PL 1 – Plenary Lecture 1

Use of microwave-assisted extraction for the production of instant coffee-like extracts from roasted coffee

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Text

The application of Microwave-assisted extraction (MAE) to roasted coffee would be a potential method for obtaining instant coffee-like extracts, providing reduced extraction times and/or improved yields as main advantages. This is possible by the use of closed-vessel systems, allowing the use of extraction temperatures higher than those possible to achieve by atmospheric boiling.

Box-Behnken design (3 factors) was used to study and optimize the coffee MAE process through response surface methodology. After filtration and freeze-drying, the coffee extracts were analysed. It was studied the influence of time of extraction (1-10 min), temperature (120-180 ºC), and mass-to-volume ratio (2-6 g/ 60 mL) on the overall extraction yield, carbohydrate content and sugar composition (GC-FID), caffeine and chlorogenic acids content (HPLC-DAD), and colour of the extracts ($K_{mix,\ 405\ nm}$). A commercial instant coffee product was used as reference concerning instant coffee properties.
MAE methodology allows to quickly obtain up to 47% w/w of coffee compounds present in the coffee powder. Temperature was clearly the main factor for differentiation of extracts content and properties, although time and m/V ratio also exerted effect on the responses. The colour of the extracts became less brown (more yellowish) with increasing extraction temperatures. The increase in extraction yield verified at higher temperatures was associated to an increase in arabinogalactan extraction, structures distinctive of instant coffee samples.

The use of pressurized conditions allows an increase of the amount of compounds extracted when compared with extractions performed at atmospheric conditions, approaching instant coffee composition. The models developed allowed to modulate the composition of the extracts, while the comparison to an commercial instant coffee sample enables to define the conditions to quickly obtain coffee extracts resembling instant coffee chemical properties (sugar content and composition, caffeine and 5-CQA content), which can be used in food/brew formulations.

Acknowledgements
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PL 2 – Plenary Lecture 2

Strategy and techniques of analysis of the volatile fraction as a tool for food control and characterization

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Text

The volatile fraction of a food and in particular its aroma is an important “signature” not only playing a fundamental role in food choice but also in its characterization, quality assessment and genuineness. Sensory analysis is still the reference criterion to define food quality; nevertheless, it still is a critical step because of i) the rather limited number of expert tasters, ii) time and number of experiments required, and iii) the limited number of daily-processable samples.

The introduction of Metabolomics in 1998 [1] and Molecular Sensory Science or Sensomics, in 2011 [2] and of the derived strategies and methods have also substantially influenced the approach to flavor analysis. In analytical terms, these disciplines imply a comprehensive and quantitative analysis of the largest possible array of low molecular weight components (<1,000 Da) in the investigated samples [3]. The application of the metabolomics strategies in the food field has involved the development of dedicated analytical approaches, the best known being Fingerprinting and Profiling, and the untargeted and targeted methods derived from them. The aim is therefore to develop inclusive instrumentation where the “sample preparation-analysis-data elaboration” sequence are on-line merged into a single step, the so-called “Total Analysis Systems” (TAS) [4]. The food volatile fraction analysis with TAS requires an automatic single-step sample preparation technique on-line combined with GC-MS system to obtain diagnostic patterns suitable for on-line data processing.

This contribution discusses the strategies to study the food volatile fraction in view of routine quality control and examples concerning cocoa, coffee and tea taken by the authors’ everyday experience.

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PL 3 – Plenary Lecture 3

A pragmatic approach to support evidence based adaptation to climate change in the cocoa industry

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Text

The global chocolate industry relies on the productive output of smallholder farming families that are already affected by climate change. A climate resilient cocoa sector will require that the millions of smallholders adapt their practices to secure a sustainable livelihood.
Climate Smart Cocoa requires by definition a more nuanced approach to determining what constitutes “good” agriculture practice by accounting for site- and time-specific variability such as climate, vulnerability and capacities of producers to identify and adopt climate smart responses when needed. Traditional guidance such as national sustainability curriculums and GAP manuals are insufficiently tailored to local variability, particularly under conditions of future climate uncertainty and volatility. We propose a framework to prioritize CSC options that is rigorous yet flexible and sufficiently lean to be applied by value chain actors in a relatively short amount of time with limited resources and resulting in an evidence-based action plan. This framework has been developed and tested over five years with Climate Smart Value Chain projects in Latin America, Africa and Asia. It has been used predominantly in value chains dominated by smallholder producers, though all of the approaches may also be applied to larger landholders and also to annual crops. The framework consists of a spatial risk scan, prioritization of responses, identification of barriers to response adoption, and business plan development for financially viable implementation.

