were revealed, mainly originating from the different "flavonoid profiles" of the teas. In order to identify those differences, sum formulae were calculated, taking both the accurate mass and the isotopic pattern of the compounds into account. With increasing molecular mass, the number of possible sum formulae in a certain mass window increases exponentially. Therefore, an autoMS/MS run was performed focusing on the largest differences between the teas as precursor ions, so that also the accurate mass and isotope pattern of the fragments could be used for sum formula generation to reduce the number of sum formula suggestions.

In this study, high resolution ESI-Q-TOF mass spectrometry, coupled to UHPLC, and data evaluation with PCA followed by sum formula generation of the detected differences proved to be a valuable tool for the characterization and quality control of tea.

Poster 5

Novel Approaches to Structure Elucidation and Confirmation in Metabolomics Studies

Sven Heiling[1], Gabriela Zurek[2], Emmanuel Gaquerel[1], Matthias Schöttner[1], Bernd Schneider[1], Aiko Barsch[2], Ian Baldwin[2]
[1] MPI Chemical Ecology, Jena, Germany;
[2] Bruker Daltonik GmbH, Bremen, Germany

Presently, the key bottleneck of metabolomics is structural confirmation and elucidation of (secondary) metabolites. Nicotiana attenuata is a well-established model system to monitor plant-herbivore interactions with metabolomics being a novel approach to investigate the underlying biology [1]. 17-Hydroxygeranyllinallool diterpene glycosides (HGL-DTGs) are abundant direct defense compounds with their mode of action being largely unknown [1-3]. New acyclic HGL-DTGs were characterized using MS and NMR after extraction of several hundred grams of raw plant material [2, 3]. Such scale is not compatible to the analytical scope of metabolomics.

Here, we present novel solutions facilitating the identification and fast dereplication process of natural products when mass spectral libraries are not yet available and the sample amount is limited.

Plant samples were prepared as described previously [1]. Chromatographic separation was carried out using an UHPLC system combined with ultra high resolution (UHR) Q-TOF MS detection. Selected plant samples were fractionated. Peaks enriched in HGL-DTGs were subjected to detailed fragmentation studies by means of direct infusion measurements.

The dereplication of HGL-DTGs is rendered difficult by the large number of in-source fragments and adduct formation, and their molecular weight of 800-1000m/z. Novel algorithms were applied for deconvolution of LC-MS chromatograms by correlation analysis to safely determine the molecular ion in the presence of adducts and in-source CID fragments. Molecular formula determination was carried out by combined evaluation of mass accuracy, isotopic patterns, adduct and fragment information. The diagnostic fragments for the HGL-DTG backbone and successive sugar units, such as $[M+H]^+$ = 271.2420m/z = $C_{20}H_{31}^+$ and 417.2999m/z = $C_{26}H_{41}O_4^+$ enabled the rapid identification of the entire compound family, which is subsequently characterized in more detail. For this, the fragmentation results have been combined with the structural information to visualize the interpretation. Simultaneously the necessary validation prior submission to a mass spectral library is achieved.

References
[1] Gaquerel, E., et al., J. Agric. Food Chem. 58 (2010), 9418-9427.
[2] Jassbi, A., et al., Z. Naturforsch. 61b (2006), 1138-1142.
[3] Heiling, S., et al, Plant Cell 22 (2010), 273-292.

Max Rubner-Institut

Poster 6

Analysis of Whisky by Electrospray FT-ICR Mass Spectrometry: Proof of Origin by Statistical Methods

Matthias Witt, Rainer Paape, Jens Fuchser, Gabriela Zurek, Aiko Barsch
Bruker Daltonik GmbH
Bremen, Germany

Whisky is a high-class consumed alcoholic beverage with a several billion dollar market. Due to the high value of this liquor counterfeiting and manipulation have been observed. Therefore, the proof of the origin of this luxury alcoholic drink is of major interest of distillers and beverage importers. Whisky consists beside water and alcohol of a variety of volatile and non-volatile chemical components, e.g. organic acids and esters, aldehydes, phenols, polyphenols and lactones. Recently the proof of origin and authenticity of whisky has been studied by electrospray mass spectrometry [1]. However, in our study we used ultra-high resolved mass spectrometry to study whiskies from different origins using statistical methods and fingerprinting.

The whiskies have been diluted 1:20 in 50% MeOH for direct infusion measurements using electrospay FT-ICR (Bruker solariX 12T) in negative ion mode. Several Scottish whiskies from two different origins as well as several whiskies from the japanese distillery Suntory have been analyzed. Using electrospray ionization the most polar components of whisky are detected. Beside dominant species like ellagic acid and gluconic acid, the mass spectra of whisky show a complex pattern with several peaks at one nominal mass resulting in several thousand peaks in a mass spectrum. The molecular formulas of more than thousand compounds have been identified. Principal component analysis (PCA) as well as cluster analysis have been performed of the full and of a part of the mass spectra with and without using the isotopic fine structure to validate the origin of the studied whiskies and to proof the relevance of the highly resolved mass spectra for the characterization of whisky. The importance of the isotopic fine structure for fingerprinting of whisky will be shown. In this study electrospray FT-ICR-mass spectrometry has been proven as a powerful tool for the characterization of extremely complex mixtures such as whisky.

[1] Moller, J. K. S., Catharino, R. R., Eberlin, M. N., Analyst 2005, 130, 890.

Max Rubner-Institut

Poster 7

Using High Resolution Accurate Mass LC-MS/MS and Statistical Data Processing for Non-Targeted Screening for Food Residues

Axel Besa[1], Andre Schreiber[2], Yun Yun Zou[2], Kai Zhang[3], Jon Wang[3]
[1] AB SCIEX, Darmstadt, Germany
[2] AB SCIEX, Concord, ON Canada
[3] FDA Center for Food Safety and Applied Nutrition, Maryland, MD USA

Recent regulations on food analysis require the screening for pesticides using confirmatory techniques, such as GC/MS and LC/MS/MS. With more than 1000 pesticides of more than 100 compound classes there is a demand for powerful and rapid analytical methods, which can detect very low concentrations of pesticides and other food relevant residues in a variety of food matrices.

