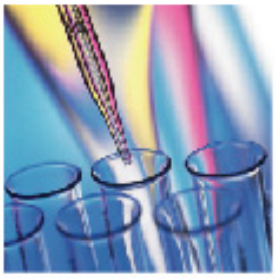
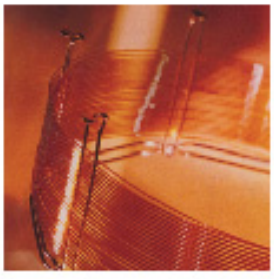
BOOK OF ABSTRACTS

3rd International Symposium on

RECENT ADVANCES IN FOOD ANALYSIS

November 7–9, 2007
Prague, Czech Republic



INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

International Association of Environmental Analytical Chemistry

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

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APPENDIX

ORAL SESSION

(L1 – L41)

L-1
OPENING LECTURE
FOOD QUALITY / SAFETY CONTROL: FP7 RESEARCH CHALLENGES

Antonio di Giulio

European Commission – DG Research, unit Food, Health and Well-beiing, Brussels, Belgium

L-2
AN OVERVIEW OF ANALYTICAL TECHNIQUES FOR DETECTION OF NANOPARTICLES IN NATURAL SYSTEMS

Karen Tiede[1*], Alistair Boxall[2], Steven Tear[3], John Lewis[4], Helen David[5]

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Nanotechnology is a fast growing market and engineered nanoparticles (ENPs) are finding widespread applications. These applications include a variety of consumer products from cosmetics, medical application to packaging materials, processing technologies and novel or functional foods. ENPs are also applied for groundwater remediation, in construction, engineering, and the electronic and chemical industry. During their manufacture and use, human exposure and the release of ENPs to the environment is inevitable. The proliferation of nanotechnology has therefore prompted concerns over their risks to organisms in the environment and on the potential direct and indirect exposure of humans. However, research to address these concerns is still in the fledgling stages and the development and application of adequate analytical techniques for the analysis of ENPs in natural samples is challenging. This presentation will give an overview of available analytical methods for the measurement, characterization, detection and chemical analysis of engineered nanoparticles in natural environments and link these techniques to possible biological and food related applications. The presentation will cover separation technologies (e.g. SEC, FFF), chemical analysis methods (including ICP-MS) and microscopy approaches (AFM, SEM, WetSEM and TEM). The application of the approaches will be illustrated using real world examples. The advantages and limitations of different approaches will be discussed and priorities for future research will be highlighted.

L-3

MICROFABRICATED FLUIDIC DEVICES (MICEOCHIPS) FOR RAPID ACCESS TO CHEMICAL AND BIOCHEMICAL INFORMATION

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There has been rapidly growing interest in microfabricated fluidic devices (microchips) over the past decade for use in chemical and biochemical experimentation. The diversity of chemical and biochemical measurement techniques that have been implemented on microchips includes various electrophoretic and chromatographic separations, chemical and enzymatic reactions, noncovalent recognition interactions, sample concentration enhancement, and cellular manipulations. In addition, the types of samples addressed by microchips has been broad in scope, e.g., small ions and molecules, single and double stranded DNA, amino acids, peptides, and proteins. These devices have low cost and small footprints while consuming miniscule quantities of reagents and can rapidly produce precise results. All of these features suggest the possibility to perform chemical and biochemical experimentation on a massive scale at low cost on a bench top, a goal being pursued by many laboratories around the world. This presentation will be an overview of our activities in this area.

L-4
ELECTROCHEMICAL DETECTION OF BIOLOGICAL REACTIONS USING A NOVEL NANO-BIO-CHIP ARRAY

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We developed an innovative electrochemical 'lab on a chip' system that contains an array of nano volume electrochemical cells on a silicon chip. Each of the electrochemical cells can be monitored simultaneously and independently, and each cell contains three embedded electrodes, which enable performance of all types of electrochemical measurements. The integration of living organisms on an electrochemical array chip that can emulates reactions of living organisms and sense essential biological functions have never been demonstrated before. In order to show the wide range of applications that can be beneficial from this device, biological components including chemicals, enzymes, bacteria and bio-films were integrated within the nano-chambers for various applications. During the measurement period the bacteria remained active, enabling cellular gene expression and enzymatic activity to be monitored on line.

The miniaturized device was designed in two parts to enable multiple measurements: a disposable silicon chip containing an array of nano-volume electrochemical cells that are housing the biological material, and a reusable unit that includes a multiplexer and a potentiostat connects to a pocket PC for sensing and data analysis 1.

This electrochemical 'lab on a chip' was evaluated by measuring various biological reactions including the microbial current response to toxic chemicals. These bacteria were genetically engineered to respond to toxic chemicals by activating cascade of mechanisms, which leads to the generation of electrical current. A measurable current signal, well above the noise level, was produced within 5 minutes of exposure to heavy metals, or organic toxicants, such as phenol or hydrazine.

In Summary, We demonstrate sensitive measurements on extremely small samples and no special cell treatment is required prior to the insertion into the chip.

[1] R. Popovtzer, et al; Novel Integrated Electrochemical Nano-Bio-Chip for Toxicity Detection in Water Nano Letters 2005, 5, 1023 - 1027

L-5
MOLECULAR ANALYSIS OF NATIVE TISSUE, FOOD PRODUCTS AND WHOLE OILS BY DIRECT INFRARED LASER MASS SPECTROMETRY

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We have employed infrared laser desorption ionization orthogonal time-of-flight mass spectrometry (IR-LDI-o-TOF-MS) to generate molecular ion profiles directly from native tissue, food products and from whole oils (Dreisewerd et al., Anal. Chem. 2007, 79, 4514). The method requires little sample preparation besides for an eventual dissection of the areas of interest and drying of particularly water-rich samples. In particular, an extraction of molecules is not necessary. The lateral resolution of the analysis is on the order of the laser focal diameter, and in the third dimension defined by the depth of material ejection of a few to ten micrometer per laser pulse. Various types of small molecules are readily detected from minute volumes of sample. Among these are carbohydrates, phospholipids, triglycerides, and flavonoids. Examples, highlighting the features of the method are presented. These include the direct analysis of fruits, tea leaves, seeds, oils, raw meat, and various examples of food products like sausages and cheese. The distribution of di- and triacylglycerols obtained from single seeds (e.g. sunflower, sesame) as well as fat-rich fruits (e.g. olive, coconut) is compared to that obtained from commercial oils. Oxidation of double bonds in the fatty acid residues was monitored in a temperature and time-controlled study.

[1] Dreisewerd et al., Anal. Chem. 2007, 79, 4514

L-6
DESI AND HYBRID MASS SPECTROMETRY

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Ambient mass spectrometry ionization techniques such as the recently introduced desorption electrospray ionization (DESI) provide a number of significant advantages over existing methods. These include facile sample preparation, reduced suppression effects, high throughput capability, excellent sensitivity and wide applicability. Femtomole detection limits have been achieved with a dynamic range of over five orders of magnitude and quantitation accuracy similar to other mass spectrometry experiments. The technique has found application in clinical diagnostics, food analysis, forensics, environmental studies and many other fields. Monitoring for explosives, pathogens and chemical agents together with screening for drugs of abuse and poisons has been carried out. The ability of the approach to monitor selected compounds in complex matrices is a significant advantage as is the capability to perform imaging studies. Other related experiments include desorption atmospheric pressure chemical ionization (DAPCI) which employs gaseous ions of volatile compounds as the primary probe beam. The experiment is carried out in a similar way to DESI except that a corona discharge source is employed. This approach has been found to give improved sensitivity for low polarity molecules of interest. Both DESI and DAPCI generate ions by bombardment with charged particles, other approaches generate ions from lasers (MALDI) and atoms (direct analysis in real time [DART]). An exciting complimentary approach, recently introduced, is neutral desorption sampling coupled with extractive electrospray ionization (EESI). Here the sample is interrogated by a stream of gas with the neutral molecules released ionized in an EESI source. Matrix effects are minimized and low memory effects are observed.

