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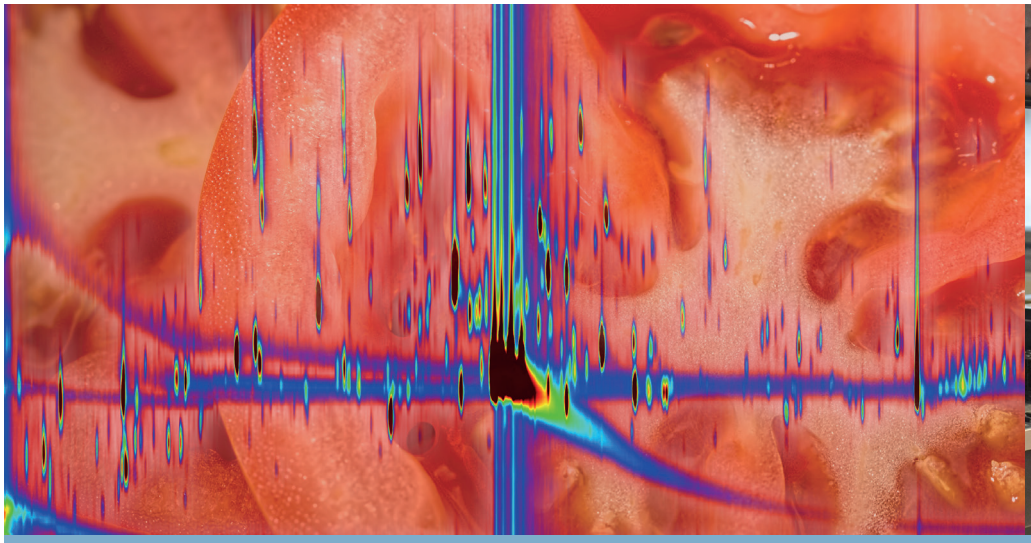

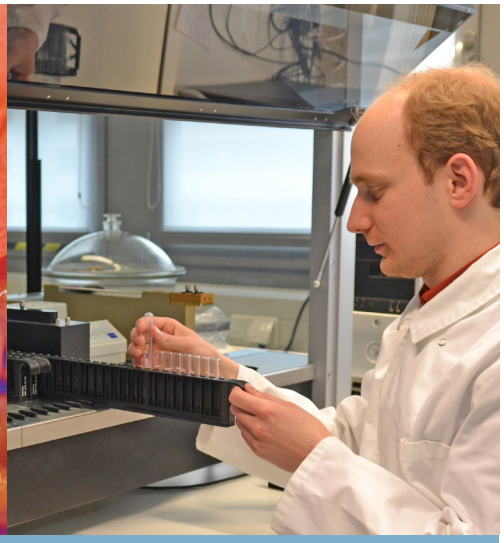

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Food Metabolomics

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Food Metabolomics: A milestone in the characterization of our food?

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

A first example regards a study aimed to investigate the dose dependent effects of consuming diets enriched in flavonoid-rich and flavonoid-poor fruits and vegetables on the urine and plasma metabolome of adults at risk of cardiovascular diseases. A single-blind, dose-dependent, parallel randomized controlled dietary intervention was conducted where volunteers were randomly assigned to one of three diets: high flavonoid diet, low flavonoid diet or habitual diet as a control for 18 weeks. High resolution LC-MS untargeted metabolomics allowed to identify several dose-dependent biomarkers, which can be considered in future studies to assess flavonoids and/or carotenoids intakes and compliance to fruit and vegetable intervention.

A second example regards a cross-over blind human trial, where healthy volunteers were given 250 ml of cloudy apple juice either natural or enriched with additional amount of apple polyphenols. This study allowed to efficiently identify the metabolic products of apple polyphenols using an untargeted metabolomic approach, and to follow their nutri-kinetic in plasma and urine after different intake. Paving the way to the design of multi-omics experiment aimed to investigate the relations between microbiota and metabolites.

A third example regards an animal study, aimed to examine the ability of selected polyphenol microbial metabolites (PMMs) to enter the brain. The time-dependent tissue distribution of a single intravenous injection of a PMM mixture in anaesthetized rats allowed to produce the quantitative profile of several PMMs in a mammalian system, proving their distribution in the brain, the main excretory organs, the heart, the blood, and the urine. Remarkably, the data obtained highlighted 10 PMMs which incrementally appear in the brain within 15 min, whereas they disappear from the blood and/or reach other organs. These data, obtained via targeted metabolomics, support the hypothesis that selected PMMs could be players in the putative connections/messengers between the microbiota and brain; suggesting further health implications for the brain–gut microbiota axis.

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Food Composition Databases 2016 and 2030: Status quo and future needs

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Food composition databases (FCDBs) provide detailed information on the nutritional composition of foods, usually from a particular country. About 10 years ago food composition data were compiled, documented and presented in many different ways, e.g. on paper, in spreadsheets and in several data management systems. The resulting tables were incompatible without considerable adjustments and only few FCDBs were available online. In 2005 the European Food Information Resource Network (EuroFIR) was launched as a partnership of universities, research institutions and small-to-medium sized enterprises to build up a framework for the compilation, management and publication of validated food composition data. Main goals of this framework were harmonisation of food description and thesauri (e.g. components, analytical methods and units) as well as standardisation of data interchange and development of a standardised food composition database management system (FoodCASE). Hereby EuroFIR could build on achievements of former initiatives like INFOODS, COST Action 99 and the EPIC-Study.

Current work of standardisation and harmonisation of FCDBs focusses on the improvement of data quality. The core task is to generate validated and traceable nutrient data. Therefore, food and value documentation, and training of nutrient data compilers is promoted. Due to limited resources, there is an essential need to distributed networking, sharing of results and avoidance of redundant work.

The German Nutrient Database (Bundeslebensmittelschlüssel (BLS)) is part of the EuroFIR network. The present version 3.02 comprises 14,814 foods, including unprocessed and processed foods, all described by 131 nutrients. Nutrient data are derived from measured values or calculations considering changes during food preparation by means of weight yield and nutrient retention factors. To improve data quality, the EuroFIR quality index was adjusted for evaluating literature data and results from own analysis projects conducted at the MRI or by cooperation partners. In addition, the BLS calculation software is merging with FoodCASE.

In the future, the FCDB network will be expanded. Machine-to-machine interaction via web services and cloud computing will facilitate database linkage of many research areas. As a result, users will be able to gather data from databases on nutrient composition, food metabolomics, food consumption or agricultural statistics with only one query. Precise data matching is the basis for this upcoming development. This enables the database servers localised in distributed networks to interact autonomously and to select and combine the data for delivering the correct query output. Therefore, interdisciplinary standardised descriptions and machine readable thesauri will be mandatory.

Food metabolomics as a rapidly growing research field may profit from the experiences and developments of the FCDB network especially with regard to data standardisation, its use for data harmonisation, and establishing a distributed technical infrastructure for data interchange.

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Metabolomics databases

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Databases are essential in metabolomics. Dozens of databases exist that differ in coverage (from generalists to focused databases), contents, search possibilities, data origin and curation, and can serve different purposes for metabolomics studies. In the identification workflow of metabolomics studies, compound-centered databases, biological databases for specific fields of research, spectral libraries, data repositories can be used for obtaining hypotheses of identification for unknowns of interest, for finding analytical and biological data that support or invalidate an hypothesis of identification, for purchasing standards or reference materials to be analysed for validation of an identification and eventually for substantiating a level of validation of an identification in a publication.

A good knowledge of the intended purposes, qualities and shortcomings of the existing resources is crucial to make good use of them. Combining different resources is certainly recommended as none of them has a comprehensive coverage and informed choices must be made according to the scientific objective. As an example, the most useful databases for nutritional biomarker discovery will be presented, with a particular focus on some resources developed in the framework of the FoodBall project (*http://foodmetabolome.org/*). In particular, FoodComEx (Food Compound Exchange, *http://foodcomex.org/*) is a new collaborative chemical library to share non-commercial standards of food-derived compounds and reference materials. FooDB (*www.foodb.ca/*) is the most comprehensive dababase on food constituents, with >26600 compounds including nutrients, natural microconstituents and man-made compounds such as additives and preservatives. PhytoHub (*www.phytohub.eu*) is a new compound database for dietary phytochemicals and their human metabolites, which is currently developed as a community research tool. Exposome-Explorer (*http://exposome-explorer.iarc.fr/*) is a new database for biomarkers of exposure to environmental risk factors for diseases that include nutritional biomarkers with populational data.

Also interesting to discuss are the recent developments to overcome the current limitations of metabolomics databases, especially their incompleteness. Experimental spectra are available for only a small proportion of known compounds. MassBank, MoNA, HMDB, and Metlin, which are the largest libraries of spectra for non-volatile small molecules, include spectral data for about 15000 compounds at best, which has to be compared to the >242000 compounds present in Dictionary of Natural Products, or to the >50 millions of compounds present in Chemspider. Mass spectra prediction is being actively developed to complement experimental data. Another trend for enrichment of compound databases is the in silico prediction of compound metabolism. For many food non-nutrients, the metabolic fate in humans is not yet known, thus predictions based on the chemical structure, knowledge of the most frequent biotransformations, and machine learning algorithms will be particularly useful in the field of nutritional metabolomics.

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The Golm Metabolome Database: From Metabolites to Metabolic Pattern Recognition

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Gas chromatography coupled to mass spectrometry (GC-MS) is one of the most widespread routine metabolomics technologies applied to the large scale screening of biological material in both in fundamental and applied studies. Applications range from assessment of the primary and secondary metabolome to volatile emission and cell wall or lipid components. The current focus is the discovery of novel diagnostic metabolic biomarkers but quantitative metabolic patterns comprising multiple known and possibly yet non-identified metabolites may be better diagnostic markers than single compounds. New developments at the Golm Metabolome Database (*http://gmd.mpimp-golm.mpg.de/*) aim to support both structural elucidation of novel yet non-identified biomarkers and to prepare for metabolic pattern matching. Biomarker elucidation is supported by novel GC-APCI-qTOFMS technology providing enhanced mass resolution and alternative fragmentation pathways. Pattern matching is supported by extension of GMD from a library of reference data for compound identification in complex samples to a data base of profile data (*http://gmd.mpimp-golm.mpg.de/profile/*) which are linked to publication and experimental meta-data, e.g., the profiled biological objects and their physiological states.