In addition food testing laboratories use latest developments in sample preparation techniques to combine the screening of pesticides with other compound classes, such as veterinary drugs and mycotoxins. Here we present a high-throughput LC-MS/MS method that combines multi-class compound screening with identification based on accurate mass MS/MS library searching. Furthermore, acquired full scan MS data was used to retrospectively screen for non-targeted and unexpected contaminants.

Food samples were extracted using a QuEChERS [1-2] procedure and injected into LC-MS/MS after dilution to minimize possible matrix effects and interferences. LC separation was performed using a Shimadzu UFLCXR system with a Restek Ultra Aqueous (100x2.1mm 3µm) column and a gradient of water and methanol and ammonium formate buffer with a total run time of 15 min. Detection was performed on an AB SCIEX TripleTOF™ 5600 system using Electrospray Ionization (ESI). TOF-MS data was used for targeted quantitation and additional screening for non-target analytes based on statistical data processing. TOF-MS/MS data was used to identify detected compounds based on mass spectral library searching. Unknown compounds, not present in the existing mass spectral library, were further studied and identified using formula finding software and online databases.

[1] Anastassiades M. et al.: J. AOAC Int 86 (2003) 412-431.
[2] J. Wong et al.: J. Agric. Food Chem. 58 (2010) 5897-5903.

Max Rubner-Institut

Poster 8

Validation of an *In Vitro* Digestive System for Studying Macronutrient Decomposition in Humans

Katrin A. Bolanz[1], Flurina Schwander[1], Martin Gijs[2], Guy Vergères[1], Reto Portmann[1], Charlotte Egger[1]
[1] Research Station Agroscope Liebefeld-Posieux ALP, Bern, Switzerland
[2] Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Bovine milk contains many essential and bioactive nutrients. To enable their action in the body, they may get metabolized during the digestion process. The investigation of physiological effects of certain nutrients *in vitro* demands a digestion process that is close to human physiology. In this study, a three step *in vitro* digestion model was developed mimicking digestion till the intestinal enterocytes participate in the process. Pasteurized milk was used as an example for a particular validation of the model via follow up of macro-nutrient digestion. For the analysis of the protein digestion gel-electrophoresis, HPLC, and LC-MS methods allowed a detailed follow-up of milk protein degradation that was similar to physiological data obtained in humans. The average peptide size after digestion of pasteurized milk was 5-6 amino acids. Interestingly, the released free amino acids from the pasteurized bovine milk after digestion were mostly essential (93.6%) and they did not correlate with the total essential amino acid content of bovine milk (44.5%). This might indicate, that there is a need for a rapid provision with essential amino acids and should be confirmed *in vivo*. The fatty acids released from triglycerides during the digestion process were detected with GC. The obtained results were in line with physiological ranges corresponding to 36% of the inserted milk fat. Additionally, though dairy products contain not many carbohydrates it was confirmed that amylase can be degraded by alpha-amylase contained in the system. This *in vitro* digestion model enables the analysis of different dairy products in regard to macro-nutrient digestion and might be also applied to determine bioavailability of nutrients and bioactive properties in combination with a cell-culture system.

Max Rubner-Institut

Poster 9

MET-EXTRACT: A Powerful Software Tool for the Automated Data Analysis of LC/MS Data Derived from *In Vivo* Isotopically Labelled Organisms

Christoph Bueschl, Rainer Schuhmacher
Universität für Bodenkultur Wien, Department IFA-Tulln, Center for Analytical Chemistry
Tulln, Austria

The extraction of high resolution LC/MS signals of true metabolites contained in complex biological samples is still a major challenge in non-targeted LC/MS based metabolomics.

The approach of *in vivo* stable isotopic labelling offers a powerful tool to circumvent these limitations. Uniformly ^{13}C labelled metabolites show identical chromatographic behaviour as their non-labelled isotopologues but can be easily differentiated by high resolution MS.

An algorithm and a program (MET-EXTRACT) have been developed to search for metabolites in *in vivo* isotopically labelled biological samples. The algorithm makes use of the chromatographic characteristics of the LC/MS data and detects MS peaks fulfilling the criteria of stable isotopic labelling. The algorithm extracts mass peaks corresponding to non labelled and stable isotopically labelled metabolites together with the number of labelling atoms (e.g. carbon) in the metabolite. Extracted mass peaks are further verified by checking the intensity abundance of mass peaks from isotopologues. If these are also present and their abundance is within a predefined ratio window, the algorithm has found a mass peak pair. In sequent steps, extracted mass peaks are used to detect chromatographic peaks in the LC/MS data. As a result of all calculations, the algorithm specifies a list of m/z values, the corresponding number of atoms of the labelling element (e.g. carbon) together with retention time and extracted adduct-, fragment-, and polymer ions which can be used for calculating the accurate mass of a substance.

19 native ^{12}C- and uniformly ^{13}C-labelled fungal standard substances were measured by high resolution LC/MS for verification. Under the described measurement conditions and parameter settings, LC/MS signals of 18 out of 19 of metabolites were correctly extracted by the developed MET-EXTRACT algorithm. For each standard substance the correct number of C-atoms was assigned. No false positives were detected by the algorithm.

Poster 10

MetaboLights

L. F. de Figueiredo, K. Haug, R. Alcántara, H. Cao, P. Conesa, P. de Matos, M. Rijnbeek, C. Steinbeck
European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, UK
R. Salek and J. Griffin
University of Cambridge, Department of Biochemistry, Cambridge, UK

MetaboLights is a database under development for Metabolomics experiments and derived information. It is projected to become the first comprehensive, cross-species, cross-technique database which combines curated reference data of pure metabolites, curated information about their occurrence and concentration in species, organs, tissues and cell types under various condition with data characterizing the experiment which lead to these findings. Protocols documenting how metabolomics experiments were conducted will also be made available.

Like all other EBI resources, the MetaboLights databases will be completely open to the public, including open access to the data. Data will be made available in publicly accepted open standards. The software will be open source. One of the main submission channels for MetaboLights will use the ISA Tools Suite (http://isatab.sourceforge.net/).

MetaboLights is not meant to replace specialist resources for Metabolomics. Rather, it will build on prior art and collaborate. We are dedicated to close collaboration with all major parties involved in the creation of this prior art, such as the Metabolomics Society, Metabomeeting and the Metabolomics Standards Initiative (MSI). The MetaboLights project group has taken the initiative to formally reform the MSI.