Since these techniques operate directly on samples complex spectral information can be produced. It is necessary therefore to provide additional mass spectrometry information to fully characterise the system. This can include accurate mass data and tandem spectra. A recently developed approach based on ion mobility mass spectrometry has recently been commercially introduced (Synapt, Waters Inc.). In this experiment ions are driven through an inert gas by means of a series of pulsed voltages. This travelling wave device offers the ability to separate mixtures based on mass, charge and rotationally averaged cross section. This separation can be combined with MS and MS/MS characterisation.

Examples of various ambient ionisation approaches will be presented together with recent results obtained from the ion mobility experiments both stand alone and combined with the ambient ionisation experiments.

L-7
NATURAL TOXINS: ANALYTICAL DEVELOPMENTS IN AN INTERNATIONAL CONTEXT

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Natural toxins in food and feed are considered important food safety issues, in particular mycotoxins, phycotoxins and plant toxins. Most developments have occurred in the last decades in the area of mycotoxins. Formal health risk assessments have been carried out and regulations for mycotoxins in food and feed have been established in approximately 100 countries. Many interlaboratory-validated analytical methods have become available through AOAC International and the European Standardization Committee. Current quantitative methods of analysis for mycotoxins often make use of immuno affinity cleanup with LC separation techniques in combination with various types of detectors, including MS. For screening purposes enzyme immunoassays are used, but other biotech-based techniques, such as sensors and tranomics are among the promising newcomers. In particular in the EU, scientific interest in mycotoxins has undergone a development from autonomous national activity towards more EU-driven activity with a structural and network character. Various organizations and programs have contributed to this development. They include the European Food Safety Authority, Scientific Cooperation on Questions relating to Food, the Rapid Alert System for Food and Feed, the creation of the EU Community Reference Laboratory for Mycotoxins and a mandate of the EC to the European Standardization Committee in methods for analysis for mycotoxins in food. Large pan-European research and networking projects with an analytical nature as “BioCop” and “MoniQA” are also important. For the phycotoxins the situation is less advanced, yet interesting developments take place. Risk assessments are currently carried out by the European Food Safety Authority for a whole series of marine phycotoxins. Whereas validated methods of analysis are scarce and rodent tests still play a key role in official methodology, joint efforts are directed towards improving this situation. Especially in the EU there is a high pressure now to replace the animal assays by chemical alternatives, for ethical reasons and because lower limits of detection are required. Analytical methods based on LC/MS, SPR sensors and functional assays are developed, e.g. by the network of National Reference Laboratories for Marine Biotoxins and in EC-sponsored projects as “BIOTOX”, and “BioCop”. The plant toxins form a category of natural toxins, least developed with respect to risk assessments, regulations and validated methods of analysis. In the EU this situation is gradually changing, as witnessed by scientific opinions of the European Food Safety Authority and the recent funding of EU large collaborative project activities on the development of analytical methodology for certain plant alkaloids of health concern.

L-8
CHALLENGES IN ANALYSIS OF MASKED MYCOTOXINS IN CEREAL-BASED PRODUCTS

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Deoxynivalenol, DON, and other toxic secondary metabolites produced by Fusarium fungi are found worldwide as contaminants of cereal grains, including wheat, barley, maize, oats, rye and rice. Alike other xenobiotics, these mycotoxins are partly metabolized by living plants including food crops. On this account, when consuming cereals or their processed products like bread or beer, humans may be exposed to not only to the native (free) mycotoxins, but also to their altered forms. Similarly, assessment of mycotoxicoses in farm animals should take into consideration possible release of additional DON from masked forms in gastro intestinal tract. In any case, little is known about the occurrence and bioavailability and further metabolism of some of these bound compounds, which additionally also escape usual analytical detection techniques used for routine control. This may lead to an underestimation of the total consumers´ exposure.

In our study, employing LC/MS-MS based method, occurrence of high levels of DON-3-glucoside (the main known DON metabolite), comparable with those determined for parent mycotoxin, were documented in a large number of processed cereal-based samples, including malts and beers. Using LC/TOF-MS for examination of the latter group of matrices, also DON di- and triglycosides have been tentatively identified. The presence of DON conjugates may explain higher DON findings obtained by various commercial enzyme linked immunosorbent assays (ELISA) compared to results of routine instrumental analyses. Preliminary experiments showed high cross-reactivity to DON-3-glucoside; also other DON derivatives (besides of acetylated DONs for which cross reactivity is declared by some producers) occurring in foods may probably cross react. In the final part of experiments, the potential of a novel MS ion source enabling Direct Analysis in Real Time (DART) was tested. This rapid, “open access” technique employing high resolution TOF analyzer, enables not only detection of target compounds in complex mixtures but also identification of unknowns by their elemental compositions.

[1] Scott, P.M. Mycotoxins transmitted into beer from contaminated grains during brewing. J. AOAC Int. 1996, 79, 875-882.
[2] European Commission (EC). Commission Regulation EC No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Offic. J. Eur. Union 2006, L 364, 5-24.
[3] Sewald, N.; Lepschy Von Gleissenthall, J.; Schuster, M.; Müller, G.; Aplin, R.T. Structure elucidation of a plant metabolite of 4-desoxynivalenol. Tetrahedron Asymmetry 1992, 3, 953-960.
[4] Savard, M.E. Deoxynivalenol fatty acid and glucoside conjugates. J. Agric. Food Chem. 1991, 39, 570-574.
[5] Berthiller, F.; Dall'Asta, C.; Schuhmacher, R.; Lemmens, M.; Adam, G.; Krska, R. Masked mycotoxins: determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography-tandem mass spectrometry. J. Agric. Food Chem. 2005, 53, 3421-3425.
[6] Berthiller, F.; Schuhmacher, R.; Buttinger, G.; Freudenschuss, M.; Adam, G.; Krska, R. Synthesis of deoxynivalenol-glucosides and their characterization using a QTrap LC-MS/MS. Mycotoxin Res. 2003, 19, 47-50.
[7] Dall'Asta, C.; Berthiller, F.; Schuhmacher, R.; Adam, G.; Lemmens, M.; Krska, R. DON-glycosides: Characterization of synthesis products and screening for their occurrence in DON-treated wheat samples. Mycotoxin Res. 2005, 21, 123-127.
[8] Berthiller, F.; Krska, R.; Dall'Asta, C.; Lemmens, M.; Adam, G.; Schuhmacher, R. Bestimmung von DON-3-Glukosid in künstlich und natürlich kontaminiertem Weizen mittels LC-MS/MS. (Determination of DON-3-glucoside in artificially and naturally contaminated wheat with LC-MS/MS). Mycotoxin Res. 2005, 21, 205-208.