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High(er)-throughput metabolite annotation

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Metabolite profiling via LC/MS can reveal “interesting” features, and subsequent tandem MS experiments provide powerful structural hints for the elucidation of these unknown mass spectral features.

Today, the identification of metabolites from MS/MS spectra relies on the comparison with authentic compounds or reference spectra. Because these are often expensive to obtain, reference libraries will never cover as many compounds as can be found in e.g. PubChem.

In-silico methods such as MetFrag (*http://msbi.ipb-halle.de/MetFrag/*) help to identify compounds with tandem MS among candidate structures obtained from general purpose compound libraries. The addition of information from spectral libraries, retention time and literature can improve the results even further.

In 2012 the experimental and computational mass spectrometry community was invited to the first CASMI, the “Critical Assessment of Small Molecule Identification”, an open contest on the identification of small molecules from mass spectrometry data. Details about the contest, participants, evaluation procedure, and the different strategies applied will be presented.

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Towards Quantitative Metabolomics

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Until quite recently, metabolomics has been a qualitative science. Indeed, the vast majority of metabolomics papers report only qualitative differences or relative differences between samples. The traditional reliance on chemometric approaches to analyze metabolomic data has prevented metabolomics researchers from exploiting one of the traditional strengths of analytical chemistry, i.e., *compound quantification*. In this presentation I will describe some of the inherent advantages of using quantitative metabolomics over qualitative metabolomics. I will also describe some of the efforts that we, and others, are making towards developing the tools, reagents and resources that can make metabolomics far more quantitative. In particular, I will highlight some recent developments in NMR spectroscopy, mass spectrometry and reagent exchange that can be used to make metabolomic measurements much more quantitative and far more reproducible. These include the development of software tools like Bayesil, BATMAN and GC-AutoFit, the creation of FoodComEx and the Human Metabolome Library (HML) as well as the development of a large number of easy-to-use quantitative metabolomics kits. I will also discuss some of the existing challenges that still remain in our effort to make metabolomics routinely quantitative.

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Volatolomics by direct injection mass spectrometry

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Volatile metabolites play a relevant role in food science and technology in most, if not all, steps of the production chain: they are, e.g., important for plant ecology and physiology (plant response and signaling upon biotic or abiotic stress), they are drivers and products of fruit changes during ripening and storage and they control to a large extent the way we perceive food before (odor), during (flavour, aroma) and after (aftertaste) consumption. Moreover, being spontaneously and continuously released, volatile compounds provide a non-invasive and rapid tool for the control of food samples and the real-time monitoring of biological and technological processes.

For these reasons, the analysis of food volatolome is of interest if, mostly in an omic approach, it can provide i) high sensitivity and large dynamic range because volatile compounds can produce biological or sensory effects at different, possibly very low, concentrations and ii) fast and non-invasive measurements both to allow the screening of large sample sets and the monitoring of rapid processes.

These issues can be efficiently addressed by different Direct Injection Mass Spectrometry (DIMS) methods developed for volatile compound analysis, Proton Transfer Reaction Mass Spectrometry (PTR-MS) in particular. The lack of specificity of these techniques, as compared with chromatographic ones, is compensated by other features: they are very fast, non-invasive and provide high sensitivity even without sample pretreatment.

This contribution, after a short description of a prototypical DIMS set-up based on PTR-MS developed for agroindustrial applications, aims at pointing out DIMS pros and cons in food volatolomics by describing few selected applications investigated at the Volatile Compound Facility at FEM.

Firstly, PTR-MS profiling of berry fruit, apple and dairy products has been used for sample sets exploration and to set classification or calibration models that link food volatolome with sensory or genomics allowing, for instance, i) the efficient identification of quantitative trait loci related to fruit volatile compounds, ii) the setting of instrumental models of sensory quality which should make "sensomic" studies realistic and iii) the identification of typicality markers.

Secondly, a fully automated system for the monitoring of volatile compounds released during biological or technological processes has been developed and used to investigate microbiological processes as bread leavening, lactic and alcoholic fermentation and spoilage during storage.

Finally, DIMS allows the investigation of the interaction of food with humans or animal models, both on a sensory and health perspective, by measuring metabolites released during food consumption (nose-space analysis) or in exhaled breath (breath analysis).

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The potential of LC-HRS metabolomics fingerprinting in food analysis: Application to the control of forbidden substances

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Growth promoting practices for cattle fattening purposes are still encountered all around the world, for instance the use of clenbuterol in pigs, recombinant growth hormone in buffalos, natural steroids in cocktails' in bovines, or hypothetic but realistic anabolic strategies consisting either on upstream disruption of the hypothalamo-pituitary axis (secretagogues, SARMs...) or even worst on direct genes modification (gene doping).

Detection of illegal practices classically relies on residue monitoring in a targeted approach and methods based on gas- or liquid chromatography coupled to (tandem) mass spectrometry are today considered as the state-of the-art. These strategies are however challenged when facing new xenobiotic growth promoting agents or new ways of application, such as the administration of low dose cocktails.

In this context, screening strategies allowing detection of the physiological response resulting from anabolic compounds administration are promising approaches to detect their misuse. Omics have shown their relevance in highlighting such physiological responses, and in particular, metabolomics studies have demonstrated the efficiency of mass spectrometric-based profiling to discriminate anabolised from control animals [1, 2].

Research studies have been designed to focus on the main suspected anabolic practices and to fit with potential practices in terms of compounds, doses and treatment lengths. Various descriptive and predictive models have been set up, allowing efficient discrimination of the treated and control populations considered [3].

The next challenge is now to free the different established statistical models from their respective given experimental conditions to elaborate models able to predict samples arising from other experimental conditions, i.e., other animals (age, sex, breeding conditions...), and dealing with other anabolic compounds, doses, treatment lengths...

Overcoming this challenge is a necessary step in the validation process of such strategy before considering any official implementation of the tool for screening purposes. The objective of the presentation is to illustrate, through the example of an efficient model dedicated to β-agonists screening, some validation strategies to assess biomarkers relevance and robustness. The implementation of suspicious thresholds is described and performances of established model are discussed with regards to EU requirements for screening methods [4]. Finally, the process toward accreditation (ISO17025) is illustrated.

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Metabolomics approaches to detect food spoilage

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'Biochemical Characterization of Fermented Cucumber Spoilage using Non-targeted, Comprehensive, Two-dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry'

Cucumbers are preserved commercially by fermentation in sodium chloride brines, which enables bulk storage for several months prior to finished product processing. Spoilage of fermented cucumbers during the bulk storage phase has been characterized by a decrease in lactic acid concentration and rise in brine pH. The unpredictable nature and sporadic incidence of this spoilage process has resulted in food waste and large economic losses for processors. We recently demonstrated that *Lactobacillus buchneri* initiates spoilage of fermented cucumbers under anaerobic conditions by metabolizing lactic acid into acetic acid and 1,2-propanediol, causing a corresponding rise in pH which destabilizes the system. Given the limited knowledge of other components in fermented cucumber that may be related to spoilage, a metabolomic approach was applied to provide deeper insight into the biochemical changes and reveal the unique metabolic capabilities of *L. buchneri*. Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GCxGC-ToFMS) metabolite profiling methods were developed and applied for nontargeted detection of volatile and nonvolatile compounds during anaerobic fermented cucumber spoilage by *L. buchneri* LA1147 and during reproduction of spoilage with natural microbiota. Among 314 volatile components detected in fermented cucumber brine, 199 had peak areas with coefficients of variation below 30%. Peak identifications (214/314) established by mass spectral library matching were 92% accurate based on 63 authentic standards. A smaller proportion of TMS-metabolite peaks were accurately identified by mass spectral match due to database limitations. Despite this limitation, univariate analysis of variance combined with hierarchical cluster analysis revealed 62 volatile and 30 non-volatile metabolites that changed during spoilage with mixed cultures and the isolated *L. buchneri* ($P < 0.01$). Decreases were observed in mono and disaccharides, amino acids, nucleosides, long chain fatty acids, aldehydes, and ketones, along with increases in several alcohols and butanoic and pentanoic acids. Most of the detected changes preceded lactic acid utilization, indicating that lactic acid is not a preferred substrate for anaerobic spoilage organisms in fermented cucumbers and that a number of energy sources remain after depletion of the typically monitored fermentable sugars. The ability to detect biochemical changes that preceded lactate utilization revealed citrulline, trehalose, and cellobiose as compounds that may signify metabolic activity of *L. buchneri* spoilage strains prior to any significant product degradation. Biochemical profiling using a non-targeted GCxGC-ToFMS metabolomic platform led to discovery of changes in several metabolites related to spoilage that were previously unknown.

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Metabolite Profiling: A Tool to Assess Safety and Quality of Crops

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Metabolite profiling based on gas chromatography-mass spectrometry (GC/MS) constitutes an effective analytical platform suitable to provide valuable information contributing to safety and quality assessments of crops. Two examples will be outlined in this presentation: (i) the elucidation of pathways and metabolic changes in induced rice and soybean mutants, and (ii) the assessment of the impact of drought on the metabolite profiles of barley cultivars.

During recent years, mutation breeding has been applied to generate low phytic acid (*lpa*) rice and soybean crops. Investigations of these materials by means of molecular mapping and metabolite profiling led to significant progress in the characterization of the *lpa* mutants culminating in the disclosure of prior unknown mutation targets. Recently, a rice mutant generated through γ-*irradiation*-induced disruption of *OsSULTR3;3,* an ortholog of the sulfate transporter family group 3 genes, has been described. The grain exhibited not only reduced concentrations of phytic acid and total phosphorus but also pronounced alterations of the metabolite profile [1].

Lpa mutants have inferior agronomic performance compared to their respective wild-type cultivars. Therefore, segregating populations have been produced by crossing primary lpa rice and soybean mutants with commercial cultivars. The impact of this cross breeding on the metabolic responses to the mutation events observed for the primary *lpa* mutants was determined. In addition, the stability of the *lpa* mutations was followed by investigating the metabolic phenotypes over several cross-bred generations.