MetaboLights aim to agree on formal data sharing agreements with major resources such as the Human Metabolome Database, the Golm Metabolome Database and the Rikken Metabolomics Platform.

MetaboLights is funded by the BBSRC as a joint project between The Chemoinformatics and Metabolism team at EMBL-EBI (Christoph Steinbeck) and The Department of Biochemistry at the University of Cambridge (Jules Griffin).

Poster 11

Metabolite Profiling of Barley – Influence of the Malting Process

Thomas Frank, Birgit Scholz, Stephanie Peter, Karl-Heinz Engel
Technische Universität München
Freising-Weihenstephan, Germany

The germination of seeds represents an important stage in the development of plants. This phase is characterized by numerous metabolic processes leading to distinct and time-dependent alterations in the metabolic profiles. One of the most important applications of the germination process in food technology is the malting of barley. The process of malting, involving steeping, germination and kiln-drying as key steps, plays a central role in the brewing industry.

The aim of the present study was to apply a capillary gas chromatography - mass spectrometry (GC-MS)-based metabolite profiling to barley seeds in the course of the malting process. Samples taken during the malting progress were subjected to an extraction and fractionation procedure allowing a comprehensive coverage of a broad spectrum of low molecular weight non-polar (fatty acid methyl esters, hydrocarbons, free fatty acids, fatty alcohols, sterols, tocopherols) and polar (sugars, sugar alcohols, organic acids, amino acids, amines) compounds. Investigation of the barley metabolic profiles by GC resulted in the detection of 587 distinct peaks of which 173 were identified by means of MS. The obtained metabolite profiling data were assessed via multivariate and univariate statistical methods. Principal component analysis revealed that the metabolic changes during the malting progress are reflected by time-dependent shifts of the scores. Analysis of the corresponding loadings showed that polar metabolites were the major contributors to the malting time-driven changes in the metabolic profiles. Quantifications based on standardized peak heights revealed dynamic changes of the metabolites in the course of the different malting stages.

Max Rubner-Institut

Poster 12

Impact of UV-B Storage and Fermentation on the Profile of Bioactive Plant Compounds in White Cabbage Leaves

Britta Harbaum-Piayda, Imke Nickelsen, Karin Schwarz
Department of Food Technology, Institute of Human Nutrition and Food Science
University of Kiel, Germany

White cabbage is an important vegetable in the human diet and especially cultivated in Northern Germany. The profile of bioactive compounds from Brassica vegetables is determined by polyphenols, glucosinolates, isothiocyanates, acorbigen and ascorbic acid.

Although many fruits and vegetables are freshly consumed, post-harvest treatments are often necessary to improve the storage behaviour and shelf life of vegetables. The fermentation process is normally used to preserve vegetables, for example olives, cucumber, or cabbage [1]. Storage processes under different conditions (e.g. low temperature, irradiation) are also responsible for chemical and quality changes of plant material [2].

In the case of sauerkraut (fermented white cabbage), salt is added to the shredded white cabbage and placed in a vessel under pressure. Microorganism activities are responsible for the decrease in pH from approx. 7 to 4 during fermentation due to the production of different acids like lactic acid, propionic acid, and acetic acid.

The aim of this work was to study the effect of post-harvest irradiation and fermentation on the profile of bioactive compounds, such as hydroxycinnamic acid derivatives, flavonoids, glucosinolates and ascorbigen in white cabbage leaves and sauerkraut.

Identification of target compounds was carried out by reversed phase HPLC-mass spectrometry (ion trap, Agilent) and quantification by reversed phase HPLC-DAD analysis at different wavelength in one run dependent on compound class (glucosinolates: 227 nm, polyphenols: 330 nm).

Fermentation of cabbage leaves leads to structural changes and a decrease in hydroxycinnamic acid derivatives by the loss of moities, e.g glucose. Microorganism activities might be also responsible for further structural changes of polyphenolic aglycones and the formation of a new pattern of degradation products in the plant material. Due to the release of the enzyme myrosinase from plant material during fermentation further changes in glucosinolate concentration were observable.

Overall, the fermentation process of plants is a complex reaction system due to microorganism activity with a high impact on chemical plant compounds, the nutritional value and quality.

[1] Caplice, E., & Fitzgerald, G. F. (1999). Food fermentations: role of microorganisms in food production and preservation. International Journal of Food Microbiology, 50, 131-149.
[2] Harbaum-Piayda, B., Walter, B., Bengtsson, G. B., Hubbermann, E. M., Bilger, W., & Schwarz, K. (2010). Influence of pre-harvest UV-B irradiation and normal or controlled atmosphere storage on flavonoid and hydroxycinnamic acid contents of pak choi (Brassica campestris L. ssp. chinensis var. communis). Postharvest Biology and Technology, 56, 202-208.

Poster 13

Metabolites and Lipids – High Throughput Analysis of Plant Tissue

Susann Irgang, Jessica Jüppner, Alvaro Cuadros Inostroza, Jan Hummel; Patrick Giavalisco
Max Planck Institute for Molecular Plant Physiology
Potsdam-Golm, Germany

Mass spectrometry has been used in research and industrial applications for over 50 years. Still, different types of samples require different types of extraction and purification methods prior to analysis. Here, we present an experimental pipeline for extracting, preparing, identifying and evaluating lipids, metabolites, proteins and starch from plant tissue, namely from the unicellular green alga Chlamydomonas reinhardtii, an important model organism for plant research.

In a first step, we modified the classical “Folch”-extraction procedure, which is based on chloroform:methanol:water, to a system relying on the use of MTBE:methanol:water. Both systems allow a liquid:liquid two-phase separation of polar and non-polar compounds, but due to the lower density of MTBE compared to chloroform, our optimized method additionally allows for the “one shot” extraction of starch and proteins which can be recovered from the solid pellet. The organic (lipid-containing) phase can be analyzed using every analytical system - we used an Orbitrap MS coupled to an UPLC. Two aliquots from the polar phase, containing primary and secondary metabolites, were either analyzed by GC-TOF MS or by UPLC-Orbitrap-MS. The standardized extraction and derivatisation protocol for the GC-TOF/MS relies on different internal standards which allow for the efficient normalization, relative quantification and annotation of metabolites. However, the annotation and relative quantification of lipids and secondary metabolites is less well established. The lack of reference compounds especially for plant tissue is still a major challenge and therefore the annotation of these compounds requires manual curation.