L-9
ELECTROCHEMICAL IMMUNOSENSORS FOR T-2, HT-2 AND DON TRICHOTHECENES

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Trichothecenes are mycotoxins mainly produced by *Fusarium* fungi and associated with outbreaks of disease both in humans and in farm animals. They are classified as Group A (T-2 and HT-2) and Group B (DON and NIV) compounds, depending on the presence of the side chain on the C7 atom. The European Commission set the maximum level for DON between 500 and 1750 ppb, with a more restrictive value for baby food (200 pbb). The maximum food levels of type A trichothecenes are yet to be established, but the JECFA has fixed a provisional maximum tolerable daily intake of 60 ng Kg^{-1} body weight for T-2 and HT-2 toxins, alone or in combination.

This work presents the development of immunoelectrochemical methods for T-2, HT-2 and DON, starting from an indirect competitive spectrophotometric ELISA test. Different monoclonal antibodies against T-2 or HT-2 have been tested in order to select those with the greatest cross-reactivity for both toxins so as to obtain an accurate quantification of the total amount of T-2 and HT-2.

Then, a 96-well screen-printed microplate was employed both as electrochemical transducer and immobilization support for HT-2-KLH conjugate. After competition for monoclonal antibodies between free analytes (T-2 and HT-2) and coated molecules, the activity of HRP labelled antiglobulins was measured using TMB as enzyme substrate and Intermittent Pulse Amperometry as electrochemical technique. LOD and EC_{50} values of the assay were 0.2 ng/ml and 0.7 ng/ml respectively.

More recently, two immunomagnetic electrochemical assays for type A trichothecenes and DON have been developed. Tosyl-activated immunomagnetic beads served as support for the immunological chain. For DON, a direct competition, using DON-HSA, biotinylated Fab anti-DON fragments and an avidin-biotin-HRP complex, was carried out. After completion of all immunochemical steps, beads were localized onto the surface of the screen-printed electrodes, with the aid of a magnet, prior to the measurement of the electroactive product by chronoamperometry or differential pulse voltammetry. The detection limit and the EC_{50} values obtained were respectively: 63 and 385 ng/ml for DON, and 4.5 ng/ml for type A trichothecenes.

Preliminary results about sample extraction will be also presented.

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L-10

RAPID SIMULTANEOUS DETERMINATION OF 6 MAJOR ERGOT ALKALOIDS AND THEIR EPIMERS IN CEREALS AND FOODSTUFFS BY LC/MS/MS

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Ergot alkaloids are mycotoxins produced by fungi of all species of the *Claviceps* genus, most notably by *C. purpurea,* which parasitize the seed heads of certain living cereals and grasses at the time of flowering. Fungal infections are most prevalent in rye and triticale that have open florets but wheat and other small grains are also potential hosts of these fungal species, which invade the sclerotia. Investigations in Germany have indicated an increase in the occurrence of *Claviceps purpurea* infections in the last 10 years. The main ergot alkaloids produced by *Claviceps* species are ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine and the group of agroclavines, the latter being less toxic. A maximum limit of 1000 mg ergot bodies per kg (0.10% (w/w)) has been introduced for feed products containing unground cereals (EU Parliament, 2002). Guideline limits of 400-500 µg/kg and 100 µg/kg in Germany and Switzerland repectively, have recently been discussed for ergot alkaloids in cereals for human consumption.

Ergot alkaloids are frequently analyzed by HPLC with fluorescence and recently also with mass spectrometric detection. Existing analytical methods for the determination of ergot alkaloids often employ substantial volumes of organic solvents, do not quantify both C8-isomers, and are tedious or are not properly validated. Recently, the European Food Standard Agency (EFSA) concluded that validated analytical methods for the quantification of ergot alkaloids are needed as a prerequisite for future survey studies.

This paper describes the accurate quantification of the 6 major ergot alkaloids- ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine and their respective epimers (ergo-inines). It involves alkaline extraction conditions using a mixture of acetonitrile and ammonium carbonate buffer followed by a rapid clean-up using dispersive solid phase extraction with PSA (primary secondary amine), and a short chromatographic LC-run (20 min) with subsequent MS/MS detection. The new method has been successfully validated for 4 different cereals and 6 processed food samples on 6 different days over a period of 4 weeks. 115 out of 120 recovery measurement results were between 69 and 105%. LOQs ranged from 0.17 to 2.78 µg/kg with expanded measurement uncertainties ranging from ± 0.56 to ± 1.49 µg/kg at a level of 5 µg/kg.

L-11
DETECTION OF TOXINS AND PATHOGENS IN LIQUID SAMPLES

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Aiming for an effective method to assess liquid samples quality (water, juices) by appraisal of the presence of pathogen microorganisms and toxins, we propose a novel electro optical approach combining differential impedance spectroscopy and Surface Plasmon Resonance, SPR, in a Flow Injection set-up. Depending on the dimensions of the target analyte, functionalized chips with immune compounds immobilized at the surface of solid electrodes, are analysed using direct, or competitive assays.

This dual method provides a rapid approach for monitoring the dynamics of biomolecular interactions and it can be used to characterize biosensors in respect to surface reactivity, surface loading and binding constants. The procedure is generally applicable to heterostructures (cells, antigens, antibodies, DNA, enzyme-layers) immobilized at the surface of solid electrodes.

Combination of differential electrical impedance spectroscopy with SPR provides inner validation and expands the analyte detection range while enabling sensor characterization during functionalization.

Both theoretical and experimental aspects on monitoring the specific and non-specific binding are addressed. The key idea is to reveal the presence of the analyte by investigating the dynamics of the (impedance and refractive index) changes at the interfaces between bulk and specific, as well as non-specific (reference) transducers during the process of antibody-antigen binding.

The chip-sample interface changes due to the specific recognition events are simultaneously monitored, on the active spots of the same chip, using a custom designed system that comprises: a multi-channel SPR module, a multi-channel, differential impedance analyzer, a fully automated injection system and a measurement chamber with flow through micro channels. In this format, the fast variations of impedance and refractive index of the sensor/specimen interface in response to specific recognition events (Ag-Ab) are monitored, while eliminating the non specific influences.

The system was successfully tested for a wide range of target analytes. Results on detection of low molecular analytes and on whole cells using reusable immunosensors will be presented.

Having in view the versatility and portability of the method and of related equipment enabling remote control of the sensing equipment, we stress on the possibility to develop this approach into an at line system for quality monitoring.

Key words: differential impedance spectroscopy, SPR, immunosensors, quality assessment quality, remote monitoring.

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L-12

STUDIES OD SECONDARY FUNGAL METABOLISM BY USING TIME-OF-FLIGHT MASS SPECTROMETRY

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Liquid chromatography-time-of-flight mass spectrometry (LC/TOF-MS) has been used for profiling fungal metabolites searching against a database of 465 mycotoxins and other compounds. Validation of this LC/TOF-MS procedure in terms of accuracy, sensitivity and repeatability was carried out by spiking mycotoxin standards into blank growth media. To demonstrate the viability of this approach to studying fungal metabolism, toxigenic fungi from reference collections were grown both on growth media and on food matrices such as dried figs and hazelnuts. After solvent extraction the crude extracts were directly analysed. The data-processing approach, based on cluster analysis, showed that ESI-TOF-MS analysis could readily identify metabolites in these crude extracts without interference of ion suppression. This approach also enabled real-time studies of the effects of various nutrients such as amino acids and sugars on metabolite production. LC/TOF-MS clearly has enormous potential for routine rapid screening for mycotoxins and other metabolites in foods.