To investigate the impact of drought on the metabolite profiles, barley genotypes were field-grown in three consecutive seasons under normal weather conditions and under induced drought stress, using a Rain-Out-Shelter. The comparative statistical assessment of the metabolite profiling data demonstrated that there are grain constituents which are significantly influenced, either consistently increased or consistently decreased, by drought stress [2]. In addition, the results revealed that free amino acids may serve as potential markers for barley genotypes differently adapted to drought stress [3].

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Untargeted GC-MS based fingerprinting to assess the impact of processing and storage in fruit- and vegetable based food products

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In the past, many studies of food quality changes during processing and storage have followed a ‚targeted' approach, i.e. focusing on a particular (set of) quality responses (such as vitamin C, color, texture,...), which have been selected at the start of the experiment. Given the progress in analytical methods and data analysis techniques, such targeted single- or multi-response studies should evolve towards more integrated studies relying on both targeted and untargeted approaches analyzing multiple responses. A zoom-in approach whereby fingerprinting studies are combined with kinetic studies allows to gain insight into complex food reactions induced by preservation and/or storage. In such a zoom-in approach, fingerprinting is used as a multivariate, hypothesis-free starting point to screen for key quality differences in food extracts of differently processed, preserved, and/or stored foods. By interpreting the identity of the selected fingerprint markers in terms of their relevance and consequences for application or connecting the markers to particular food reactions, in a subsequent kinetic study mechanistic as well as quantitative insight into the effect of extrinsic processing variables on quality changes can be obtained.

In the present presentation, the potential of a combined GC-MS based fingerprinting and kinetic approach will be illustrated by several examples (i) to compare the process impact of equivalent thermal and high pressure-high temperature processing of vegetable purees (Kebede *et al.*, 2013a-b, 2014), (ii) to evaluate quality changes during storage, including accelerated shelf-life testing of vegetable and fruit-based products (Kebede *et al.*, 2015a-c, Wibowo *et al.*, a-c) and (iii) to investigate the impact of process sequences on the volatile profile of a (mixed) vegetable system (Koutidou *et al.*, 2016).

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Process-induced chemical reactions and metabolites in food

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Process-induced chemical reactions during heating or storage of food can impact the quality of the final product in a positive (flavour, colour) or negative (food-borne intoxicants) way. For protein-containing foods, the Maillard reaction (also referred to as "glycation") is of particular importance (1). In the course of glycation reactions, amino compounds react with reducing carbohydrates to so-called Amadori products, which may be degraded to dicarbonyl compounds during prolonged heating, giving rise to the formation of advanced glycation endproducts (AGEs). Corresponding lysine and arginine derivatives as well as 1,2-dicarbonyl compounds such as 3-eoxyglucosone or methylglyoxal are of significant quantitative relevance. With a "conventional" Western diet, up to 2000 mg of Amadori compounds, 25-75 mg of AGEs and 20-200 mg of dicarbonyl compounds are taken up per day, in particular due to bakery and pasta products (2). It is noteworthy, that vegetarians have a significantly higher intake of dietary glycation compounds when compared to meat eaters.

Despite this quantitative relevance, only very limited information is available concerning the metabolic handling of glycation compounds and resulting physiological consequences. AGEs in food are discussed as potential risk factor, because corresponding reactions also occur in the human body, contributing to the pathophysiological consequences of diabetes (3). However, corresponding "risk-motivated" studies are very often based on poor analytical characterization of individual glycation compounds. To date, no clear relevance of dietary glycation compounds for certain medical conditions has been attributed to chemically and analytically distinct, rational structures in vivo. On the basis of the current data, therefore, no benefit of restricting AGEs by avoiding heated foods in the diet can be seen.

In the shadow of a debate on possible health risks it may not be overlooked that recent papers have shown interesting positive aspects of dietary glycation compounds (antioxidative effects, inhibition of pathophysiologically relevant enzymes, prebiotic stimulation of intestinal microbiota etc.). According to a current hypothesis of anthropological research, the use of fire for food preparation was a decisive evolutionary advantage in human development (4). In other words: Humans consume heat-induced glycation compounds since several hundred thousand years. A holistic approach, using the competences of chemistry and biology, may show in the future how metabolism of *Homo sapiens* adapted to a physiological use of process-induced glycation products.

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Metabolomics as a tool to assess food authenticity

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Food fraud has been in the news the last couple of years, although it is by no means a new topic. Even if there is no official (European) definition, it is understood to be an intentional substitution, addition, or misrepresentation of food, motivated by economic gain. Examples range from addition of melamine in milk powder to selling horsemeat as beef, from selling cheap wine for expensive brands or regular eggs as organic, or from blending olive oil with other oil or grinding spices added with botanically correct low-value by-products. Food fraud as such is only in rare cases a direct food safety issue, but once the legal line is crossed, 'mild', undetected cases may develop into more serious cases. Moreover, food fraud can lead to significant economic losses, brand damage, loss of consumer confidence in entire food sectors, and it disturbs the market for honest competitors. Obviously, the public should be sure that measures are taken to prevent food fraud, and indeed more and more measures are taken. The direct measures are improved labelling and tracking and tracing systems and managerial systems throughout the food chain, but it is believed these measures should be reinforced by analytical measurements.

Traditionally, analyses to detect food fraud are single-marker measurements, such as determination of protein content in milk or meat to detect addition of water or specific compounds in specific honey or saffron as integrity marker. These single-marker measurements are not available for many types of food fraud, and even if they are, they can be sensitive to manipulation. In the case of milk protein content, which is determined by total nitrogen measurement rather than protein, this has led to the well-known melamine-incident. Moreover, these single-marker methods rarely can detect anything but a very specific type of fraud.

Metabolic profiles are influenced by many factors, including variety/breed, conditions during growth (production system, climate/weather, various stress responses), maturation/ripeness stage and processing conditions. Therefore, metabolic profiles carry information on the product's history, which could also be used to verify authenticity of a product. All that is needed is a solid database of authentic products and preferably examples of adulterated products, measured by suitable technique(s), and a sound statistical method to estimate group membership in a multivariate way.

We show an example where we use the profile of volatile organic compounds as determined by Proton Transfer Reaction- Mass Spectrometry (PTR/MS) in Dutch cumin cheese to discriminate between a protected designation of origin (PDO) cheese and "lookalikes", which provides a good discrimination between both groups.

Accredited targeted and non-targeted 1H-NMR based Methods for Authenticity and Quality Control of Food

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In a context where fraud on food is increasing using more and more sophisticated methods, the need for holistic, targeted and non-targeted analytical approaches has emerged. High resolution 1H-NMR spectroscopy offers unique screening capabilities for food quality, authenticity and safety control by combining non-targeted and targeted screening in one analysis.

1H NMR analysis, thanks to its high reproducibility, allows the acquisition of spectroscopic fingerprints of the samples. This allows the creation of reference databases which can be used unlimited in time and across laboratories.

Combined with multivariate statistical analyses it is possible to assess the authenticity of the sample (e.g. geographical origin, botanical variety or vintage) and to detect any deviation to the reference database. Thus, even new frauds can be uncovered with this technique.

In addition, 1H NMR is a primary method for quantification and allows the determination of concentrations of a high number of components, including sugars, organic acids, amino acids, (poly-)phenols or fermentation parameters. Reference concentration distributions can be created thanks to the reference database in order to give further indication on authenticity and quality of the tested samples.

The strategy applied and the results obtained will be presented with examples from the screening of honey, wine and fruit juices. These methods achieved ISO 17025 accreditation for the quantification and even for the statistical analyses regarding authenticity control.

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The effect of potassium fertilization on the metabolite profile of tomato fruits

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The macronutrient potassium (K) is essential for several physiological functions in plants like translocation of assimilates, activation of enzymes, maintenance of turgescence, and stomata regulation [1]. Consequently, K supply has also a major impact on the concentrations of metabolites in plant tissues, for example amino acids, amines and organic acids [2, 3]. However, the impact of K supply on tomato fruit metabolite profile – and thus fruit quality - has not been investigated so far.

The cocktail tomato cultivars Primavera, Resi and Yellow Submarine were grown in an outdoor pot experiment and fertilized with 5 increasing K doses. Ripe fruits were sampled in the mid-harvest season and by combining whole-fruit segments to pooled samples. After freeze-drying, grinding, methanol extraction and derivatisation, an untargeted metabolome analysis was conducted using GC×GC-MS [4]. Mineral contents of the fruits were determined with ICP-OES.

As shown by ICP-OES analysis, K levels in fruits increased in a dose-dependent manner due to increasing K supply while concentrations of other minerals, for example calcium, were not or only slightly affected. On the basis of 244 consistently detected and reproducibly quantified metabolites, the untargeted metabolome analysis revealed a substantial K effect in the metabolite profiles of Primavera and Yellow Submarine (about 60 metabolites significantly changed). In contrast, the levels of only 11 metabolites were changed in the cultivar Resi. Despite these general cultivar-specific differences, especially citric acid and α-ketoglutaric acid were consistently upregulated by K fertilization in all cultivars. In case of most other compounds like succinic, threonic and quinic acid, uridine, putrescine, isopentylamine and phosphorylethanolamine, asparagine, phenylalanine, methionine, lysine and several sugars, however, the profile of the response to K fertilization was cultivar-specific: Differences were observed either concerning the direction of concentration change (increase or decrease) or the profile of the metabolic response (linear, dose-dependent change or non-linear changes with an intermediate minimum or maximum). In summary, although we could confirm that K fertilization has a consistent and dose-dependent effect on TCA cycle, K influences also several other metabolic pathways in a complex and cultivar-specific way.

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Max Rubner-Institut

Metabolomics and metabolic flux analysis of fruit during postharvest storage

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Advanced omics-techniques are increasingly being used to investigate physiological causes of postharvest diseases and disorders. Typically huge datasets are produced and the challenge is to extract meaningful information using an ever-growing arsenal of software tools for statistical analysis, modelling, and visualisation. The goal is usually to identify patterns of changes at the transcriptomic, proteomic or metabolomic level. This often leads to large lists of up- or downregulated genes, differentially expressed proteins and contrasting metabolite fingerprints that are difficult to interprete beyond the trivial. Gene interactions and signalling pathways further complicate the analysis.