As an initial proof of concept we present metabolic- and lipidomic- data derived from Chlamydomonas reinhardtii cells exposed to different abiotic stresses, supporting the concept that the measurement of several compound classes from a single sample is essential for systems-based high throughput analysis of biological samples from various types of tissues.

Max Rubner-Institut

Poster 14

Controlling the Aroma Generation During Hazelnut Roasting by Sensobolomics and Comparative Metabolome Marker Analysis (COMMA)

Johannes Kiefl
German Research Center for Food Chemistry
Freising, Germany

Roasting is the key process converting raw hazelnuts into a semi-manufactured product with a characteristic aroma. In a previous study, the heat-induced aroma generation in Italian hazelnuts of the variety Tonda Romana was studied by means of the molecular sensory science approach revealing that the pairs 2- and 3-methylbutanal (malty), 2-acetyl- and 2-propionyl-1-pyrroline (roasty, popcorn-like), 2,3-pentandione and 2,3-butandione (buttery), 2-furanmethanethiol and 2-thiophenemethanethiol (coffee-like) as well as 5-methyl-(E)-2-hepten-4-one and 3-methyl-4-heptanone (fruity, hazelnut-like) are among the most important aroma-active compounds. However, further sensory experiments indicated that different aromas are obtained by changing the roasting regime as well as the hazelnut variety. Hence, the aim of the present study was to analyze the heat-induced aroma generation by means of targeted and untargeted approaches, and to correlate the data with the overall sensory impact of the respective samples. The targeted analysis of hazelnuts' Sensobolome comprises the identification and quantitation of key odorants across a larger sample set. The development of a new comprehensive quantitative approach on basis of GCxGC-TOF-MS in combination with stable isotope dilution assays will be presented in detail. Recombination experiments finally verified the effectiveness of the Sensobolomics approach to understand the generation of hazelnut aroma on a molecular basis. Then, by means of GCxGC-TOF-MS, the heat-induced changes of the total volatile hazelnut metabolome were investigated employing the so-called COMMA approach. This way, marker compounds are located by application of an untargeted comparative analysis, and the data are correlated with the overall aroma as well as the outcome of the Sensobolomics approach. The results showed that a combination of both methods is a useful tool in understanding and controlling the aroma generation during hazelnut roasting.

Max Rubner-Institut

Poster 15

Absolute Quantification of 20 Major Dairy Proteins by Selected Reaction Monitoring

Déborah Mathis, Flurina Schwander, Katrin Kopf-Bolanz, Lotti Egger, Reto Portmann
Agroscope Liebefeld-Posieux Research Station ALP
Bern, Switzerland

Proteins form a major class of the milk components, consisting of more than 100 individual different types that can be regrouped into caseins, whey proteins, proteins associated with the milk fat globule membrane, and others. Methods for the quantification of the total caseins or whey fractions are mostly based on fractionation procedures, but a method for the absolute quantification of the individual proteins is still lacking. For nutritional research, the absolute amounts of the individual dairy proteins are particularly interesting, because peptides being generated during human digestion may have specific biological functions. For instance, they are thought to influence the inflammation of the gut and to help in the defense against pathogenic bacteria, yeast, and viruses.

We developed a mass spectrometry-based method (selected reaction monitoring), which allows the simultaneous quantification of twenty individual proteins in one run. The quantified proteins include all caseins, the major whey proteins, and the major proteins from the milk fat globule membrane. The method involves tryptic digestion of the sample producing peptides, which are separated with liquid chromatography before being sprayed into the mass spectrometer. For each protein, a proteotypic peptide is chosen, whose response is linear to the amount of protein contained in the sample. Comparison with the responses of isotopic labeled variants of each proteotypic peptide allows the absolute quantification of the individual proteins in dairy products.

The method is suited for various fields and applications, as for example comparisons of individual protein contents between different milk fractions, different milk products, or between milks produced under different conditions (differing in cow breed, lactation time, age, or feeding regime).

Max Rubner-Institut

Poster 16

Modification of Composition and Antioxidative Potential of Wine Flavour During Fermentation

Michel, O[1], Raddatz, H.[1], Henle, T.[2]
[1] University of Applied Sciences Trier, Trier, Germany
[2] Dresden University of Technology, Dresden, Germany

Previous researches of wine assumed that the antioxidative potential of must and wine bases mainly on the effect of contained polyphenols. Recent developments of terpenoid, volatile compounds showed that these compounds have a significant antioxidative potential too [1], [2], [3]. Main flavour compounds of wine belong to the chemical groups of unsaturated monoterpenes, aliphatic and aromatic alcohols, lactones, esters and carbonyl compounds [4], [5]. On the one hand they originate from the natural flavour composition of the used must; on the other hand they are built by wine yeasts during fermentation.

Aim of our research was to document the modification of flavour composition during fermentation and to determine the related antioxidative potential in wine and flavour extracts using different tests.

For analyses typical wines of the Mosel producing area like Scharzhofberger Riesling or a mixture of Spätburgunder and St. Laurent (rosé wine) were used. For recovery of flavour extracts simultaneous distillation extraction by Likens-Nickerson was used; the identification of compounds took place by gas chromatography mass spectroscopy. For determination of antioxidative potential in must, wine and flavour extracts the following methods were implemented: determination of amount of total phenolics and total flavonoids, determination of monomer index, trolox equivalent antioxidant capacity assay (TEAC II), 2,2-diphenyl 1-pikrylhydrazyl assay (DPPH), ferric reducing antioxidant power assay (FRAP), ribose degradation (Fenton reaction), iron complexation and photochemoluminescence (PCL).

Results showed that flavour compounds do have a significant antioxidative (radical scavenging and reductive) potential that originates from nonphenolic, volatile compounds. In rosé wine this effects probably originates from the high amount of furfural and benzeneacetaldehyde. Flavour of Riesling wine principally consists of acetals and esters. During fermentation the amount of these substances decreases. But caused by low amounts in wine the antioxidative potential of the flavour compounds compared to the effect of total phenolics is low.