PL 4 – Plenary Lecture 4

Cocoa Bean Proteomics

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Amongst other compounds, cocoa flavour is dependent on peptides that are formed during fermentation of cocoa beans from proteins such as albumin and vicilin, and potentially others. Enzymes crucial in specific metabolic pathways can add to the plethora of compounds important for cocoa flavour generation. Thus, studying the proteome of cocoa genotypes with different flavour profiles should potentially reveal some of the proteinaceous protagonists in cocoa flavour generation. Moving on from the traditional gel electrophoretic analysis of cocoa proteins, we used bottom-up label-free UHPLC-ESI MS/MS for comparative (quantitative) analysis of the proteomes of four different genotypes. From this analysis the abundance of ~60 proteins was found to be significantly different with a fold change of ≥2 among the four cocoa genotypes analysed. PCA analysis allowed clear separation of the genotypes based on their proteomic profiles. Genotype-specific abundances were recorded for proteases involved in the degradation of storage proteins and release of flavour precursors. Different genotype-specific levels of other enzymes, which generate volatile compounds that could potentially lead to flavour-inducing compounds, were also detected. Overall, this study shows that UHPLC-MS/MS data can differentiate cocoa bean varieties, and thus be linked to differences in their flavour profile.

PL 5 – Plenary Lecture 5

EGCG Alters Transcriptional Rhythm and Circadian Gens Expression in Vascular Smooth Muscle Cells Induced by AngⅡ

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Text

Circadian clock system plays a critical role in human health. Rhythmic blood pressure during day and night is one of important biomarkers for healthy human. Vascular smooth muscle manages the contraction and relaxation of blood vessels, and thus regulates blood pressure. Therefore, investigating the biological rhythm of vascular smooth muscle cells (VSMCs) is important for homeostasis of blood pressure and health.

First, circadian gene expression model of VSMCs induced by AngII was successfully set up. Transcriptome sequencing, qPCR, western blot, and biorhythm fluorescence detection were employed to investigate the EGCG, a main catechins from tea, modulating circadian genes expression in VSMCs induced by AngII. Based on the transcriptome sequencing data, analysis tof KEGG pathway of DEGs revealed that EGCG down-regulated an array of differentially genes expression in circadian rhythm signal pathways, whereas *Dec1* and *Dec2* were up-regulated. The qPCR results demonstrated that EGCG significantly reduced the rhythmic amplitude of the *Bmal1*, *Per2* and *Rev-erbα*. Meanwhile, EGCG prolonged the periods and delayed the phases of the *Bmal1*, *Per2* and *Rev-erbα*. In addion, western blotting results showed that EGCG significantly prolonged the cycle, down-regulated the amplitudes and delayed the phases.of BMAL1 and REV-ERBα protein rhythmic expression. The results from fluorescence detection showed that EGCG reduced the amplitude and also delayed the rhythm phase of the PER2 expression in the aorta and mesenteric artery isolated from the PER2::LUC mice.

In conclusion, our results are first time to find that EGCG down regulated an array of circadian gene expression and decreased the rhythmic amplitude induced by AngII in VSMCs. The potential mechanisms of EGCG-regulated rhythm of circadian genes is to stimulate the over expression of *Dec1* and *Dec2*, to down-regulate of the transcriptional activation of the BMAL1-CLOCK heterodimer, and consequently down regulated other circadian genes expression. The pathophysiological significance of EGCG down regulated circadian gene expression and decreased the rhythmic amplitude induced by AngII in VSMCs is under investigation.

PL 6 – Plenary Lecture 6

Investigating Cocoa Bean fermentation by Mass spectrometry Imaging

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PL 7 – Plenary Lecture 7

The biochemistry of cocoa flavour: A holistic analysis of its development along the processing chain

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PL 8 – Plenary Lecture 8

New healthy food design of coffee and cocoa

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KL 1 – Keynote Lecture 1

Coffee and health in the era of personalized nutrition: evidence and challenges

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Text

Several epidemiological studies and meta-analyses indicated that coffee consumption is associated with a reduced risk of all-cause mortality and many diseases such as type-2 diabetes, non-alcoholic fatty liver disease, hepatocellular carcinoma, and chronic liver disease. On the other hand, the association between coffee consumption and colorectal cancer risk is still controversial whereas positive associations between coffee consumption and risk of pancreatic cancer, fracture in women and preterm delivery during pregnancy, were found.