In this presentation we will introduce metabolic pathway modelling as powerful novel tool to extract information from large scale omics datasets and dissect pathways related to postharvest diseases and disorders with unprecedented resolution. The methodology is based on metabolomics and chemical reactor engineering and combines first principles based models for biophysical phenomena such as transport of metabolic gases with kinetic equations for metabolite conversion and regulation at the metabolomic level through mechanisms such as allosteric control and mass action. We will show how fluxomics techniques that combine advanced metabolomics experiments involving labeled substrates with computational algorithms are essential to identify and characterise such metabolic pathway models. We will also show how such models can be extended to incorporate regulation at the transcriptomic and posttranslational level. We will illustrate the concepts with examples of pathway models for ethylene biosynthesis during tomato ripening and the respiration metabolism in hypoxic conditions such as during controlled atmosphere storage of pome fruit and identify future research needs in this area.

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We are all individuals: metabolically!

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We are all different! This applies not only to our physical appearance but also our metabolism and how we respond to our diet. Our metabolic phenotype is the expression of a (meta)genome x environment interaction and is closely linked to the individual health-disease trajectory. Differences between individuals are these days mainly explored via GWAS (genome-wide association studies) that link genetic heterogeneity to disease susceptibility and risks. Recent GWAS also analyzed metabolite profiles in context of SNPs/haplotypes and for the plasma metabolome even on a genome-wide basis. We all know that proper phenotyping is most critical for any analysis of the genome-phenome relationship and because proper phenotyping is costly, tedious and demanding, this is in most cases insufficient.

Almost all intervention studies conducted in the life and/or biomedical sciences try to recruit their patient or volunteer cohorts as homogenous as possible to have the least variation in outcome and a normal distribution with little extremes. And, even extremes – called outliers – are frequently eliminated by statistical means for having more homogenous data. This means that the most interesting phenotypes are likely not found in scientific literature. Amongst the methods used to reduce further the variation amongst volunteers within a human study a fasting period (typically overnight fasting) is included before collecting samples. I shall be demonstrating how much variability can be found behind an extremely homogenous group of young male volunteers when challenges are imposed. I shall also use data from the NutriTech study to demonstrate how robust the metabotypes are in an individual despite marked differences between individuals. Although NMR, GC- or LC-MS/MS based methods are able to deliver high density metabolite data for phenotyping, putting these into a biological context is not trivial - in particular when only body fluids are available.

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Untargeted metabolomics as a tool to assess food consumption? Perspectives and challenges

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Quantifyng food consumption is at the heart of nutrition science since accurate food intake data are necessary to understand any relations between food intake and health.Questionnaire- or diary based assessments have reached a high standard but they still have some caveats, especially the biases related to subjectivity and to the assessor and confounding due to the influence of several phenotypic traits on the accuracy of the answers provided by volunteers. A well-known example is the under-reporting of foods perceived as unhealthy in overweight and obese subjects. The need for more objective instruments to assess food intake is therefore clear.

Using biomarkers of intake has a relatively long history for nutrients but non-nutrient biomarkers for specific foods have come up more recently. The major technological breakthrough has been the application of metabolomics to nutrition studies. A large number of compounds have been observed as potentially specific to certain foods or food groups. This has created a new science frontier of groups identifying and evaluating the validity of potential new food intake biomarkers. At present (summer 2016) there seem to be potential food or food group specific markers for foods in all major food groups, which is highly promising.

The validation task for these biomarkers is daunting and standardising this effort has only just started. Of course the analytics need to be validated according to standards in analytical chemistry but also the nutritional and biological aspects need validation. Could other, possibly less common foods, provide the same compounds and create ambiguity? Are all varieties of a food giving the same biomarker response at the same intake level? What is the optimal time point or period for sample collection? How should we assess intake of complex, processed foods? And so on; the challenges are certainly lining up! And most importantly, can this area provide the quantitative answers that are so much needed in nutritional science?

It is obvious that a great effort will be needed before this emerging area has provided all the promised tools and the first goal should therefore be to provide a qualitative instrument to assess the quality of individual questionnaires. On the other hand we have fast advances in chemical analytics, well-established kinetical models and new efficient approaches to estimate biomarker robustness so chances are quite good that this area could venture into quantitative answers for nutrition within a 5-10y time span.

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Sugar profiling analysis in nutrition

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Various diseases and metabolic disorders as well as diet have been shown to influence the sugar profile in urine and blood. However, most studies so far focused on a few common and well-known sugar species. A more comprehensive method to analyse the sugar profile in human body fluids would be desirable as sugars could serve as markers of food consumption or as disease-biomarkers. Metabolomics methods aim for a comprehensive profile but are often not specific enough to separate closely related sugar isomers. An additional problem is the broad concentration range of different sugar species in human body fluids and the sensitivity to detect minor sugar species.

Our objective was the development of a semi-targeted GC-MS profiling method that allows the detection of known as well as unknown sugar species in urine and plasma or serum. The use of a Scan/SIM-approach and typical mass fragments enabled the detection of known and unknown sugar species within a broad concentration range. The method was applied to two human studies. We analysed urine samples from the KarMeN study (Karlsruhe Metabolomics and Nutrition), an observational human metabolomics study with healthy participants on an unrestricted diet. Additionally plasma samples from a human study employing healthy, prediabetic and diabetic participants, who ingested a drink containing 50 g maltodextrin19, were analysed. Urine samples were normalised to osmolarity and then evaporated, while a protein precipitation followed by a lipid extraction was used for plasma samples before evaporation. Thereafter, samples were methoximated, trimethylsilylated and then analysed.

A wide range of different sugar species was detected in urine as well as in plasma (55 and 33 different known and unknown sugar species, respectively in urine and plasma). In urine samples 38 were identified, while in plasma samples 23 were identified. The reproducibility of the internal standards was better for the measurement of urine samples as compared to plasma (average of the variation coefficients of 14 and 5 measurement days respectively: urine: 4.0-5.4 %; plasma: 9.0-10.5 %). A large inter-individual variance was observed in the urine samples of the KarMeN study. Based on the data set generated, markers for the consumption of dairy products could be identified and distinct sex-specific differences were found. The maltose level in male and female urine showed a significant difference ($p < 0.0001$). The sugar profiles in fasting plasma of healthy, prediabetic and diabetic participants could clearly separate volunteer groups and major differences in the response to the ingestion of maltodextrin were observed as well. The results from both studies show that the semi-targeted sugar profiling method gives valuable insights in the human sugar profile and thus enables the detection of possible markers of dietary exposure or of disease-biomarkers.

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The KarMeN-Study: Biomarkers of age, sex, and diet

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Introduction
Metabolomics is a promising tool to investigate the effects of diet on human health. However, background variation of the metabolome has to be taken into account.

Objectives
The goal of this study therefore was to investigate in healthy humans whether age and sex are associated with metabolite patterns, and if food intake is reflected in the metabolome.

Method / Design
KarMeN (Karlsruhe Metabolomics and Nutrition) is a cross-sectional study that was performed at the Max Rubner-Institut in Karlsruhe, Germany. 301 healthy male and female participants (age range 18 – 80 years) were thoroughly characterized based on anthropometric, physiological, functional and biochemical parameters. Fasted blood and 24h urine samples were collected and analysed by targeted and untargeted GC×GC-MS, GC-MS, LC-MS and NMR. Food intake was recorded using a 24h dietary recall, capturing the same 24h for which urine was collected. Predictive modelling was applied to find associations between age or sex and the metabolite profile using the following machine learning algorithms: SVM, glmnet and PLS. Principal component analysis was used to derive a dietary pattern based on data from the 24h dietary recall. Correlation between current diet and 24h urine metabolome was investigated using Kendall correlation analysis adjusted for age, sex, body mass index and energy intake. The Bonferroni method was used to correct for multiple testing.

Results
Based on metabolite profiles from plasma obtained with different analytical platforms, it was possible to identify metabolite patterns which can predict age in men and women. These patterns include ornithine, hippuric acid, and choline. Additionally, in women, classification according to age (based on their menopause status) was possible from plasma metabolome data. Besides a number of unknown analytes, some metabolites important for this prediction could be identified, such as ornithine, serine, or glucuronic acid. Classification of participants according to sex was possible with >95% accuracy based on plasma metabolite profiles. Metabolites important for correct classification included creatinine and branched-chain amino acids. Furthermore, we identified a dietary pattern representing a "Western diet" for high positive loadings or representing a "prudent diet" for high negative loadings, respectively. "Western diet" was associated with high consumption of e.g. meat, potatoes, animal fats and alcohol accompanied with low consumption of e.g. fish, cereals and cereal products, fruits, vegetable fats and whole meal bread; the "prudent diet" showed opposite associations. We observed 17 metabolites as being significantly correlated with this pattern pointing in particular to the tyrosine metabolism.

Conclusions
Age and sex are associated with metabolite patterns in healthy humans. This needs to be considered in studies looking for the effect of food and diets on the human metabolome. Independent of age and sex, prudent and Western dietary habits are associated with variations in urinary metabolite patterns.

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Metabolomics as a tool in the EPIC study

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The European prospective Investigation into Cancer and Nutrition (EPIC)-cohort was initiated in the late 1980s by a group of young researchers, who collaborated to establish a large scale cohort study in Europe. At that time, first results from the Nurses' Health Study were published and it turned out that a long-term observation of study participants was giving new insights and was highly wanted. The planning of the EPIC study fit well into the at that time existing initiative "Europe against cancer". This initiative was established by the previous political leaders in the European Union for a time period of 10 years in order to increase European collaboration.

The EPIC group was not only repeating approaches being shown to be successfully conducted in the 1990s such as using food frequency questionnaires tailored to the source population but was also introducing novel aspects. One of these aspects has been the collection of blood from most of the study participants and to storage of this blood in liquid nitrogen for further use. During recruitment for the EPIC study in the years 1992 – 1999, blood from more than 360,000 study participants were stored in this way. The low temperature of liquid nitrogen makes it possible that this blood can also nowadays been utilized

The stored blood was used in different ways when linking blood parameters with disease risk. In recent years, traditional blood analyses (hormones, metabolic biomarkers) were extended by novel techniques. The introduction and use of the novel techniques were dependent on the groups that were proposing study proposals for certain research questions, including taking care of the funding of the laboratory analyses.