L-13

THE EUROPEAN APPROACH TO IMPROVING ANALYSIS OF DSP AND PSP MARINE TOXINS

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Over the last few decades the consumption and trade in shellfish has increased across the globe. From an industrial and health perspective this is a very positive trend. However, in parallel to this has been an increase in the observed frequency and diversity of toxic algal blooms. These toxin producing algae are accumulated by most shellfish species and can cause serious health problems for the consumers.

An important classes of algal toxins is the diarrhetic shellfish poisons (DSPs). This is a highly diverse groups of compounds which cause similar clinical effects on the consumer. Another important class of toxins is the Paralytic shellfish poisons (PSPs) which when consumed can lead to death. For both the DSP and PSP toxins there is a lack of reliable analytical methods for the determination of their presence and concentration in shellfish samples. This is a major concern for regulators.

In response to this problem the European Commission funded, as part of work 6, several research projects, Biocop, Biotox and Detectox, aimed at delivering new and improved means of performing rapid and reliable marine toxin analysis.

In a joint presentation the project co-ordinators will give an overview of the objectives and progress to date in these projects in areas relating to toxin purification, production of standards and reference materials. In addition, detailed deion of improvements in analytical techniques such as ELISA, biosensor, functional assays and mass spectrometry will be presented. The progress in validation of these methods will be described in detail.

L-14

UNRAVELING THE FLAVOR OF COCOA BY APPLICATION OF THE MOLECULAR SENSORY SCIENCE APPROACH

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The characteristic flavor of cocoa is formed as a result of biochemical reactions during fermentation of the cocoa fruit and, secondly, by complex thermoconversion reactions inside the dried cocoa beans during roasting. But, although many studies have been performed, in particular aimed at identifying volatile constituents, there is a lack in knowledge on the key flavor compounds, i.e. the compounds responsible for the characteristic odor and taste profile of roasted cocoa, and, also on their precursors and formation pathways. This knowledge is, however, an important prerequisite to optimize the flavor of cocoa during processing and, also to minimize the formation of unwanted constituents, like acrylamide or biogenic amines.

On the basis of the application of state-of-the-art methods in molecular sensory science, first, the key aroma and taste compounds of roasted cocoa were characterized and quantified. Then, the time course of the formation of each “bioactive” molecule during fermentation and roasting was monitored. Because roasting was performed on the unfermented as well as on samples fermented for 2, 3, 5 and 7 days, the results allowed to clearly differentiate between biochemical and thermal formation pathways of each single compound. A new method for the quantitation of some rare biogenic amines was developed to study, in particular, the influence of cocoa processing on their amounts. A new formation pathway of biogenic amines during roasting will be presented.

L-15

GAS CHROMATOGRAPHY – OLFACTOMETRY (GC-O) IN THE DETERMINATION OF KEY FLAVOR COMPOUNDS IN FOOD PROCESSING AND STORAGE

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Human nose is one of the most sensitive detectors in gas chromatography that can be used for the analysis of flavor compounds. The hyphenation of gas chromatography with olfactory detection (GC-O) has become an indispensable tool in the analysis of odoriferous compounds, especially since the introduction of quantitative approaches in GC-O.

The application of GC-O in the determination of food odorants generated during food processing and storage will be discussed using: *i)* oat flakes, *ii)* muesli and *iii)* low-fat extruded potato snacks as examples.

Volatiles were extracted using SPME, SAFE and SDE techniques, identified and quantified by GC/MS. To perform GC-O analyses volatiles were isolated using SAFE technique, and AEDA (Aroma Extract Dilution Analysis) was performed to quantify them. Samples were also characterized by sensory profile analysis.

Oat flakes due to the high fat content are susceptible to lipid oxidation. In order to assess the effect of heat treatment on the flavor and volatile compounds of oats, raw oats, kiln and dried oats, dehulled oats and oat flakes were analyzed. The most abundant compound in headspace was hexanal - its concentration varied from 176.1 to 1671.9 μg/kg depending on the processing stage. However, the key aroma compounds of oat flakes identified using GC-O were 2-methyl-3-furanthiol, methional, dimethyl trisulfide, 1-octen-3-ol and 2-methyl-3,5-dimethyl pyrazine.

To identify rancidity markers for stored model muesli its ingredients (oat, corn and wheat flakes, hazelnuts, sunflower seeds and flakeseeds and raisins) were subjected to accelerated storage test at 50^{o}C and flavor GC/MS and GC-O profiles were compared.

Low-fat potato snacks subjected to roasting in their production represent an abundant source of heat generated flavors. Development of flavor in all production stages was monitored using GC-O. Among characteristic compounds, methional, benzenemethanethiol, 2-acetylo-1-pyrroline, benzacetaldehyde, butanal and 2-acetylpyrazine were considered as main contributors to its aroma.

GC-O was used, together with deive sensory profiling to asses the influence of flavor precursors: cysteine and cystine addition on the formation of different flavor notes in snacks. High addition of cysteine (1%) resulted in a formation of undesirable odor and taste notes. On the contrary, addition of cystine gave product with pleasant odor and taste, slightly changed into bread-like notes with the highest FD values for butanal, 2-acetyl-1-pyrroline, benzenemethanethiol, methional, phenylacetaldehyde, dimethyltrisulfide, 1-octen-3-ol, 1,5-octadien-3-one and 2-acetylpyrazine.

L-16

DIRECT THERMAL DESORPTION–COMPREHENSIVE GAS CHROMATOGRAPHY–TIME-OF-FLIGHT MASS SPECTROMETRY USING A PROGRAMMED-TEMPERATURE VAPORIZING INJECTOR AS AN ANALYSIS TECHNIQUE FOR EDIBLE OIL CHARACTERIZATION

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Frying is one of the most commonly used methods of food preparation. As is to be expected, the prolonged use of oil for this purpose causes changes in its chemical composition due to oxidation processes. This oxidation is initially characterized by the emergence of a sweetish and pleasant odour, which unfortunately becomes progressively worse until attaining the characteristic smell of rancid fat.

In literature, several methods have been proposed to evaluate the quality of edible oils. Thermo-analytical methods such as differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA) and derivative thermal gravimetric analysis (DTG) are used for this purpose [1]. Another analytical technique often used in the quality evaluation of edible oils is gas chromatography (GC). Because of the complex nature of such samples, the GC chromatograms often are very crowded.

Comprehensive two-dimensional GC (GC×GC) has a much increased peak capacity and hence offers clear advantages for the characterization of complex samples. To quote one example, GC×GC was applied for essential oil analysis by Marriott *et al.* [2]. The use of a mass spectrometer (MS) is highly desirable for the identification of the numerous compounds that are (partly) resolved by the GC×GC separation. At present a time-of-flight mass spectrometer (ToF MS) is the instrument of choice to achieve this. Since GC×GC can easily generate several thousand peaks in one run, manual compound identification is nearly impossible when analysing non-routine samples.