In most cases across epidemiological studies, the positive effects of coffee on health outcomes were confirmed after adjusting data for genders and smoking habit whereas other behavioural factors that may have a role in disease development such as physical activity level or dietary habits as well as genetic make-up of individuals were completely ignored or not sufficiently considered. All these factors together with the type of coffee consumed (caffeinated or decaffeinated coffee, from Arabica, Robusta or blend of them, filtered or espresso) might play a role in short-term and long-term metabolic effect of coffee. Indeed, different coffee types vary in the composition of bioactive compounds including caffeine, phenolic compounds, lignans, trigonelline, N-methylpyridinium, minerals, vitamins, proteins, lipids and melanoidins. These compounds, at different extent, may modulate health through many physiological mechanisms taking place in the alimentary canal and several organs. Along with antioxidant protection exerted by coffee consumption, mounting evidence in animal models indicates that nutrient metabolism and sensing in the gastro-intestinal tract, gut microbiota composition and activity, microbial metabolite profiles and permeability may also play a role in the effects of the beverage on health. Long term randomized controlled intervention studies involving populations well-characterized for genetic traits, lifestyle and metabotype, and consuming coffee with a standard composition in association to specific dietary regimes are warranted to clarify the causal role of coffee consumption in disease prevention and the underlying mechanisms.

KL 2 – Keynote Lecture 2

Is it feasible the use of coffee cascara as novel sustainable and healthy ingredient/s?

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KL 3 – Keynote Lecture 3

Tea chemistry – what do and what don't we know?

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Text

This presentation will review the current status of tea chemistry. The focus will be on the following aspects:

- Taste compounds of the – depending on the tea in question
- Authentication of tea and tea products

Our knowledge of tea constituents has changed rapidly within last decades. One reason for that is the availability of better separation systems (e.g. UPLC) and more sensitive and sophisticated detectors, such as different types of mass spectrometers (ion trap, qTOF, FTICR). This lecture reviews our knowledge of tea constituents with respect to the taste of tea beverages. Relevant compounds mentioned are flavonol glycosides, theaflavins, thearubigin compounds in different types of tea, the catechins and the amino acids, foremost theanine and glutamic acids. Important are e.g. the bitter/astringent and the umami taste. Other compounds possibly relevant in tea chemistry and taste, are the phenolic acids including derivatives (gallic acid, theogallin and chlorogenic acids). For a number of compounds quantitative data are scarce or missing. Gaps in our current knowledge will be discussed along with possible concepts of future work. A major problem in black tea chemistry is the quantification of thearubigins as the separation with conventional chromatographic methods is not possible. The authentication of Matcha will be covered as regards the changes yielded by shading. Another aspect briefly discussed in the presentation will be the current status of health claims of tea constituents along with safety aspects from the literature.

KL 4 – Keynote Lecture 4

New LC-MS/MS-based molecular networking strategy for the identification of phenolics urinary metabolites in cocotea human trail

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Text

Dietary phenolic compounds are often transformed by gut microbiota prior to absorption. This transformation may modify their structures, producing novel gut floral metabolites associated with numerous health benefits[1]. Traditional approaches for assessing dietary exposure of cocotea (cocoa, coffee and tea) products provided very little information about the modification and fate of dietary phenolics after ingestion, mainly due to the limitation of complex sample nature and their data analyses[2].
In order to overcome such limitations, a cocotea based human trials were conducted where we used molecular networking approach for the identification of various structural analogues of dietary phenolics. Molecular networking is a newly introduced tandem mass spectrometry (MS/MS) based approach in the field of metabolomics, mainly used for interpreting the metabolomic 'dark matter' of the natural products and can be applicable to a variety of human samples[3].
To demonstrate the utility of this approach, we have applied it to a diverse collection of humans (*n*=34) urine samples, who consumed large amount of cocoa, coffee and tea products over 48 h period. This approach illustrated the power of the new strategy, allowing the identification of new analogues of bioactive metabolites of cocotea products after consumption. The new (unknown) gut microbial metabolites were identified based on tandem MS spectral similarity if compared to the literature known compounds.
In addition to this tool, multivariate statistical analyses using LC-MS and NMR data were also implemented for the investigation of the characteristic metabolic variability between control and cocotea dosed volunteers' samples.

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