Different techniques of metabolomics are therefore being utilized by the different EPIC groups and applied to the stored blood aliquots. The approaches include mainly targeted and untargeted metabolomics based on mass spectroscopy, and also recently nuclear magnetic resonance spectroscopy (NMR). Particular interest found the targeted approach with the BIOCRATES kits. The EPIC Potsdam group invested relatively early in metabolomics and examples of this research will be shown, in respect to exposure as well as outcomes. Other very interesting work is done at the International Agency (IARC) in respect to the food metabolome and such profiles in urine and blood.

It is obvious that the various techniques of metabolomics will help to get a much better understanding of diseases processes and their potential prevention. Also the use of such techniques to estimate food intake will help to improve dietary assessment methodologies.

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Poster Abstracts

Profiling of *Thymus capitatus* and *Teucrium capitatum* secondary metabolites and odor volatiles collected from temperate and semi-arid regions in Palestine

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Herbal medicine has been used over hundreds of years and it is estimated that approximately a quarter of modern medicines are directly or indirectly derived from higher plants. The chemical composition of herbs varies depending on the species, harvesting season, and location of growth. Especially for the species *Thymus capitatus* and *Teucrium capitatum*, used in folk medicine, health beneficial effects are associated with specific growing locations.

Since it is unclear, if the different biological effects are resulting from changes in single (classes of) metabolites or changed metabolite profiles, it is of importance to identify and characterize metabolomic changes.

Non-targeted analysis of secondary metabolites and odor volatiles was performed using UHPLC-QToF-MS and GC-MS and aimed to explore and differentiate different compounds and potential key classes of metabolites affected by species and growing location. Compound spectra were collected in positive and negative ionization modes using an electrospray ionization source (ESI $^{+/-}$) and electron impact ionization (EI) over a broad mass to charge range to detect a large number of plant metabolites. After peak picking, alignment of the detected features, integration, and peak area calculation, the data were subjected to statistical and multivariate analysis. This step aimed to reduce the dimensionality of the complex dataset brought on by the plant matrix. Visualization tools enabled the discrimination between the groups. The compounds, which significantly differed between the samples, were then tentatively identified by in-house and public databases.

The variations observed for specific metabolites might contribute to the different effectiveness's known in folk medicine.

It is expected, that a combination of volatile and non-volatile profiling will be further employed especially for species authentication and geographical origin discrimination.

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Characterization of low phytic acid *(lpa)* soybean mutants and their crossbreds by means of metabolite profiling

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Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate) represents the major storage form of phosphorus in soybean (*Glycine max* L. Merr.). It can form indigestible chelates with divalent and trivalent cations and is therefore considered as an anti-nutrient in food and feed. In addition, it is a major source of phosphorus pollution in agriculture. Therefore, various efforts have been made to obtain low phytic acid *(lpa)* crops, e.g. by mutation breeding.

The generation of an *lpa* soybean mutant has been achieved via γ-irradiation resulting in a 2bp deletion in the D-*myo*-inositol 3-phosphate synthase gene 1 (*MIPS1*) [1]. The application of a GC-MS profiling approach revealed that the reduction of phytic acid was accompanied by consistently decreased contents of *myo*-inositol, raffinose, stachyose and the galactosyl cyclitols galactopinitol A and galactopinitol B, compared to the wild-type [2].

Unfortunately, the lowered phytic acid contents of *lpa* mutants are accompanied by inferior agronomic performance compared to their respective wild-types. To overcome this drawback, the primary lpa soybean was crossed with a high-performance commercial cultivar. The progenies were selected based on their content of inorganic phosphorus and the use of molecular markers and subsequently subjected to a GC-MS metabolite profiling procedure.

Univariate and multivariate statistical assessment of the data demonstrated metabolic differences depending on the generation, e.g. regarding the contents of sugar alcohols. However, the cross-breeding with the commercial cultivar did not have a significant impact on type and extent of the differences in metabolite concentrations initially observed between the primary *lpa* mutant and the corresponding wild-type.

These metabolite profiling data are encouraging from a breeder's point of view. They demonstrate that desired nutritional and environmental effects, such as the reduction of the content of phytic acid and lowered amounts of raffinose and stachyose, achieved by induced mutation are stable despite the required cross-breeding step and can be maintained over several generations.

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MRI
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Metabolite profiling of wheat varieties under drought stress

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Wheat supplies one third of the world's grain populations. Nowadays, more and more evidence shows that regular consumption of wholegrain may prevent several chronic diseases, such as cardiovascular disease, diabetes, and certain types of cancer, since wholegrain wheat is rich in bioactive phytochemicals. Wheat production is affected by water deficit by about 65 million ha worldwide, and this is expected to increase in the future due to climate change. Not only the agronomic performance and yield, but also the nutritional and technological grain quality is affected by the environmental factors. In order to evaluate the effect of drought stress on wheat quality, and especially the profile of phytochemicals, metabolomics based investigation of three wheat varieties under different watering conditions was performed in this study.

The wheat varieties, namely Plantahof, Arina and Zinal, were grown in three watering conditions: well watered during whole growing period, early water stressed (water stressed from sowing until flowering, from flowering until maturity well watered) and late water stressed (water stressed from flowering until maturity). Phytochemicals of whole grain under different drought stress were extracted with methanol, and further analysed with an UPLC-HR-Q-TOF-MS--based untargeted metabolite profiling approach. Multivariate statistical analysis was utilized to find the characteristic markers, enabling us to differentiate between various drought stress in each wheat variety. Furthermore, these markers were tentatively identified. For instance, *p*-coumaric acid was higher in the grains from early water stressed wheat Arina compared with the ones under well watered and late water stressed treatments. Our preliminary results indicate that, within one variety, the metabolite profile is significantly impacted by the watering conditions. In addition, the difference between wheat varieties is larger than the influence of drought stress treatments (environment factors) on their metabolites profiling level.

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LC-MS-based profiling of winter wheat – method development and validation

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Wheat was one of the first domesticated food crops and its cultivation reaches far back into history. Today, wheat is grown on more land area than any other crop including rice, maize or potatoes and continues to be one of the most important food sources for humans. The essential role of wheat as a foodstuff comes along with the indispensability to ensure sufficient and secure crop production for worldwide nutrition.

The AWECOS (**A**ssessment of **w**heat cropping systems from an economical, **eco**logical and the **s**ociety's perspective) project aims to assess different breeding strategies for winter wheat. The project identifies advantages and disadvantages of eight different genotypes (high-yield, disease resistant and drought-tolerant cultivars) cultivated with different plant protection strategies (organic vs. conventional farming systems) at five locations around Germany. The assessment will focus on economical, ecological and socio-economic impacts, as well as on the quality of wheat samples.

To investigate cultivar- and cropping system-specific metabolite changes in winter wheat, UHPLC/ESI-QTOF-MS-based metabolite profiling studies are in progress. To cover a wide range of metabolites with different polarity, three analytical methods will be used to provide information about polar, semipolar and nonpolar compounds. In the chromatographic method specific UHPLC columns (HILIC, C18 and C8), suited for metabolites of different polarity, are used.

Comprehensive long-term analysis of metabolites in complex biological matrices like winter wheat is a challenging task for quality assurance. To ensure reliable metabolomic data with high reproducibility, validation strategies become an important part during method development of non-targeted metabolomic studies. To optimize the analytical conditions overall and investigate parameters like accuracy, precision, recovery, matrix effects, test compounds, which cover a broad spectrum of chemical diversity, were used in the validation process. The test compounds provide important data about the methods and instrumental setup and build the basic information for the next step the profiling of wheat samples. Furthermore the validation involves assessment of chemometric models and normalization methods which are applied to the huge data sets.

With the established methods and optimized analytical conditions cultivar- and cropping system-specific metabolite changes can be analysed and interpreted with knowledge about the variability of precision, accuracy and suitability of the instrumental system.

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Comprehensive metabolite profiling of onion bulbs *(Allium cepa)* using liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry

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Onion *(Allium cepa)* represents one of the most important horticultural crops and is cultivated under different climatic conditions nearly worldwide. Beside other species of the genus Allium, in particular onion represents a vegetable with good storage ability and a versatile application potential. Onion bulbs are used in different forms as food and spice in many dishes in almost all cultural areas and can be processed into a wide range of products which are used for seasoning and flavoring of food. In addition, onion has been recognized as a medical plant since ancient times and represents a rich source of putative health-promoting phytochemicals.

Onion bulbs accumulate a diverse set of primary and secondary metabolites, which define their nutritional, sensory and technological properties. Relevant compound classes of the onion metabolome include fructooligosaccharides, amino acids and derived peptides, S-substituted cysteine derivatives and derived peptides, flavonoids, phenylpropanoids and saponins. To comprehensively profile these mostly polar and semi-polar metabolite classes an analytical approach on basis of (U)HPLC/ESI-QTOFMS and two chromatographic methods was developed and validated. Because of the enzymatic turnover of S-alk(en)ylcysteine sulfoxides which is activated upon tissue disruption in onion, a novel extraction method for fresh onion tissue with low-temperature quenching was established. In addition, chromatographic and mass spectral data of more than 120 metabolites was assembled in the course of this work.

On the poster, details regarding the developed analytical scheme, the extraction method, the identified metabolites and method validation data will be presented. The developed protocols were exemplarily applied to compare the metabolite profiles of six onion cultivars by targeted and non-targeted approaches.

