SESSION III

THURSDAY, MAY 25th, 2017

CHAIRPERSONS:

Irena Vovk and Danilo Corradini

10.

Chromatographic and hyphenated techniques in the development of functional food enriched with xanthophylls

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Current trend in food industry is to develop new functional foods and functional food ingredients. This can be achieved by addition of health promoting ingredients during food processing or by enrichment during farming (breeding). Chromatographic and hyphenated techniques are indispensable in the process of development of functional food ingredients and functional food products. The most critical steps are selection of raw materials rich in bioactive ingredients, optimisation of extraction procedures, quality control of the final products, etc.

We will present the role chromatographic and hyphenated techniques in development of functional foods enriched with xanthophylls zeaxanthin and β-cryptoxanthin (provitamin A). Zeaxanthin and its isomer lutein are both equally important and essential micronutrients in prevention of age related diseases such as macular degeneration. Due to the lack of dietary sources (including rare food supplements) of zeaxanthin, and due to its low bioavailability from available sources, we designed a new functional food - zeaxanthin and β-cryptoxanthin enriched egg by feeding 30 laying hens with zeaxanthin and β-cryptoxanthin enriched feed. For this purpose we developed and validated HPLC methods for the determination of carotenoids in plant materials, in feed and in egg yolks (8 permitted in animal feed in the EU). For identification of other bioactive ingredients (e.g. flavonoids) in the used plant materials we developed several methods based on HPTLC-densitometry, HPTLC–MS/MS, HPLC–UV and HPLC–UV–MS^n.

11.

Capillary electrophoresis and HPLC of biomolecules in agro-food matrices

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The plethora of natural organic compounds produced in plants by secondary metabolism comprise food ingredients conferring specific sensorial characteristics and/or beneficial effects on human health. The identification and quantification of these target compounds in plants and agro-food matrices is a challenging task, continuously requesting the development of more robust, efficient and sensitive instrumental analytical techniques. This communication discusses fundamental and practical aspects of both reversed phase high performance liquid chromatography (RP-HPLC) and capillary zone electrophoresis (CZE) employed for the analysis of plant secondary metabolites occurring in food matrices. The two analytical separation techniques might display complementary capability in separating secondary metabolites, as it is discussed for the analysis of phenolic compounds in plant extracts. The different selectivity exhibited by RP-HPLC and CZE in separating phenolic compounds has been ascribed to the concomitant presence of hydrophilic, hydrophobic and ionogenic groups displayed by most of these compounds, which is expected to influence to different extents the separation mechanisms operating in CZE and in RP-HPLC of molecules bearing multifunctional moieties. The presentation evaluates the influence of various operational parameters and experimental conditions employed in CZE and in RP-HPLC on the separation performance of phenolic compounds, which are widely distributed in the plant kingdom, form an integral part of human diet, and have a remarkable position as active components in functional foods and food supplements. Also discussed is the practical application of either CZE or RP-HPLC to the study secondary metabolites in transgenic food of plant origin as well as the determination of phenolic compounds in agro-food matrices during the transformation of raw ingredients into food and in the production of foods that have a potentially positive effect on health and disease prevention beyond basic nutrition.

12.

New analytical technologies for natural product research

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The progress in natural product research has been very fruitful for many decades for various areas of modern life, including medicine, cosmetics and nutrition. However the development of reliable analytical methods for the monitoring of the active constituents, as well as for the detection of low concentrations of compounds, which could be important for e.g. the identification of allergenic effects, are necessary.

Advances in analytical technologies play an important role. Especially sample preparation including enrichment and separation, have greatly facilitated the state-of-art research. Although numerous methods have been developed, still many challenges remain because of the complexity of the sample matrix and the diversity of the analysed products. In general the development of complete analytical methods for a natural product includes sampling, sample preparation, separation, detection and data evaluation.

We are focusing on the development of novel analytical methods for phytopharmacy, phytocosmetics and phytonutrition. New isolation, enrichment and purification tools based on solid phase extraction technologies were developed in order to reduce the complexity of the plant sample, remove interfering components and detect traces of analytes. The developped methods improve the speed and accuracy, allowing high selectivity, high throughput, robustness and automation. Great attention is paid to the development of macroporous, monolithic separation columns with tailored properties such as surface area, surface chemistry and porosity, to achieve high efficiency and selectivity.

Furthermore, online sample preparation and LC-MS methods play an important role in the analysis of natural products. They permit the identification and quantification of the target analytes. Apart of MS, other spectroscopic technologies such as NIR are studied.

On the other hand, the Austrian Drug Screening Institute (ADSI) as a translational research institute, is a research enterprise of the University of Innsbruck, which offers screening technologies for phyto-relevant companies and academic research institutions.

13.