This contribution reports the use of a programmable temperature vaporizing (PTV) injector as a Direct Thermal Desorption (DTD) device coupled to GC×GC–ToF MS for the characterization of edible oils. Multi-step thermal treatment is applied to obtain information on the original oil, the volatiles formed upon heating and the degradation products formed if heated to excessive temperatures. Data processing is done by use of modern software features as automatic peak finding, mass spectral deconvolution and ing [3].

[1] A. Gouveia de Souza, J.C. Oliveira Santos, M.M. Conceição, M.C. Dantas Silva, S. Prasad, Brazil. J. Chem. Eng., 21 (2004) 265
[2] Ph. Marriott, R. Shellie, J. Ferguson, R. Ong, P. Morrison, J. Flavour Fragr., 15 (2000) 225
[3] W. Welthagen, J. Schnelle-Kreis, R. Zimmermann, J. Chromatogr. A, 1019 (2003) 233

L-17
CHEMICAL METHODS FOR THE IDENTIFICATION AND QUALITY CONTROL OF HONEY

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Among various products obtain from an apiary the most recognized one, by consumers is honey. Polish beekeepers are famous from the production of the high quality honey with excellent taste as well as healing and nutrition properties. According to polish quality standards, there are three types of honey depending on its origin namely, honey makes from the plant nectar, from honeydew and from nectar-honeydew. The quality standards also provide other honey classification that is based on the type of plant from which this product is obtained from. Those honeys are as follows: rape nectar, acacia nectar, lime nectar, buckwheat nectar, heather nectar, polyfloral nectar, nectar-honeydew, deciduous trees honeydew and finally coniferous honeydew. Each of them contains specific certificate of its botanical and geographical origin evidenced by the presence of hundred thousand pollens from various plants suspended in it. Therefore, the classification of honey is made by the specification of the percentage of leading pollen from the plant of origin. The honey can be recognized as a specific of given plant only if the percentage content of the pollen in this plant is equal or higher to the values they can be found in polish quality standards. Traditionally, this botanical and geographical classification is done by the analysis of pollen content. However, this method is very time consuming and depends on the experience and abilities of the particular expert. Hence, other than traditional methods of the honey analysis should be developed in order to help in fast, economic and effective as well as unambiguous honey identification.

The identification of the specific substances – markers allows to create the chemical profiles of the given honey that can be treated as its fingerprint trace. The main factor in honey characterization is its aroma. Unifloral honeys possess highly characteristic aromas, due to the presence of specific volatile organic components deriving from the original nectar sources. In the presentation, the study considering the classification of the chemical compounds (e.g. terpenoids and phenols) characteristic for the particular type of honey will be undertaken.

The other problem that can be again addressed to the honey quality is its contamination. Continuous exposure of the bees on the influence of various sorts of chemical compounds has also impact on the quality of the produced honey. Because of the common use of the honey and its medical properties it is important that the active substances present in the drugs used for the bees treatment and also other chemical pollutants should not be found in the honey even in the trace amounts. However, those substances nowadays produced by motorization industry or used in the intensive cultivation for crop protection are easily accumulated in the environment. In result, they are also present in the pollen and nectar being the base of the bees food and finally can be transferred to the hive. Additional pollutants are introduced to the hive even by beekeepers themselves as acaricides or antibiotics used for bees protection. Therefore, it is important to consider the monitoring of pollutants, during the quality assessment of the honey present in the country market.

L-18
FOOD PROCESS CONTAMINANTS

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The processing of food within a manufacturing environment ideally furnishes products that are nutritional, safe, tasty, of consistent high quality, and that can be successfully marketed through their functionality, convenience, and affordability. Undesired compounds that are formed as adventitious by-products during thermal processing of foods cannot be fully avoided, and are also formed in our everyday preparation of food in the home. Substances such as for example acrylamide, furan, and 3-MCPD/3-MCPD esters have recently received much attention due to potential food safety concerns, and several major research projects have been initiated to provide a solid basis for risk assessment (e.g. Heatox project).

After the announcement of the occurrence of acrylamide in foods in April 2002, the CIAA (Confederation of the European Food and Drink Industries) established an “Acrylamide Technical Expert Group”, with the main objective of co-ordinating the industry-driven research and sharing the results of the different research initiatives. The results of this group are documented in several peer-reviewed publications, including acrylamide analysis, mechanisms of formation, and mitigation in different foods. A major milestone in terms of identifying options for interventions that may lead to a reduction of acrylamide in certain foods has been the establishment of the CIAA “Toolbox”. The CIAA “Toolbox” reflects the results of several years of industry cooperation to understand acrylamide formation and potential mitigation steps. Its aim is to provide brief deions of those steps evaluated and, in many cases, already implemented by food manufacturers and other partners in the food chain. A total of 13 parameters, grouped within the four major Toolbox compartments, have been identified, that can be applied selectively by each food producer in line with their particular needs and product/process criteria.

Furan is formed mainly during the thermal decomposition of carbohydrates and thus encountered in canned products, several boiled/cooked vegetables and coffee. However, the exposure levels and health risks in the human diet are not yet clear. Model studies have revealed three main precursor systems producing furan upon thermal treatment, i.e. polyunsaturated lipids, ascorbic acid, and Maillard precursors. The key formation pathways of the aforementioned compounds will be discussed.

3-Monochloropropane 1,2-diol (3-MCPD) was originally identified as a processing contaminant of acid-hydrolysed vegetable proteins (HVP), and recently also in several foods subject to thermal treatment. The unexplained traces of 3-MCPD in several heat-treated foods have stimulated fundamental model studies to assess the possible involvement of hydrolytic enzymes such as lipase in the formation of chloropropanols. Consequently, an alternative pathway has been proposed, pointing to a more important role of the esterified 3-MCPD (mono- or diesters of fatty acids, also termed 3-MCPD esters). These fatty acid mono- and diesters of 3-MCPD can be chemically formed from triacylglycerols, and their contribution to the exposure of “free” 3-MCPD will be elaborated.

The progress made in understanding how these compounds are formed in foods, in particular possible mitigation options, will be discussed, focusing on the three aforementioned compounds that today can be considered as a high priority. However, any measures that are introduced to mitigate process contaminants must be carefully assessed, as they may lead to potentially more serious risks and/or compromise the health/quality benefits of the food.

[1] The CIAA Acrylamide Toolbox. ISSN 1782-1584, 23 Sept 2005, http://www.ciaa.be/ciaa.
[2] Robert, M.C., Oberson, J.M., Stadler, R.H. J. Agric. Food Chem. 2004, 52, 5102-5108.
[3] Becalski, A., Seaman, S. J. AOAC Int. 2005, 88, 102-106.

L-19

GC–TOFMS, HIGH-SPEED vs. HIGH-RESOLUTION: ADVANTAGES AND LIMITATIONS

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Hyphenation of gas chromatography (GC) with mass spectrometry (MS) allows identification and quantification of a wide range of trace amounts of GC-amenable compounds in complex matrices. Until now, low-resolution (unit mass) mass spectrometric detectors employing either single quadrupole or ion trap mass analysers have been used in most of the food analysis applications.

Recent progress in instrumentation design (optics mainly) as well as the use of fast recording electronics (which were not available or were too expensive until a few years ago) together with improvements in signal-processing strategies has led to the renaissance of time-of-flight (TOF) mass analysers for the determination of a wide range of both target and non-target organic components occurring in various biotic matrices.