A non-targeted approach for discrimination of Sri Lankan teas by UPLC-QTof/MS and chemometrics

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Tea *(Camellia sinensis L.)* is one of the most popularly consumed beverages worldwide. It reportedly contains many compounds beneficial to health and has been used as a natural medicine for thousands of years. The two most popular types are green (favoured in Asia) and black tea (favoured in the western countries). The different growing seasons, geographical regions, processing techniques and fermentation methods create many varieties of tea, some of which have premium value compared to the others. The expansion of the consumer market, which has increased demand for "manufactured" food as well as "pure" food such as tea, has encouraged adulteration because of the prospects for increased profit. The adulteration of tea has become a common problem. Mixing tea-leaves with leaves of some other plants (elder, hawthorne and sloe), addition of the dust of the tea leaves and sand, chemical enhancement of green tea (with Prussian blue and sulphate of lime or gypsum), and simply re-dried and resold tea-leaves, are some of the main examples of tea adulteration.

Sri Lanka ranks as the world's fourth-largest producer of tea. Specific climatic conditions favour the production of high-quality tea, well known as Ceylon tea. Black tea accounts for about 95% of local consumption in Sri Lanka. It has been found that additives (e.g. potassium permanganate) have been added to poorer quality teas in some instances to improve their colour and apparent quality. An untargeted metabolomics approach was developed to investigate the possibility of distinguishing Sri Lankan teas from different geographical origins, and detecting varieties that had been adulterated. Authentic tea samples were obtained directly from four production sites in Sri Lanka (green (Talawakelle, Hanatana, Ratnapura, and Passara) and black (Talawakelle)), infused in water/methanol, sonicated and analysed by ultra-performance liquid chromatography – quadrupole time of flight mass spectrometry (UPLC-QToF MS).

Results indicated that the characteristic fingerprint reflected inherent characteristics of green and black teas collected from different regions, and enabled reliable discrimination between various tea types. Some of the metabolites that contribute to discrimination of the sample groups were tentatively identified using Progenesis QI and database search.

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Changes in the metabolite profile of rice upon disruption of the sulfate transporter gene *OsSULTR3;3*

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Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate), the major storage form of phosphorus in cereals, is considered as an anti-nutrient for monogastric animals and humans; it also contributes to phosphorus pollution of the environment. Therefore, various cereals like maize, barley, wheat and rice have been subjected to mutation breeding for generating low phytic acid *(lpa)* crops.

Recently, it was demonstrated that reduction of both phytic acid and total phosphorus in a rice mutant obtained by γ-irradiation was due to a disruption of *OsSULTR3;3,* an ortholog of the sulphate transporter family group 3 genes [1]. The application of a GC-MS based metabolite profiling approach revealed that the significant reduction of phytic acid was accompanied by consistent changes of other metabolites, e.g. a reduced content of cysteine, increased concentrations of various amino acids, organic acids and other nutritionally relevant compounds, such as γ-aminobutyric acid.

To improve the agronomic performance of the *lpa* mutant, cross-breeding with a high performance cultivar was performed. The objectives of this study were (i) to investigate the impact of cross-breeding on the metabolic phenotype of the *lpa* rice mutant, and (ii) to assess the stability of the mutation by following the metabolic phenotypes over several generations.

Statistical assessment of the data via multivariate and univariate approaches demonstrated that type and extent of the metabolic differences observed between the primary *lpa* mutant and the respective wild type were not significantly affected by the cross-breeding step. The observed increases or decreases of certain metabolites also remained stable over several generations.

The elaborated metabolite profiling data provide an important analytical basis for further implementations of mutation breeding in the generation of *lpa* crops.

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Targeted metabolomics approach for the characterization of wild *Vitis* genotypes

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Wine is one of the most popular beverages in the world which is exclusively produced from *Vitis vinifera* varieties due to the superior quality of their grapes However, today a large amount of pesticides are used in viticulture in order to protect grapevine from their pathogens with a strong impact on environment and human health. For this reason, research has focused on the development of new interspecific hybrids using wild American genotypes in order to introgress their resistant traits to pests and diseases in *V. vinifera* cultivars. Despite this, little is known regarding the metabolic profile of wild genotypes.

The aim of this work was to characterize the grape composition of two hybrids varieties (41B and K5BB) and five American genotypes (*V. andersonii, V. arizonica Texas, V. champinii, V. cinerea* and *V. californica*) in six different vintages. Also *V. vinifera* cultivars (Pinot Noir and Cabernet Sauvignon) were taken into consideration as references. A targeted metabolomics strategy was used for the investigation of simple phenolic compounds, anthocyanins, proanthocyanidins and lipids. Grape skins anthocyanins were analyzed using LC-DAD [1]. In three wild genotypes less than 5% of the total anthocyanins detected were diglucosides. In the four remaining genotypes, diglucosides accounted for more than 40% of the total. Maximum acceptable limit for diglucosides contained in wine is 15 mg/L [2].

LC-MS/MS methods were used for the study of phenolic compounds and lipids [3,4]. The results obtained showed that three wild genotypes contained higher average amount of total phenols and that the one out of seven non-*V. vinifera* genotypes contained a higher content of total lipids compared to *V. vinifera* cultivars. Analysis of proanthocyaninds by LC-MS showed that wild genotypes were mainly rich in oligomers and short-chain polymers [5]. Heatmap analysis was used to point out the differences in genotypes' content for the different metabolites studied.

This work demonstrates the existence of a significant genotypic diversity between the grape composition of *V. vinifera* and other species. The information gained could be very useful for the future grapevine breeding.

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Metabolomics approaches to study the influence of sulfur dioxide treatments on the chemical composition of white wines

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Sulfur dioxide is commonly added to must and wine in order to protect it from oxidation 1. Consumption of high contents of SO2 is potentially detrimental to human health2. In a context of societal concern about food and wine preservation, along with the search for environmentally friendly productions, the reduction of sulfite input plays a major role in wine industry. Metabolomics could be used as an effective tool to followup the production processes and stability of food products exemplified here in wines3,4.

To improve the understanding of the impact of sulfur dioxide on the molecular composition of wine, a series of bottle aged white wines made from the same must, but in three different concentrations of SO2 (0, 4 and 8 g.hL-1) added to protect the grape must at pressing, were analyzed by excitation emission matrix fluorescence (EEMF)5 and ultrahigh resolution mass spectrometry (FT-ICR-MS) 3,4. The consequence of the initial addition of SO2 on the subsequent chemistry of wine after more than 7 years of bottle ageing was investigated by multivariate statistical methods.

Spearman rank correlation was applied to link the statistically modeled EEMF components (parallel factor analysis (PARAFAC)) and the exact mass information from FT-ICR-MS, and thus revealing the extent of sulfur-containing compounds which are correlated to fluorescence fingerprints. Different metabolite classes, including amino acids and phenolic compounds, were affected by SO2 and significant dose-dependent molecular changes were observed.

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Effect of Pulsed Electric Field-assisted vinification on New Zealand Sauvignon Blanc grapes: Using GCxGC-qMS analysis as an untargeted global metabolomics approach

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Pulsed Electric Fields (PEF) processing applies high voltage electric pulses across biological materials placed between two conducting electrodes for short times (typically in the range of micro- to milli-seconds). PEF, at electric field strengths above 0.3-0.5 kV/cm, can lead to irreversible cell membrane electroporation of plant tissues and facilitate the release of cell contents. In wine making, this processing can be used to enhance grape maceration. Since PEF might change the global metabolite profile of macerated grapes and ultimately the resulting wine. The aim of this research is to study the untargeted metabolite profile of wines produced from untreated and PEF-treated Sauvignon Blanc grapes, using comprehensive two-dimensional gas chromatography-mass spectrometry (GCxGC-qMS). As a complement, a targeted analysis of specific phenolic compounds using reversed phase HPLC-DAD was performed. This is the first time that the effect of PEF treatment on metabolite extraction during vinification has been investigated for white grape variety.

The results showed that Sauvignon Blanc wines produced from untreated and PEF-treated grapes had distinctly different metabolite profiles. For example, the concentration of the organic acids malic, citric, malonic, shikimic, α-ketoglutaric, L-threonic, fumaric, glutaric, citramalic, isocitric acids and the phenolics protocatechuic, vanillic, syringic, chlorogenic and ferulic acids in the wine obtained from PEF-treated grapes was higher, as compared to the control wine. The amino acids composition was not greatly affected by the PEF pre-treatment of grapes; however the sugars glucose and fructose were found at lower concentrations in the wine from PEF-treated grapes.

Overall, the global metabolomics-based approach was found to be a suitable technique for detecting and identifying changes in either targeted or non-targeted metabolites in the wine produced from PEF pre-treated grapes. Therefore, this technique offers an opportunity to further understand how these changes at the molecular level could impact on the characteristics of wine.

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Establishment of methods for food authenticity detection by non-targeted metabolomic profiling analysis by UPLC-IMS-HR-Q-ToF MS

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Food quality and traceability are issues of wide relevance to costumer protection and manufacturers. The quality of food products is often linked to their origin as, for example, French wine or Spanish ham, which have a substantial influence on the market price of the product. Another factor causing differences in the intrinsic value of food is its way of production e.g. by organic or conventional farming. Thus, to guarantee the traceability of these items will be essential, not only for costumers but also for manufacturers and retailers. To detect food fraud in the manufacturing and supply chain specific and robust analytical methodologies are needed.

For example, stable isotope analysis (HR-IR-MS) is a classical method for authenticity detection evaluating differences in isotope ratios of the main elements. Depending on the geographic region and the practice of cultivation there are differences in the isotope ratios of elements like H, C, N and O giving proof of the authenticity of the origin and way of cultivation. Unfortunately, this methodology is limited concerning specificity and robustness and may be affected by artificial irrigation and fertilizers.

The aim of the FOODOMICS project is the establishment of a valid methodology for authenticity testing by metabolomic profiling via UPLC-IMS-HR-Q-ToF MS. This project is funded by the German Federal Ministry of Food and Agriculture (BMEL).

Authentic plant-derived commodities (apples, potatoes and tomatoes) of different botanical and geographical origin grown under organic and conventional farming conditions were obtained. After a QuPPe-like extraction step sample extracts were subjected to UPLC-IMS-HR-Q-ToF MS in HDMSE ESI+/- mode. For the evaluation of discriminative models, a metabolomic fingerprinting approach by multivariate data analysis (PCA and OPLS-DA) was accomplished using EZInfo3.0 software (Umetrics Inc., Sweden) and Progenesis QI v2.2 (Nonlinear Dynamics, UK).