Identification of bioactive peptides in food matrices by multidimensional liquid chromatography and bioinformatics

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Bioactive peptides are amino acids chain generated from the hydrolysis of proteins that exert in the body a biological activity, while being completely inert in the parent protein. Bioactive peptides can be generated mainly in three different ways: directly in the food during processing, *in vivo* during digestion and *in vitro* selected a specific enzyme. In the literature, more than 40 activities have been studied [1], such as antioxidative, antimicrobial, anticancer.

Bioactive peptides can have a wide range of sizes and physicochemical properties giving a very complex matrix. In fact, while trypsin generates peptides that share similar properties (e.g. charge state or length) other enzymes, even when used alone, have semi-specific cleavage site, like pepsin or chymotrypsin or completely unspecific (e.g. Alcalase, Protamex or Flavorzyme).

Nowadays, multidimensional LC is the most effective analytical tecnique in simplifing the complexity of the peptide matrices. The sequential use of different HPLC columns (mainly with orthogonal mechanism [2]) led to obtain higher resolution and higher peak capacity that is useful for this kind of analysis.

In a typical peptidomic protocol, each collected fraction, obtained from the chromatographic dimension, is usually assayed and the most asctive ones are further analysed by nanoRPLC coupled with an Orbitrap mass spectrometry for peptide sequencing [3]. After database search, the identified peptides are further mined by *in silico* analysis using bioinformatic softwares, which provided a bioactivity score later used to select candidates for chemical synthesis. Otherwise, scaling up the chromatographic system, using preparative columns, is possible to isolate and purify the most active peptides. This could simplify the entire procedure making the protocol less time consuming and expensive since no bioinformatic and chemical synthesis would be required.

References:

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14.

How to determine aromatic hydrocarbons in bees and bee products using chromatographic techniques?

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Among the many factors affecting bee survival and the quality of bee products, one of the most important are aromatic hydrocarbons (benzene, toluene, ethylbenzene, xylenes) and polycyclic aromatic hydrocarbons (for example naphthalene or benzo(a)pyrene). Aromatic hydrocarbons in environment originate from industry, transport and other combustion processes.

Determination of aromatic hydrocarbons in bees and bee products using chromatographic techniques involves the preparation of a sample (isolation of analytes from the matrix) and chromatographic analysis.

The studies covered by this presentation are subject to extraction of monocyclic and polycyclic hydrocarbons. Extraction of monocyclic compounds was carried out using headspace technique and solid-liquid extraction by shaking with organic solvents (hexane and acetone). Extraction of polycyclic hydrocarbons was carried out in a traditional solid-liquid system by shaking and in the Soxhlet apparatus. For bee samples artificially contaminated with hydrocarbons' standards, extraction efficiencies were determined. The analyses were performed using a gas chromatography technique coupled to a mass spectrometer.

In addition, the degree of accumulation of polycyclic aromatic hydrocarbons in bee products exposed to traffic pollutants was checked. The content of polycyclic aromatic hydrocarbons (naphthalene, acenaphthylene, fluorene, phenanthrene and fluoranthene) in honey and propolis samples exposed for 30 days near Polish road no. 7 (near Myślenice) was much higher than the contents of these compounds in samples kept at the same time far away from communication lines (in a forested area situated about 4 km from road no. 7). A particularly large difference (44-fold) in the total hydrocarbon content of the tested hydrocarbons was observed for the multiflorous honey.

The presented results compare the different methods of preparation of and bee and bee products samples for analysis of monocyclic and polycyclic aromatic hydrocarbons using gas chromatography technique.

15.

Being uncertain in thin layer chromatography – some thoughts about latent details in the retention estimation

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During the investigation of thin-layer chromatography retention behavior, the most frequently used approach is to note R_F values up to two decimal places and R_M values up to three. Every parameter derived from the retention (for example ΔR_M or lipophilicity) is often presented in similar accuracy to give a reference value. Most of TLC chromatographers are aware that the real uncertainty of these results is higher, but the problem is complex and studying the mathematical dependences in error propagation often leads to surprising results. The presentation would go as deep as possible into basic details of TLC retention uncertainty, giving answers to several legitimate, but difficult (and uncovered directly in the literature) questions, such as:

1. What is the real uncertainty of R_M value?
2. Which R_F range correspond to low absolute, and which to low relative uncertainty?
3. When computing ΔR_M, what is the role of R_M covariance in the error value?
4. What is the practical importance of pure error and lack-of-fit error in lipophilicity estimation?
5. Can we estimate the intercept of extrapolated retention uncertainty without taking into account the slope uncertainty?
6. What is the dependence between uncertainty and the regression type?
7. How many accurate significant digits can we obtain during typical retention experiments?

Depending on the problem complexity, various solutions can be considered. In most complex problems, the best solution is the Monte Carlo simulation or bootstrapping the results.