The effort to exploit the unique features of the rapidly developing TOFMS technique resulted in the introduction of two types of spectrometers differing in their basic characteristics:

- Unit mass resolution instruments that feature a high acquisition speed (up to 500 spectra/s), which predetermines their use as detectors coupled to fast and ultra fast GC or comprehensive two-dimensional GC (GC×GC)
- Instruments with only a moderate acquisition speed (max. 20 spectra/s), but having high mass resolving power (>7,000 FWHM), which allows a greater ability to resolve the analytes from the matrix components. Additionally, mass measurement accuracy (<5 ppm) permits estimation of the elemental composition of the detected ions.

In recent years, both systems have been demonstrated as a powerful and highly effective analytical tool in various fields of food analysis [1–6].

In this presentation, examples of (GC×)GC–TOFMS performance in food analysis applications, including determination of acrylamide and its volatile markers—pyrazines in heat-processed foodstuffs, will be illustrated. Advantages and limitations of both TOFMS systems will be discussed.

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[1] T. Čajka, J. Hajšlová, LC GC Eur., 20(1) (2007) 25–26, 28–31.
[2] T. Čajka, J. Hajšlová, J. Cochran, K. Holadová, E. Klimánková, J. Sep. Sci. 30(4) (2007) 534–546.
[3] L. Dunovská, T. Čajka, J. Hajšlová, K. Holadová, Anal. Chim. Acta 578(2) (2006) 234–240.
[4] T. Čajka, J. Hajšlová, R. Kazda, J. Poustka, J. Sep. Sci. 28(7) (2005) 601–611.
[5] T. Čajka, J. Hajšlová, J. Chromatogr. A 1058 (2004) 251–261.
[6] J. Zrostlíková, J. Hajšlová, T. Čajka, J. Chromatogr. A 1019 (2003) 173–186.

L-20

QUANTITATIVE DETERMINATION OF LYSINE, CARBOXYMETHYLLYSINE, PYRRALINE AND FRUCTOSELYSINE BY ISOTOPE DILUTION LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY TO EVALUATE GLYCATION IN FOODSTUFFS

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The heat-mediated reaction of side chain amino groups with reducing sugars or sugar decomposition products leads to various modified residues which can be classified as “early” or “advanced” modifications. For instance, in the presence of glucose and lysine, the resulting “early” product is known to be fructosyllysine (Amadori product). The Amadori product may undergo several degradation steps, giving rise to various reactive species which further react with lysine residues to form stable derivatives known as “advanced endproducts”, such as carboxymethyllysine and pyrraline. These modifications alter the nutritional value (by decreasing the lysine content) of the products and some of them are claimed to promote the induction of inflammatory mediators. Therefore, particular attention should be devoted to the developement of reliable analytical methods aimed at monitoring these compounds in foodstuffs.

The purpose of our work was to develop a method for the simultaneous measurement of lysine, carboxymethyllysine, pyrraline and fructosyllysine in various foodstuffs using isotope dilution liquid chromatography-tandem mass spectrometry. Commercial 1,2,3,4,5,6-$^{13}C_6$-1,6-$^{15}N_2$-lysine (upmass shift: +8 Da) was used as internal standard for the quantification of lysine, while 1',2'-$^{13}C_2$-carboxymethyllysine (+2 Da), 1,2,3,4,5,6-$^{13}C_6$-1,6-$^{15}N_2$-pyrraline (+8 Da), and 1,2,3,4,5,6-$^{13}C_6$-1,6-$^{15}N_2$-fructosyllysine (+8 Da) were prepared by chemical synthesis for the measurement of the pertaining lysine modifications.

Lysine, carboxymethyllysine, pyrraline and fructoselysine were monitored as protein residues in infant formulae and human milk, while as free amino acids in food products such as butter, fruit juice and biscuits. Infant formulae were digested enzymatically to avoid the degradation of both pyrraline and fructosyllysine occurring in hydrochloric acid 6N. Particular attention was devoted to the optimization of the enzymatic conditions to ensure an efficient digestion of the proteins, and avoid potential trends in the measurement due to an uncomplete cleavage of the peptidic bonds. The method was validated based on the stability of the analytes, the linearity of the response and its performance, evaluated with the limit of detection, repeatability, reproducibility and trueness. The selectivity of the detection was ensured by recording two characteristic transitions per compound (for both the non-labelled analyte and its stable isotope labelled analogue) using the selected reaction monitoring mode. The transition leading to the most intense signal was used for the quantification, while the second one aimed at confirming the certainty of the detection.

Our measurements were interpreted based on values reported in the literature, and emphasis was laid on the method performance.

L-21

GROUP-TYPE AND FINGERPRINT ANALYSES OF ROASTED FOOD MATRICES BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY – QUADRUPOLE MASS SPECTROMETRY (GCxGC/QMS)

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"*Comprehensive two-dimensional gas chromatography – GCxGC* is a multidimensional analytical technique introduced by Liu and Phillips in 1991 [1] to analyze very complex matrices because of its high separation power. By definition [2] in GCxGC the separation power of two, or more, different columns connected in series is applied to a complex sample to exploit a resulting separation capability that is the product of the theoretical peak capacity of each chromatographic dimension. On the basis of the specific column configuration and experimental condition adopted it is possible to obtain separations providing different information: the so called "target-type" analysis approach gives analyte identification (or identity confirmation) and quantitation with a suitable level of confidence while the "group-type" analysis is useful to investigate chemically correlated groups of compounds giving "structured" 2D chromatograms where components are distributed on the chromatographic plane on the basis of their physico-chemical characteristics. The latter approach, known also as "fingerprint-type" analysis, provides a 2D-fingerprint of the sample suitable for sample matching and emphasizes differences and similarity.

This study aims to evaluate potentials and benefits of Comprehensive GCxGC, coupled to conventional quadrupole mass spectrometry (qMS) operating in fast scanning, in group-type and fingerprint-type analysis of some roasted food matrices. Coffee (*Coffea arabica*), cocoa (*Theobroma cacao*) and hazelnuts (*Corylus avellana L.*) have been analyzed with different column configurations by adopting suitable orthogonal stationary phase combinations [3, 4] in order to maximize sample component separation and group type correlations. The key-aroma compounds and chemically correlated groups of components, mainly produced by roasting and assumed as specific markers of thermal processes, have been located in the chromatographic plane and used to discriminate samples submitted to different technological treatments.

[1] Z. Liu, J. B. Phillips, J. Chromatogr. Sci. 1067 (1991) 227.
[2] J. C. Giddings, Anal. Chem 39 (1967) 1027
[3] C. Cordero, P. Rubiolo, B. Sgorbini, M. Galli, C. Bicchi, J. Chromatogr. A 1132 (2006) 26
[4] P.Q. Tranchida, P. Dugo, G. Dugo, L. Mondello, J. Chromatogr. A, 1054 (2004) 3

L-22a
ADVANCES IN THE ANALYSIS OF POPs IN FOOD

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Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) have been found in food since about half a century. Although most of these contaminants have been phased out, residues are still being found, emphasizing the persistent character of these POPs. On top of that, chemicals that have been unknown for many years have been identified in food and proposed for including them in the list of POPs. Examples are various brominated flame retardants such as polybrominated diphenylethers (PBDEs) and the perfluorinated alkyl compounds (PFCs) such as perfluoroctylsulphonate (PFOS). This type of compounds could be detected because over the last half century the sensitivity of our methods has improved more than six orders of magnitude. Although this is a great progress in analytical chemistry, the risks of the trace amounts of POPs that are being found nowadays should not be overestimated until effects at those (or slightly higher) levels are being found as well. Most of these 'new' POPs are complex mixtures that require highly selective methods for their determination. Both gas chromatography (GC) and high performance liquid chromatography (HPLC) in combination with mass spectrometric detection can be used for a reliable identification and quantification. Combined methods such as comprehensive multidimensional GC (GCxGC) with for example Time-of-Flight MS, preferably –because of the halogen atoms present in most POPs – combined with electron capture negative ionisation (ECNI) will even provide more information.