Subsequently chemical markers specific for the geographical and botanical origin and the respective farming practice were characterized using a metabolomic profiling workflow using an EM_RT_CCS coordinate system finally leading to the identification of marker compounds. The validation is carried out using representative sample sets originating from multiple crop years.

The individual discriminative models developed by metabolomic fingerprinting and profiling approaches represent valuable tools in authenticity testing of plant-derived commodities like apples, potatoes and tomatoes from different regional or botanical origin and organic or conventional farming practice. A metabolome database with selective marker compound entries has been established.

Classification of the botanical origin of honey by 1H NMR in combination with chemometric methods and new data fusion approaches

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The authentication of the botanical origin of honey is particularly challenging, since the composition not only depend on the botanical provenance, but also on factors such as geographic area, soil types, climatic conditions or even on storage quality. Hence, an analytical approach covering a multitude of parameters in parallel on the one hand, paired with strong discrimination power on the other hand is required here. Targeted analysis methods for marker substances commonly fail here, as quite often there simply are no characteristic substances present. In this context, non-targeted honey analysis by ^{1}H-NMR spectroscopy, combined with multivariate statistical analysis was applied as a powerful tool for quality assessment, which provides fast, simple and low-cost per analysis screening of honey samples. The NMR fingerprint of a honey sample is processed by means of chemometric techniques, typically by principal component analysis (PCA).

A second approach is the low-level data fusion of orthogonal data, obtained from multiple spectroscopic techniques, e.g. NMR and FT-MIR, in order to increase the discriminative power. This was performed in custom MATLAB routines by combining non-targeted analyses of honey samples by ^{1}H-NMR and FT-MIR.

Quality assessment of olive oil based on volatile compound fingerprinting using HRGC-IMS analysis

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The evaluation of extra virgin olive oil (EVOO) with respect to quality and authenticity has been in the focus of a plethora of studies in the past years and still poses a challenge for researches. In particular, sensitive, rapid and low-cost analytical methods are highly relevant for enforcement purposes to ensure EVOO authenticity. Non-targeted headspace analysis of volatile organic compounds (VOCs) by high resolution gas chromatography (HRGC), coupled to ion mobility spectrometry (IMS) provides an fast and effective approach for the discrimination of EVOOs of different geographical origins. This is especially due to minimal sample preparation and short run times, paired with the orthogonal separation power of retention time vs. drift time in GC-IMS. The resulting product-characteristic three-dimensional fingerprints can be evaluated by multivariate statistics tools implemented in custom MATLAB routines.

All analyses were carried out on a prototype headspace high resolution GC-IMS system with a G.A.S. IMS detector. A defined amount of the olive oil sample was spiked with internal standards and then subjected to headspace analysis. The chromatographic separation was carried out on a mid-polar GC column, using nitrogen as a carrier gas. To interface the GC and the IMS, a heated transfer line was kept at 120°C. The drift tube was operated at constant voltage of 246 V cm^{-1} and a temperature of 90°C under flow of nitrogen at 150 mL/min. Resulting orthogonal data (retention time x drift time) were evaluated with the LAV software by G.A.S. (Dortmund) and with custom MATLAB routines.

Headspace high resolution capillary gas chromatography (HS-HRGC) coupled to IMS is a very promising method for the generation of volatile composition profiles for the discrimination of extra virgin olive oils (EVOOs) of different geographical origins. Different algorithms were evaluated in MATLAB, as well as data unfolding strategies. The generated data allowed a non-targeted separation of EVOO samples with the same origin, but different qualities, as well as a tentative separation based on geographical origin. In combination with novel multivariate data analysis techniques, the data generated proved to be superior to the currently used IMS hyphenation techniques. Together with a simplified sample preparation and fast analysis times, HS-HRGC-IMS delivers a cost-efficient experimental setup.

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The effect of potassium fertilization on the metabolite profile of tomato fruits

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Tomatoes are economically the most important vegetable worldwide, with 161.8 million tons produced in 2013 [1]. The tomato plant requires relative high amounts of nutrients during growth and fruit development [2]. Therefore, it is important to determine the demand of cocktail tomatoes and understand respond to low nutrient supply. In contrast to nitrogen and phosphorous, the impact of potassium (K) fertilization on crop yield and quality is often underestimated although K is very important for several physiological functions, as translocation of assimilates, activation of enzymes, maintenance of turgor, and stomata regulation [3]. In this study we fertilized cocktail tomatoes with different K levels and investigated the metabolic response with the aid of an untargeted metabolome analysis.

In an outdoor pot experiment (substrate: peat) three cocktail tomato genotypes (Primavera, Resi and Yellow Submarine) were cultivated. They were fertilized weekly with five increasing K doses (0.4, 0.7, 1.5, 2.2 3.7 g K2SO4). Ripe fruits were harvested at mid-season and whole-fruit segments were combining to pooled samples. Samples were freeze-dried, milled with methanol extracted and derivatized for an untargeted metabolome analysis by using GC×GC-MS. Potassium concentrations were determined with ICP-OES. Amines and organic acids were analyzed with HPLC.

In the tomato fruits the K concentration increased with rising K fertilization. With increasing K fertilization most metabolite changes were strongly cultivar-dependent. Primavera and Yellow Submarine showed approximately 60 changed metabolites, while in Resi only 11 metabolites were changed. However, in all cultivars certain metabolites of the citric acid cycle (citrate, succinate and α-ketoglutarate) were positively influenced by increasing K doses. Furthermore, the amine putrescine was decreased in all cultivars with rising K, although not significantly in Yellow Submarine. While amino acid concentrations were not changed in Resi, in Primavera some amino acids were increased (Ile, Ala, Phe) and others were decreased (Met, Asn, Lys, Cys) in abundance. In fruits of Yellow Submarine concentrations of certain amino acids were only reduced (Leu, Ile, Tyr, Ala, Phe, Met, Asn).

In all cultivars low K supply lead to a decrease of the substance the citric acid cycle. The primary cause of metabolic disorders in low-K plants is the direct inhibition of pyruvate kinase activity by low cytoplasmic K [4]. Pyruvate is an important substrate for citric acid cycle. Higher levels of putrescine in low K plants, can be explained by fact that polyamines is an alternative to K for adjusting charge balance [5]. Interestingly many substances, like the amino acids and sugars, show a cultivar dependent and not uniform reaction to different K doses. Thus the metabolic changes in cocktail tomato plants induced by different K doses varies greatly between the cultivars. Therefore an efficient nutrition supply is cultivar and parameter dependent in cocktail tomatoes.

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Metabolomics-basen discovery of early maillard reactions

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Maillard reactions refer to non-enzymatic reactions of reducing carbohydrates with amino compounds (amino acids, peptides or proteins). They play a crucial role in determining the aroma, taste and color of thermally processed food [1, 2]. Most knowledge of Maillard reactions to date has been deduced from the results of experiments in sugar / amino acid model systems [2]. Furthermore, the discovery of the entire complexity and coherences arising from even simple model systems is still a challenge in analytical chemistry [3].

In this particular study we present a methodology that exposes the amazing complexity of Maillard reaction products (MRPs) formed in aqueous model systems. Non-targeted ultrahigh-resolution mass spectrometry (FT-ICR-MS) in combination with mass difference network analysis enables a comprehensive investigation of non-volatile MRPs and the prediction of possible chemical transformations [4].

Compounds arising from amino acid and sugar degradation were considered independently in order to identify pools of specific MRPs resulting only from the degradation of Amadori rearrangement products [5]. The investigation of different treatment times, amino acid and sugar precursors allowed the visualization of a time dependent and precursor specific evolution of MRPs. More than 700 distinct elemental compositions could be identified in a thermally processed ribose / lysine model system whereas more than 50% of the identifications were found to be specific to the amino acid lysine. Many of the identified compounds have formulas consistent with important precursors in the formation of aroma, taste and color compounds which also occur in real food samples.

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Microbial metabolites and the exploration of the adherence to a Mediterranean dietary pattern by 1H-NMR-based untargeted metabolomics approach

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The assessment of dietary pattern compliance is an essential component in nutritional research and epidemiology. To our knowledge, only dietary indexes or scores through dietary questionnaires have defined the adherence to the Mediterranean dietary pattern. Biological biomarkers are potentially powerful tools to assess the Mediterranean Diet (MedDiet) adherence and a better understanding of its health benefits.

We aimed to identify a biomarker associated with high adherence to a MedDiet pattern through a 1H-NMR-based untargeted metabolomics approach.

In this cross-sectional study, a total of 122 individuals from the PREvención con DIeta MEDiterránea (PREDIMED) study were included and assigned to high or low MedDiet adherence categories (H-MDA and L-MDA, respectively) by using the MedDiet Adherence Screener score (called MEDAS). An 1H-NMR-based untargeted metabolomics approach was applied to analyse urine samples. Multivariate and univariate statistical analyses were performed to determine the metabolite differences between H-MDA and L-MDA groups. A stepwise logistic regression model and receiver operating characteristic (ROC) curves were used to develop and validate a prediction model for H-MDA for identify the discriminant biomarkers.

We identified a total of 35 metabolites that were discriminant between H-MDA and L-MDA. The group of H-MDA showed higher concentrations of metabolites related to the intake of fruits and vegetables, fish or white meat, antioxidant metabolites, microbial metabolites and lower concentration of metabolites related to energy metabolism pathway (glucose, lactate and succinate). The prediction model included the microbial metabolites phenylacetylglutamine, p-cresol sulfate and 4-hydroxyphenylacetate. It has a strong ability to discriminate between H-MDA and L-MDA (90% specificity, 95% sensitivity and 97% AUC). Moreover, the prediction model showed significant correlation with the intake of vegetables, fruits, legumes and fish ($r=0.3$, $p<0.05$).

In conclusion, the results remark the role of microbiota and microbial metabolites in the study of the biomarkers related to high adherence to a Mediterranean dietary pattern which is rich in vegetable-derived foods. The study of these biological biomarkers is a more specific tool and complementary to traditional indexes/scores.This study may assess and aid to nutritional epidemiology in future associations between adherence to dietary patterns and prevention of diseases.