Literature:
[1] Chizzola R, Michitsch H, Franz C., J Agric Food Chem 2008, Chem 56 (16), 6897 – 6904,
[2] Kulisic T, Krisko A, Dragovic-Uzelac V, Milos M, Pifat G., Int J Food Sci Nutr 2007, 58 (2), 87 – 93,
[3] Tuckey NPI, Forster ME, Giesig SP., J Food Science 2009, 74 (4), C333 – C338,
[4] Karagiannis S, Economou A, Lanaridis, J Agric Food Chem 2000, 48, 5369 – 5375,
[5] Ong PKC, Acree TE, L Agric Food Chem 1999, 47, 685 – 670

Poster 17

Stress Effect on the Metabolism of Zebrafish (Danio Rerio)

M.Y. Mushtaq[1], R.M. Marcal[2], D. Champagne[3], F. van der Kooy[1] Y.H. Choi[1], R. Verpoorte[1]
[1] Natural products laboratory, Institute of Biology Leiden University, The Netherlands
[2] Lab. Ethnopharmacology and Pharmacodynamic, DFS
[3] Institute of Biology, Department of Integrative Zoology, Leiden University, The Netherlands

Zebrafish model is gaining its place as a bio- or clinical activity test alternative to conventional rodent model due to many of its advantages such as low cost and easiness to maintain and Also, the zebrafish is genetically more closely related to human than other invertebrate models like Caenorhabditis elegans and Dorsophilla melanogaster. In these days many research reports show that the physiological functions of zebrafish are similar as found in higher vertebrates so a wide range of diseases can be investigated in this model without high-cost conventional rodent model. So far Zebrafish has been being extensively investigated in toxicological, genetic or medical studies. However, more information on metabolism of the fish needs to be more studied although metabolites are the final products of gene expression. Particularly, zebrafish as itself, metabolism or effect of external- or internal factors are not clear yet, which should be solved before using the zebrafish model as real bioactivity assay. In order to do metabolism study metabolomics may be a potential tool as functional genomics so it can help us to understand the complete overview of organism physiological responses.

Here we try to apply acute stress model in zebrafish to have better insight into the impact in terms of metabolomics. Stress is a natural phenomenon as organism faces different kind of stresses throughout whole life but it has been able to cop these stresses for short period of time. In case of acute stress organism has been able to cop it by adaptive mechanism. So as part of our work we apply fifteen minutes of acute confinement stress, zebrafish were then given five minutes for open field test and light box test. After that they were collected and then analyzed for metabolomics by applying1H nuclear magnetic spectroscopy as primary tool. Data was analyzed by applying diverse multivariate data analysis including PCA, PLS-DA, and Orthogonal signal correction. Among them the OSC PLS-DA showed a separation of not only between stress and non stress but also between zebrafish applied to open field test and applied to light dark box test. The difference is associated with increase in format in stresses zebrafishes while alanine, taurine were high in zebrafish applied to light box test regardless of stress contrary to Tri methyl amine oxide which seems to be higher in zebrafish exposed to open field test.

These results show that any change in the environment even for small period of time has its effect on the function of the body. This research provides an insight of how different mechanisms work under different environments to maintain the balance of the body. It will also help to established zebrafish as model in term of metabolomics.

Poster 18

A Simple HPLC-Based Approach to Quantify Spoilage of Minced Beef Stored in Different Temperatures and Packaging Systems

Anthoula A. Argyri, Agapi I. Doulgeraki, Vasiliki A. Blana, Efstathios Z. Panagou, George-John E. Nychas
Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Technology, Agricultural University of Athens, Athens, Greece

The current practice employed in the evaluation /monitoring of meat quality relies heavily on regulatory inspection and sampling regimes (European Commission, 2005). Moreover, there is no general agreement on early criteria indicative of changes in meat quality. Thus, despite the more than 50 chemical, physical and microbiological methods, which have been used over decades for the detection and measurement of meat spoilage, no definitive biomarkers have been identified. The existing methods have limitations, as they are time-consuming, provide retrospective information and cannot be used for on- or at-line monitoring. Also, due to continuously developing technologies in food processing and preservation (e.g., vacuum packaging (VP), modified atmosphere packaging (MAP), active packaging) identifying spoilage indicators is a complex issue. It is, therefore crucial that valid objective spoilage indicators and/or methods to monitor freshness and safety be developed which can ensure quality irrespective of the stakeholders perspective (i.e., consumer, industry, inspection authority, or scientist).

The present study describes the potential use of metabolomics derived from HPLC data. For this reason minced beef stored (i) aerobically, (ii) under modified atmosphere packaging (MAP), and (iii) under MAP with the presence of the volatile compounds of oregano essential oil (MAP/OEO) at 0, 5, 10, and 15 ºC and the shelf life was investigated. The microbial association of meat and the temporal biochemical changes were monitored. Microbiological analyses, including total viable counts (TVC), Pseudomonas spp., Brochothrix thermosphacta, lactic acid bacteria, Enterobacteriaceae, yeasts/moulds, were quantified, in parallel with sensory assessment, pH measurement and HPLC analysis of the organic acid profiles. Spectral data collected by HPLC were subjected to statistical analysis, including Principal Components Analysis (PCA) and Factorial Discriminant Analysis (FDA). This revealed qualitative discrimination of the samples based on their spoilage status. Partial Least Square Regression (PLS-R) was used to evaluate quantitative predictions of TVC, Pseudomonas spp., Br. thermosphacta, lactic acid bacteria, Enterobacteriaceae, yeasts/moulds. Overall, the metabolic profile of organic acids, determined by HPLC analysis, was found to be an effective method to evaluate the spoilage and microbial status of a meat sample regardless of storage conditions. This could be a very useful tool for monitoring quality of meat batches during transport and storage in the meat food chain.