However, the increasing number of chemicals that we need in our modern society causes an increasing number of residues in our food, most of them of a complex nature. Therefore, analytical methods are challenged and pushed to their limits. Also, the enormous amount of information for example provided by GCxGC-ECNI-ToF-MS is actually too much to be digested by analysts and authorities. Therefore, a different approach should be considered. In Toxicity Identity Evaluation (TIE) or Effect Directed Analysis (EDA) the effect of a compound or a group of compounds in a bioassay is the key element. After an effect is found, several fractionation steps and more checks with other bioassays follow before finally the effect-causing contaminant is being identified. This approach has shown its first successes but needs to be developed further until it can be standardized.

L-22b
PERFLUORINATED COMPOUNDS IN FOOD: EMERGING ISSUE

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Perfluorinated compounds (PFCs) have received considerable attention from scientists and policy makers in recent years. PFCs consist of a large group of compounds of which PFOS (perfluorooctanesulfonate) and PFOA (perfluorooctanoic acid) are best known. Other compounds belonging to this class have different chain lengths and/or functional groups (see [1] for an overview). PFCs have been and are still being used widely as surfactants for production of fluorinated polymers, for metal plating, in photographic industry, in fire fighting foams and as fat, and water repellents for textiles, paper and leather. PFCs are widely distributed in the environment and many are persistent. PFOS accumulates in fish, whereas PFOA is water soluble. Following concerns on potential adverse effects, US-EPA has initiated various actions including human risk assessments on PFOA (http://www.epa.gov/oppt/pfoa/).

The surfactant type properties give PFCs a very distinct behaviour in the environment and potentially also in the food chain. For analysis of PFCs, laboratories can no longer rely on methods that were once developed for lipophilic contaminants. PFCs call for a different analytical approach in order to meet these different properties. This paper aims at reviewing the current state of the art of PFC analysis in food. Initial methods were developed for fish (liver) mainly and relied on ion-pair extraction (with poor performance). Recent studies used medium polar solvents (acetonitrile, methanol) to extract a wide range of PFCs from fish samples, combined with a clean-up step using Envicarb [2]. Instrumental analysis is generally performed by HPLC-ESI-MS/MS [1]. Milk was analysed in some studies and is generally analysed using solid phase extraction (SPE) [2]. The same method is used for the analysis of the more water-soluble (short chain) PFCs in water [2]. Nearly no methods are available for meats, vegetables, fruits and cereal based products. To enable a human exposure assessment for PFCs, methods for these food commodities should be developed. Current methods may be adapted to fulfil these tasks. Initially, many laboratories struggled with the accuracy of their measurements [3]. Results of the 1st world-wide interlaboratory study were poor for a fish and water sample. However, with the commercial availability of good quality standards (native and mass labelled) and the increased knowledge on the physico-chemical properties this situation is situation has been improved. Therefore, a new international interlaboratory study was organized during the summer of 2007 to check the progress in the comparability of the PFC analyzing laboratories.

[1] de Voogt P. and Saez, M., Analytical chemistry of perfluoroalkylated substances, Trac-Trends in Analytical Chemistry 2006, 25, 326-342.
[2] van Leeuwen S. P. J. and de Boer, J., Extraction and clean-up strategies for the analysis of poly- and perfluoroalkyl substances in environmental and human matrices, Journal of Chromatography A 2007, 1153, 172-185.
[3] van Leeuwen S. P. J., Karrman, A., Van Bavel, B., de Boer, J.,Lindstrom, G., Struggle for quality in determination of perfluorinated contaminants in environmental and human samples, Environmental Science & Technology 2006, 40, 7854-7860.

L-23

COMBINED METHODOLOGY FOR BROMINATED DIOXINS (PBDD/FS), PBDES AND PBBS, ALONG WITH PCDD/FS, PCBS AND CHLORINATED NAPHTHALENES (PCNS)

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Methods for the analysis of polychlorinated dioxins and furans (PCDDs, and PCDFs) and polychlorinated biphenyls (PCBs) based on modified silica and carbon clean-up are well established and comprehensively validated for the analysis of food, animal feed and other matrices. The method achieves the analytical standards of EU protocols (2002/69/EC and 2002/70/EC) that are used to determine the compliance of food and animal feed with maximum permissible levels of chlorinated dioxins and biphenyls in these commodities. The methodology provides WHO–TEQ data for dioxins and PCBs as well as individual concentrations for toxic PCDD/F congeners, >50 commonly occurring PCBs, individual tri- to hexa- brominated dioxins (PBDDs and PBDFs), polybrominated biphenyls (PBBs), polybrominated diphenylethers (PBDEs), and polychlorinated naphthalenes (PCNs). A wide range of ^{13}Carbon-labelled surrogates (currently 17 PCDD/Fs, 17 PCBs, 7 PBDEs, 2 PBBs, 6 PBDD/Fs and 4 PCNs) allow accurate internal standardisation. Measurements are carried out using high resolution GC coupled mostly to high resolution mass spectrometry (HRMS). The methodology has been validated by the frequent use of reference materials, and successful participation in international inter-comparison exercises. A large number of different food types have been analysed for these compounds using this methodology over several years. Typical congener profiles for various food matrices, and validation data for all compounds is presented.

L-24

CRITICAL ASSESSMENT OF DIFFERENT APPROACHES TO ANALYSIS OF PRIORITY PESTICIDES IN BABY FOOD

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Altogether 24 toxic pesticides and their metabolites are listed in the Annexes I and II of Commision Directive 2003/13/EC on processed cereal-based foods and baby foods for infants and young children, setting maximum residue limits (MRLs) even lower than 10 $\mu g\ kg^{-1}$ applied for residues of any other modern pesticides in such commodities. Pesticides representing various structure groups are involved in this "black list", and therefore it was challenging to determine them in a single analytical run. In this study conventional GC coupled to high-speed "time-of-flight" (TOF-MS) or single quadrupole mass analyzer (MS-q) and orthogonal two-dimensional gas chromatography (GCx GC) coupled with TOF MS were employed to analysis of fruit-based baby food extracts prepared either by "classic" ethyl acetate extraction followed by gel permeation chromatography (HPGPC) purification step or recently introduced QuEChERS approach employing dispersive SPE. Both sample preparation approaches provided satisfactory recoveries ranging from 65 to 95%, repeatability was as RSD 3-26%. GC x GC/TOF MS and GC/MS-q (LVI used to sample introduction in this case) proved to withstand tough legislation demands for control of priority pesticides with LODs ranging from 0.2 to 7 $\mu g\ kg^{-1}$ (all below respective MRLs).