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Two complementary metabolomics studies to identify biomarkers of banana intake

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Banana is a widely consumed fruit in the world. According to FAOSTAT, this fruit has an intake of 33.01g/capita/day worldwide which places it above other popular fruits such as apples (25.7g/capita/day). However, in spite of its popularity there are to date no biomarkers of intake reported in the literature and little is known about the long term intake effect in human health. The identification of novel biomarkers of banana intake is of great value to provide more reliable data about its consumption than the one given by FFQ and 24HR. In order to achieve the identification of such compounds, we assessed two different studies. First, we studied the 24h urine samples from 12 volunteers participating in the BioBanaTom study. BioBanaTom is a crossover, randomized controlled trial were subjects consumed three different test foods 1) 240g of banana 2)300g of tomato and 3) Fresubin 2kcal as control. Blood and urine samples were collected during the trial at different time points. Along with the latter, we also assessed the spot urine samples from 20 high consumers and 20 low consumers of banana that participated in the French cohort SU.VI.MAX2.

The metabolomics profiles are compared using univariate and multivariate statistical methods. The identification of discriminant compounds is performed by tandem mass fragmentation with a high resolution LTQ-Orbitrab Mass spectrometer and by an extensive inquiry of different online databases. We have detected 78 discriminative metabolites for the intake of banana in the BioBanaTom study while in the SU.VI.MAX2 study we detected 3 metabolites that are highly discriminative for high consumers of banana. The discriminant ions associated to habitual banana intake will be identified and compared to the discriminant ions observed in the BioBanaTom study after acute consumption of banana.

Non-targeted 1H NMR metabolite profiling for food biomarker detection

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Specific and sensitive food biomarkers are necessary to support dietary intake assessment and link nutritional habits to potential impact on human health. Non-targeted 1H NMR spectroscopy profiling of human urine may aid detection of such biomarkers in a noninvasive way [1]. We performed a multistep nutritional intervention study to suggest new biomarkers for coffee consumption [2]. 1H NMR metabolite profiling was combined with multivariate data analysis and 2-furoylglycine detected as a novel putative biomarker for coffee consumption. In a second step, 2-furoylglycine was relatively quantified in the urine of coffee drinkers and consequently its origin, metabolism, and excretion kinetics investigated. Potential precursors were identifd as different furan derivatives in coffee products, which are known to get metabolized to 2-furoylglycine. Maximal urinary excretion of 2-furoylglycine occurred 2 h after consumption ($p = 0.0002$) and returned to baseline after 24 h ($p = 0.74$). In order to investigate the specificity of this biomarker, coffee substitutes such as tea and chicory coffee were also investigated but not found to increase 2-furoylglycine excretion. The elucidated biomarker might therefore be a promising acute biomarker for the detection of coffee consumption in human urine.

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A multi-omics approach to characterize the metabolic effects of fermented dairy products on healthy men

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Humans eat fermented foods since more than 7'500 years, first to increase shelf life, then to improve taste. This tradition now translates into dietary patterns in which up to a third of human diets is made of fermented foods, not least because of their impact on health. Dairy products represent a major fraction of fermented foods in western societies. Characterizing the interaction of fermented dairy products with humans requests a holistic approach that takes into account all three elements of the triad composed of food, lactic acid bacteria (LAB), and the human host.

A double-blinded, cross-over clinical intervention was conducted on fourteen healthy men fed a yoghurt containing the widely used probiotic *Lactobacillus rhamnosus* GG and a non-fermented milk. Mass spectrometry-based untargeted metabolomics was conducted on blood serum of the subjects to assess their postprandial response as well as their fasting status after a two-week intervention. The postprandial metabolome of the subjects after a high-fat metabolic challenge, known to induce a transient inflammatory response, was also evaluated at the end of the intervention phase. Finally, the metabolome of the dairy products as well as the metagenome of the yoghurt were measured.

The serum metabolomes of the subjects were able to differentiate milk from yoghurt ingestion. In particular, we have identified products of milk fermentation that were transferred to human blood upon ingestion of the yoghurt. Some of the metabolites differentiating the consumption of the two products under postprandial conditions were also discriminative under fasting conditions after two-week intervention. Differentially produced metabolites indicating a specific impact of yoghurt consumption on the endogenous metabolism of the subjects were also identified.

Taken together, this multi-omics work allows us to link the genome of the fermenting LAB, the metabolome of the test products, as well as the metabolic response of the subjects.

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The Karlsruhe Metabolomics and Nutrition (KarMeN) Study: Protocol and Methods of a Cross-Sectional Study to Characterize the Metabolome of Healthy Men and Women*

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The human metabolome is influenced by various intrinsic and extrinsic factors. A precondition to identify such biomarkers is the comprehensive understanding of the composition and variability of the metabolome of healthy humans. Sample handling aspects have an important impact on the composition of the metabolome; therefore, it is crucial for any metabolomics study to standardize protocols on sample collection, pre-analytical sample handling, storage, and analytics to keep the non-biological variability as low as possible.

The main objective of the KarMeN study is to analyze the human metabolome in blood and urine by targeted and untargeted metabolite profiling (gas chromatography-mass spectrometry [GC-MS], GC×GC-MS, liquid chromatography-mass spectrometry [LC-MS/MS], and1H nuclear magnetic resonance [NMR] spectroscopy) and to determine the impact of sex, age, body composition, diet, and physical activity on metabolite profiles of healthy women and men. Here, we report the outline of the study protocol with special regard to all aspects that should be considered in studies applying metabolomics.

The project was funded in 2011 and enrollment was carried out between March 2012 and July 2013. A total of 301 volunteers were eligible to participate in the study. Metabolite profiling of plasma and urine samples has been completed and data analysis is currently underway. Metabolite analyses related to age, sex, resting energy expenditure, TMAO and diet will be presented at this conference.

We established the KarMeN study applying a broad set of clinical and physiological examinations with a high degree of standardization. Our experimental approach of combining scheduled timing of examinations and sampling with the multiplatform approach (GC×GC-MS, GC-MS, LC-MS/MS, and1H NMR spectroscopy) will enable us to differentiate between current and long-term effects of diet and physical activity on metabolite profiles, while enabling us at the same time to consider confounders such as age and sex in the KarMeN study.

Trial Registration: German Clinical Trials Register DRKS00004890; *https://drks-neu.uniklinik-freiburg.de/drks_web/navigate.do? navigationId=trial.HTML&TRIAL_ID=DRKS00004890* (Archived by WebCite at http://www.webcitation.org/6iyM8dMtx)

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Associations of plasma and urine TMAO with actual diet of healthy individuals in KarMeN

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Trimethylammonium-N-oxide (TMAO) and related methylated amino compounds have been widely discussed in the context of nutrition and health. On the one hand, TMAO has been linked to consumption of specific food such as fish, meat and eggs. On the other hand, TMAO was found to be associated with clinical aspects such as cardiovascular disease risk or renal impairment. Moreover, TMAO is significantly associated with age. In fact, the specificity of TMAO for either clinical or nutritional aspects is still not clear.

Several food sources may influence plasma or urine TMAO concentrations in different ways. Natural TMAO and trimethylamine are present in fish and seafood. Precursor compounds such as choline or carnitine origin mainly from animal food, e.g. meat and eggs, and microbial degradation in the intestine and subsequent oxidation by flavin containing monooxygenase finally leads to formation of TMAO.

However, it is not clear how far plasma and urinary TMAO are determined by current diet or other parameters such as age or lean body mass, and how significant such associations are in a healthy study population without dietary intervention. Therefore, we investigated to what extent concentrations of TMAO and related compounds can be traced back to current food intake, using data from the well-characterized cross-sectional study KarMeN (Karlsruhe Metabolomics and Nutrition).

TMAO was analysed by LC-MS in plasma and by 1H-NMR in urine. Food consumption was assessed by 24h recall. In a first step, parameters were screened by partial Spearman correlation controlling for age, sex, lean body mass index and glomerular filtration rate (GFR) to identify food groups that are correlated with plasma and urinary TMAO. In a second step, associations with identified food groups as independent variables were investigated in multivariate linear regression models for parameters with Spearman's $\rho > |0.15|$. The effect size of different parameters on TMAO variation was estimated based on individual regression coefficients in the multivariate model.

In general, the investigated parameters could explain less than 25% of the TMAO variations. The influence of age was below 9% in plasma and below 5% in urine. Lean body mass and GFR contributed to less than 3%. Several food groups were significantly associated with TMAO, including fish and meat. Interestingly, association with fish was much higher in 24h urine compared to plasma, whereas a significant association with meat was found only in plasma, but not in urine. From our preliminary data, we propose distinct differences between direct TMAO intake from fish and indirect TMAO intake from other animal sources containing the metabolic precursors choline and carnitine.

Resting energy expenditure is not associated with distinct plasma and urine metabolite profiles in healthy humans

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Background
Lean body mass (LBM) substantially impacts human metabolism and is the major determinant of resting energy expenditure (REE). Differences in REE between men and women mainly result from sex related differences in LBM. So far, little is known if REE and LBM are reflected by a distinct human metabolite profile. Therefore, we aimed to identify plasma and urine metabolite patterns that are associated with REE and LBM of healthy men and women.

Methods
We investigated 301 healthy male and female subjects (18 – 80 years) under standardized conditions in the cross-sectional KarMeN study (Karlsruhe Metabolomics and Nutrition). REE was determined by indirect calorimetry and LBM by dual x-ray absorptiometry. Fasted blood and 24h urine samples were analyzed by targeted and untargeted metabolomics methods using GC×GC-MS, GC-MS, LC-MS and NMR. Data were evaluated by predictive modelling of combined data using different machine learning algorithms, namely SVM, glmnet and PLS.

Results
For the participants of the KarMeN study LBM correlates with REE (r = 0.877; linear regression). However, the applied machine learning algorithms did not reveal a metabolite profile predictive for REE or LBM, when analyzing data for men and women, separately. When evaluating data of men and women combined, as it has been described by others, we were able to predict REE and LBM with high accuracy (>90%). This, however, was a clear effect of sex, which is supported by the high degree of overlap in identified important metabolites for LBM, REE and sex, respectively.

Conclusion
We conclude that studies in healthy humans applying metabolomics need to consider sex specific data evaluation.

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