This work was supported by the EU Projects ProSafeBeef (Food-CT- 542-2006-36241 / 6th FP) and Symbiosis – EU (no 543 211638 / 7th F P)

Max Rubner-Institut

Poster 19

Assessment of Beef Spoilage Using HS/SPME-GC/MS

A.A. Argyri [1,2], A. Mallouchos[1], E. Z. Panagou[1], G.-J.E. Nychas[1]
[1] Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Technology, Agricultural University of Athens, Athens, Greece
[2] NAGREF, ITAP, Lycovrisi, Greece

The correlation between microbial growth, sensory analyses and chemical changes during spoilage has been continuously recognized as a means for quantifying muscle tissue quality as well as the degree of spoilage. In this study minced beef stored aerobically, under Modified Atmosphere Packaging (MAP) and under MAP with the presence of the volatile compounds of oregano essential oil (MAP/OEO) at 0, 5, 10, and 15 ºC. The microbial association was assessed in parallel with sensory analysis, pH measurements and the evolution of the metabolic products as well as the compounds occurring in the meat substrate by using HS/SPME-GC/MS (headspace solid phase microexraction-gas chromatography/mass spectrometry). In particular the microbiological analyses, was related to measurements of total viable counts (TVC), Pseudomonas spp., Brochothrix thermosphacta, lactic acid bacteria (LAB), Enterobacteriaceae, yeasts and moulds, while the headspace analyses revealed a large number of volatile compounds, including aldehydes, alcohols, ketones, esters, hydrocarbons and terpenes, were identified at each storage condition, whilst more than 100 of them were further semi-quantitatively determined.

The data collected by GC/MS were correlated with microbial counts and sensory scores to estimate the shelf life of the mince, aiming mainly at the early detection of spoilage. Correlation of the volatile compounds, developed during the storage of beef under aerobic and MAP conditions, with the spoilage sensory status of the samples and subsequent qualitative classification of the samples was performed with principal component and factorial discriminant analysis (PCA and FDA, respectively), whilst quantitative predictions of the different microbial groups was performed using the Partial Least Squares-Regression (PLS-R). Both parameters i.e. temperature and packaging, were found to have a great impact on the evolution of volatiles during storage that resulted in distinct chemical profiles. Indeed the findings indicated that the profile of beef stored under aerobic conditions was quite different from the profile obtained from samples stored under MAP. The volatile profile obtained from samples stored under MAP/OEO, differed significantly from the two aforementioned conditions, since the compounds of the essential oil overlapped the compounds of meat. By analysing the GC/MS data with PCA, many of the identified compounds were correlated with the sensory scores, depicting possible spoilage indicators such as 2-Pentanone, 2-nonanone, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl hexanoate, ethyl propanoate, ethyl lactate, ethyl acetate, ethanol, 2-heptanone, 3-octanone, diacetyl, and acetoin. The FDA and PLS-R models indicated that the dynamic changes of the volatile metabolic compounds being present in the meat substrate during storage could provide predictions about the microbial populations and the sensory scores. The overall results the HS/SPME-GC/MS analysis of the volatile profile of meat combined with chemometrics, may be considered as a potential method to predict the spoilage of a meat sample regardless the storage conditions (e.g. packaging and temperature).

This work was supported by the SYMBIOSIS-EU Project (no 543 211638 / 7th F P)

Max Rubner-Institut

Poster 20

FraGenomics: Genetical Genomics for Improving Strawberry Fruit Nutritional Quality

L. Ring, J. Muñoz-Blanco, W. Schwab
Biotechnologie der Naturstoffe, Technische Universität München, Freising, Germany

Strawberry (Fragaria x ananassa Duch.) is a very popular fruit throughout the world, especially in Europe and North America. It is also a rich source of phytochemicals such as polyphenols which are known to have a positive effect on human health. The main aim of this project is to get better knowledge on genetic control of the biosynthesis of these metabolites and thus to optimize breedings programs by means of genetical markers.

Therefore different varieties and segregating populations were analyzed via LC-MS^n determining 20 major compounds. While the sum of all quantified metabolites did not differ by more than factor 2 between the genotypes, specific compounds revealed variances about factor 8 or even more, e.g. pelargonidin-glucoside malonate was only present in half of the samples. According to these metabolite data comparative microarray assays were performed by the Spanish cooperation partner. For each metabolite the variety with highest content was compared with the one which showed the lowest relative concentration. Gene expression between the respective pairs varied up to factor 900. The candidate genes with highest variance in expression are now being tested concerning their contribution to polyphenol biosynthesis by transient silencing or overexpression studies during fruit ripening.

Furthermore an un-targeted approach of LC-MS data is taken to elucidating differences in metabolic profiles of the varieties. Using statistical methods such as principle component analysis (PCA) the most affecting substances can be found and, if necessary, further investigated by NMR-spectroscopy after fractionation via HPLC-MS^n.
Additionally proteomic data of selected genotypes are obtained by 2D gelelectrophoresis. After software based evaluation spots are picked followed by sequencing via LC-MS^n.

Poster 21

Evaluation of Drinking Water Purification Processes by Micro-Pollutant Detection Using a Comprehensive LC/Orbitrap Mass Spectrometry Approach

Jérôme Cotton [1], Mylène Marie [1], Geoffrey Madalinski [1], Victor Sabarly [1], Sébastien Masclet [2], Jean Perot [2], Christophe Junot [3] and Bruno Corman [1]
[1] Profilomic, CEA-Saclay, Gif sur Yvette, France
[2] SAUR, Direction Recherche et Développement, Les Cyclades, Saint Quentin en Yvelines, France
[3] Laboratoire d'Etude du Métabolisme des Médicaments, DSV/iBiTec-S/SPI, CEA-Saclay, Gif sur Yvette, France

Industrial waste, phytosanitary compounds and drugs often pollute environmental waters at the origin of drinking water. The presence of all these compounds at different concentration levels in natural resources is a real environmental and public health concern. Thereby, the monitoring of water quality by an untargeted metabolomic approach was investigated in this study. The presence of xenobiotic compounds was detected by acquiring chemical fingerprints of river waters. Samples were taken upstream of a drinking water treatment plant located in Brittany (France) and after water treatment processes. This work was conducted in collaboration with the company SAUR, one of the main operators for local authorities in the areas of water and waste mangement in France.

After concentrating the samples thousand fold using HLB Oasis SPE cartridges (Waters, St Quentin en Yvelines, France), analyses were performed by LC/FTMS on an ultra-high resolution Orbitrap mass spectrometer fitted out with an electrospray ionization source (Exactive, Thermo Fisher Scientific, San Jose, CA, USA).

This comprehensive approach highlighted several chemicals detected upstream of the drinking water treatment plant including drugs, pesticides, fungicides and industrial products. Although 85% of these molecules were effectively removed by the treatment system, 15% were not or poorly retained by these processes and detected at trace levels. These results will allow SAUR to achieve drinking water quality beyond the French and European regulatory frameworks as well as to better direct its prevention policy in terms of environmental pollution.

Max Rubner-Institut

Poster 22

Comparative Cow Milk Metabolomics: A GC-MS Pilot Study on the Effects of Roughage, Farming-Style and Season

Nicolas Schauer*, Stephan Mosler, Jenifer Wohlers, Daniel Kusche, Martin Lehmann*, Ton Baars
Department of Biodynamic Agriculture of Kassel University, Witzenhausen, Germany
***Metabolomic Discoveries GmbH, Potsdam, Germany**

The complex composition of metabolites in milk and its determinants are of growing interest. Applying the wide sensitivity range of GC-MS, we piloted whether various production factors such as intensity and origin can be differentiated in milk extracts. In a crossover study in a single herd, most of the metabolites differed for hay of pasture vs ley, 14 of which significantly. Five of these compounds were fat synthesis related, reflecting the high energy content of hay of ley. ¬ In a two-factorial study at farm level in winter and summer, milk from „Organic“ and „Conventional“ farms were compared with regard to extensive/intensive feeding, either dominated by grass/hay or silages of grass and maize plus concentrate. 21 metabolites were found to vary significantly. Large differences between summer and winter reflect the feeding of grass. Under conventional farming many sugars are elevated in milk. High roughage-to-concentrate ratio lowered carbonic acids, while hippuric acid was pronounced. An exemplary indicator set of 10 analytically robust, most significantly affected metabolites clearly differentiated the four conditions, in winter and summer, respectively.

Max Rubner-Institut

Poster 23

LC-qToF-MS Based Metabolomics to Study Effects of Climate Conditions on the Phytochemical Content and Composition in Brassica Species

Gesine Schmidt, Sidsel F. Hagen, Helle Olsen, Grethe Iren A. Borge Nofima AS,
Norwegian Institute for Food, Fisheries and Aquaculture Research
Ås, Norway

Vegetables of the cabbage family (Brassica) like broccoli, cauliflower, kale, kohlrabi, turnips, and rapeseed are an important dietary element for people living in the northern hemisphere. Brassica vegetables are rich in potentially health-promoting phytochemicals such as vitamin C, glucosinolates, flavonoids, hydroxycinnamic acids, and carotenoids. Many members of the Brassica family are cool-weather crops that grow best at temperatures between 18 and 23 °C. In addition to genetic variation, the plants show large variations in the development of their phytochemicals depending on climate factors such as light intensity, length of day, effective UV radiation, air and soil temperature, and water availability.

The Nordic countries have an interest in utilizing their long photoperiods and special solar radiation to produce local foods with optimized health and sensory quality traits. This interest has initialized several research programs to study the effects of climate on the phytochemical content and composition in various fruits and vegetables, especially of the Brassica family. In order to explore these effects, a metabolomics approach will be used. We are currently establishing protocols for high-throughput analyses of a wide range of phytochemicals in Brassica vegetables and other fruits and vegetables relevant to the Nordic diet. Optimal sample (plant material) storage and processing, extraction procedure, UHPLC-qToF-MS, HPLC-(IonTrap)-MS/MS and GC-MS/MS methodology, the reproducibility and comparability of different instruments, as well as compound identification and quantification are currently being investigated and will be presented.

Max Rubner-Institut

Poster 24

Metabolomics of the Wheat Pathogenic Fungus *Fusarium Graminearum* by *In Vivo* Stable Isotopic Labelling and LC/MS

Rainer Schuhmacher[1], Christoph Büschl[1], Bernhard Kluger[1], Franz Berthiller[1], Roman Labuda[2], Georg Häubl[2], Gerald Lirk[3], Stephan Winkler[3], Rudolf Krska[1]

[1] Center for Analytical Chemistry, Department for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna, Tulln, Austria

[2] Romer Labs Diagnostic GmbH, Tulln, Austria

[3] School of Informatics/Communications/Media, FH OÖ Upper Austria University of Applied Sciences, Hagenberg, Austria

In vivo stable isotopic labelling offers a powerful tool for LC/MS based metabolomics research by introducing a spectral feature that is only observable for true metabolites of the investigated organism.

This work presents the successful application of an efficient approach for the assignment of the metabolome odf a filamentous fungus by LC-high resolution MS. The plant pathogenic fungus *Fusarium graminearum* was cultivated in parallel on similar nutrition media, which only differed in the isotopic composition of the carbon energy source (e.g. glucose), either solely in form of native ^{12}C glucose or U-$^{13}C_6$ glucose. 1+1 mixtures of these biological samples were then measured with LC/MS.

The presented work makes use of the MET-EXTRACT algorithm and software programme, which was recently developed in our laboratory. This programme automatically detects MS signals originating from isotopically labelled metabolites using a brute force approach. The isotope patterns for monoisotopic and fully labelled ion molecules are confirmed and EIC-chromatograms of these isotoplogues are compared by means of the Pearson correlation coefficient. Moreover, the programme was used for an automated search for matching metabolites in “Antibase” [1] a database, containing a list of more than 33,000 microbial metabolites.

The presentation will illustrate the complete methodical approach from cultivation of biological samples to data interpretation. Several hundred metabolites have been assigned and partly identified or annotated for the investigated strain of *F. graminearum*. Moreover, metabolite production was monitored as a function of time and the biological and technical precision of the presented approach have been evaluated.

[1] Laatsch, H. (2007). AntiBase 2007: The Natural Product Identifier. Wiley-VCH Verlag GmbH. ISBN-13: 9783527319756, ISBN-10: 3427319751.

Max Rubner-Institut

Poster 25

NutriChip – A Technological Platform for Nutrition Analysis to Promote Healthy Food

Flurina Schwander[1], Katrin Bolanz[1], Reto Portmann[1], Charlotte Egger[1], Martin Gijs[2] , Guy Vergères[1]
[1] Research Station Agroscope Liebefeld-Posieux ALP, Bern, Switzerland
[2] Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

A balanced diet is important for the well-being and healthy aging of humans. Chronic metabolic diseases, e.g. obesity, is one of the major problems nowadays. Its prevention requires a deep knowledge of how different food affect human metabolism. The multidisciplinary project NutriChip, funded by the Swiss federal program Nano-Tera, aims to develop a chip-based technology that allows the screening of health promoting properties of food products, focusing on the bioavailability and immunomodulatory properties of their nutrients. Dairy products were chosen as model food since their anti-inflammatory effects both in vitro and in vivo were demonstrated. Dairy products are digested in vitro using a three step enzymatic digestion model. The nutrients released are subsequently applied apical to CaCo-2 intestinal cells cultured in a transwell system mimicking absorption in the gut. The transported nutrients are finally analyzed on their ability to modulate the inflammatory response of activated monocytes. This cellular co-culture model will be transferred and integrated into a microfluidic chip-system and coupled to a high resolution fluorescence detection system to routinely screen food products on their bioactivity via detection of inflammatory cytokines (IL-6, TNF-α). This in vitro system is going to be validated by conducting several human nutritional trials. The food samples after in vitro digestion and the blood samples obtained from the clinical intervention trials will both be screened with metabolomic and proteomic approaches for the identification of novel biomarkers.

Max Rubner-Institut

Poster 26

Eight Carbon Containing Volatiles Shape the Variability in Aroma of the Black Truffle *Tuber Uncinatum*

Richard Splivallo, Nayuf Valdez, Nina Kirchhoff, Marta Castiella Ona, Petr Karlovsky
Molecular Phytopathology and Mycotoxin Research, University of Goettingen, Goettingen, Germany

Because of their rarity and their captivating aromas, truffles, symbiotic fungi that develop belowground, keep on fascinating food connoisseurs. Truffle aromas are made of tens of volatile compounds that are small hydrocarbons containing alcohols, aldehydes and sulphur atoms. Truffle aromas present an important variability within a single species. The causes of this variability and whether it influences how humans perceive truffle aromas is not known.

Our aim here was to investigate aroma variability in the black truffle *Tuber uncinatum*, a species found throughout Europe. To investigate how aroma varied between distinct geographical locations, we collected 196 truffles fruiting bodies from 15 truffle grounds and 7 European countries. Furthermore to investigate the variability in aroma due to seasons, we sampled one truffle ground in Switzerland 3 times per year for 2 consecutive years by recording the exact positions of the fruiting bodies in the field. A volatile fingerprint was generated for each sample by SPME-GC/MS.

Statistical analysis of the volatile fingerprints demonstrated that regardless of the collection place or season, most variability in the aroma of *T. uncinatum* could be attributed to eight carbon containing volatiles. Furthermore sensory analysis (sniffing tests) revealed that humans classify the aroma of the black truffle *T. uncinatum* by large according to the C8-VOCs content of their fruiting bodies.

Our data also illustrated that in the *T. uncinatum* truffle field in Switzerland, truffles producing different levels of C8-VOCs clustered around distinct host trees. This suggests that the synthesis of the major eight carbon-containing volatile 1-octen-3-ol is controlled by genotypic variability and not by maturation as argued by others. Indeed genotypic fingerprinting revealed that C8-VOCs production capacity correlated with genotype.

These results demonstrate that the plasticity of truffles aroma major constituents is by large under genetic control. They additionally pave the way to use breeding techniques to select for the most desirable truffle aromas.

Max Rubner-Institut

Poster 27

Discrimination Between Feeding and Rearing Systems of Iberian Pigs by ^{1}H-HRMAS NMR Spectra of Dry-Cured Ham

Palmira Villa[1], Juan A. Ordóñez[2], Rosa Escudero[2], Elena Soto[2], M. Isabel Cambero[2*]
[1] CAI de Resonancia Magnética Nuclear. Universidad Complutense de Madrid, España
[2] Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Facultad de Veterinaria, Ciudad Universitaria, Universidad Complutense de Madrid, Madrid, España

Metabolomics is a powerful tool not only in biological system but also in food science. In our case, we present here an interesting application for the classification of the Iberian pig as an example of traditional productions. Iberian Pig is an autochthonous pig breed that eats acorns and pasture in a free-range system (*montanera*) in the Mediterranean forest. The fattening phase under *montanera* leads to a special composition of the Iberian pig fat, and consequently its meat products reach high market prices. Due to geographical limitations of the Mediterranean forest, only about 15% of the animals are reared under *montanera*. Nowadays, most Iberian pigs are reared indoors and fed with mixed diets thus their meat products obtain lower market prices. Due to the outstanding economic importance of the fattening phase in Iberian pig production, several chemical tests have been proposed to differentiate the products coming from each of the rearing systems. High-resolution magic angle spinning (HR-MAS) ^{1}H-NMR spectroscopy allows direct non-destructive analysis of intact tissues to obtain highly resolved spectra that can provide detailed and complex information on the biochemical composition. In this poster, we explore the potential of this spectroscopy combined with multivariate data analysis to distinguish between dry-cured ham from Iberian pigs reared in *montanera* or in confinement. With this aim, samples of femur bone marrow were analyzed. ^{1}H-HRMAS NMR spectroscopy was performed at 500.13 MHz using a Bruker AMX500 spectrometer 11.7 T. The samples were placed within a 50 µl zirconium oxide rotor and a standard solvent suppressed spectra (NOESYPRAST) was done.

Discrimination between samples from animals fed in *montanera* or in confinement with different diets (formulated and acorn) was achieved by applying principal components analysis to ^{1}H-HRMAS NMR spectral data as unsupervised method of classification. It was concluded that the analysis with ^{1}H-HRMAS NMR together with multivariate analysis is a powerful tool to be used for the traceability of Iberian meat products.

Acknowledgments. Financed by AGL2010-19158, CARNISENUSA-CONSOLIDER-INGENIO-2010 (CSD2007-00016).

Max Rubner-Institut
Federal Research Institute of Nutrition and Food

Address Haid-und-Neu-Straße 9, 76131 Karlsruhe
Phone +49 (0)721 6625 201
Fax +49 (0)721 6625 111
E-Mail mrc@mri.bund.de
Internet www.mri.bund.de