This work was realized as a part of the European Commission-funded Integrated Project Food-CT-2004-06988 "BIOCOP (New Technologies to Screen Multiple Chemical Contaminants in Foods)" co-ordinated by Queen's University (Belfast, Ireland), and also within the scope of research projects MSM 6046137305 supported by the Ministry of Education, Youth and Sports of the Czech Republic

L-25

COMPREHENSIVE SCREENING OF ORGANIC CONTAMINANTS IN THE FOOD CHAIN USING UPLC OR GC(×GC) AND FULL SCAN MASS SPECTROMETRIC DETECTION

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Within the EU food safety is an important issue. During production and storage of food, organic contaminants like residues of pesticides and veterinary drugs, natural toxins and environmental or processing contaminants may enter the food chain. For many of these contaminants legal limits in food are established or under evaluation. Throughout the food chain, samples are taken to monitor and control the presence of organic contaminants, to verify compliance with these limits and to ensure food safety. For monitoring purposes selective and sensitive methods are needed and chemical analyses are commonly applied. Within the respective fields of analysis, a clear trend towards multi-compound methods can be observed. In the field of pesticide residue analysis this has been well developed and methods covering many hundreds of target analytes exist [1]. More recently, similar developments are taking place in the field of veterinary drugs [2], mycotoxins [3] and environmental contaminants [4]. In all cases, methods for these contaminants are based on chromatography with mass spectrometric detection. An obvious continuation of the current trend is to integrate multi-compound methods from different groups of contaminants into even more generic and comprehensive (screening) methods. This not only makes screening for organic contaminants much faster, it also greatly reduces cost, in terms of €/analyte.

In the present contribution, approaches will be presented for developing comprehensive methods for combined screening and determination of multiple groups of organic contaminants. All steps of the analytical method, i.e. extraction, clean up, chromatographic separation and detection, are reconsidered. For generic extraction, existing multi-methods from the fields of pesticides and mycotoxins, and new approaches, are evaluated for their suitability to extract compounds varying widely in physical/chemical properties from different kinds of matrices. The (im)possibilities for clean up will be discussed. The inherently limited selectivity during sample preparation needs to be compensated for by the instrumental analysis of the extracts. This means the use of more advanced chromatographic separation such as UPLC and comprehensive two dimensional GC. The key aspect in generic methods is sensitive and selective detection. With the high number of analytes targeted for (>1000), full scan MS detection is essential. Several options are considered, with emphasis on time-of-flight (ToF) MS.

Example applications of the comprehensive methods developed will be presented. These include a method based on UPLC-ToF-MS for the combined determination of pesticides, mycotoxins and plant toxins in cereal-based matrices and the determination of veterinary drugs, pesticides, natural toxins and environmental contaminants in milk. Comprehensive analysis based on GC(xGC)-ToF-MS is applied to the determination of pesticides and environmental contaminants in fat-containing matrices.

The present contribution shows that, through the use of efficient chromatographic separation combined with advanced full scan mass spectrometric detection, chemical analysis is a feasible option for comprehensive screening and determination of organic contaminants in the food chain.

[1] H.G.J. Mol, A. Rooseboom, R.van Dam, M. Roding, K. Arondeus, and S. Sunarto, J. Anal. Bioanal. Chem (in press 2007) (DOI 10.1007/s00216-007-1357-1)
[2] A. Kaufmann, P. Butcher, K. Maden and M. Widmer; Anal. Chim. Acta 586 (2007) 13
[3] M. Sulyok, F. Berthiller, R. Krska, R. Schuhmacher, Rapid Commun. Mass Spectrom. 20 (2006) 2649
[4] E. Pitarch, C. Medina, T. Portolés, F.J. López, F. Hernández, Anal. Chim. Acta 583 (2007) 246

L-26

DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF TEN COCCIDIOSTATS IN EGGS AND FEED USING LIQUID CHROMATOGRAPHY / TANDEM MASS SPECTROMETRY

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Coccidiostats are mainly used in poultry production as feed additives or veterinary drugs to prevent coccidiosis. The application to laying hens is not permitted; therefore no residues may be present in eggs. During food surveillance residues of anticoccidial drugs especially Lasalocid and Nicarbazin are periodically detected in eggs. These residues are caused by contamination of unmedicated feedingstuffs due to carry over of agents during feed production.

To guarantee the absence of residues of these substances in eggs and feed, effective measures of control have to be carried out. For this aim analytical methods with low limits of detection (below 1 µg/kg) for all licensed coccidiostats are necessary.

A sensitive LC-MS/MS-multimethod for ten coccidiostats (Decoquinat, Diclazuril, Ethopabat, Lasalocid, Maduramycin, Monensin, Narasin, Nicarbazin, Salinomycin, Semduramicin) was developed. Different extraction procedures for egg and feed were tested (ASE, protein precipitation with acetonitrile, enzyme proteolysis). For further clean-up different RP-18 solid phase materials from different producers were examined. Various elution solvent mixtures were also investigated in regard to recovery and purification efficiency. Best results were obtained by accelerated solvent extraction with acetonitrile and SPE clean-up with methanol-water mixtures.

Liquid chromatography was performed on a C_8 stationary phase protected by a guard column. Previous tests of different reversed phase HPLC columns showed this column to offer the best retention properties and peak separation. A gradient of water and acetonitrile was applied containing 0,1 % acetic acid. The analytes were ionized in positive or negative electrospray mode and two ions each were chosen for multiple reaction monitoring (MRM).

The analysis of some coccidiostats below 1 µg/kg was impeded by high ion-suppression both in egg and feed material.

These matrix effects were studied by simultaneous introduction of a standard solution through a “t”-coupling system and injection of blank sample extract.

Therefore additional clean-up steps were tested and introduced to analyse samples at the sub-ppb level.

The now developed method is capable to detect trace levels of ten coccidiostats in feeds and whole eggs. After optimization of the purification procedure it can be used to quantify the amount of residues of coccidiostats in eggs caused by contaminated feedingstuffs.

Without performing the complex clean-up procedure it is possible to control the approved minimum and maximum content of these coccidiostats in animal feed by the developed LC-MS/MS method.

L-27
BIOASSAY DIRECTED ANALYSIS OF AH-RECEPTOR AGONISTS IN CITRUS FRUIT

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Citrus fruit and citrus fruit products, like grapefruit, lemon and marmalade were shown to be able to induce Aryl hydrocarbon receptor (AhR) mediated activity, as detected with the DR CALUX® bioassay. The Ah-receptor pathway plays an important role in the toxic effects of dioxins, PCBs and other aromatic hydrocarbons. So far it is unclear which compounds in citrus fruit are responsible for the AhR-mediated activity and whether these compounds can cause effects comparable to e.g. dioxins.

The present study aimed at identifying unknown Ah-receptor agonists in citrus products based on bioassay directed analysis. Marmalade was extracted with hexane and cleaned up via an Al_2O_3-column. The extract was fractionated via HPLC on a C-18 column. Fractions were solvent-exchanged into dimethyl sulfoxide by nitrogen evaporation and subsequently tested on the DR CALUX® and re-injected on the HPLC to control the fractionation. Extracts were shown to contain furocoumarins and polymethoxyflavones, but standards of the latter compounds showed hardly any response in the bioassay.

Identification of fractions most active in the bioassay via LC/MS pointed to bergapten (a furocoumarin) as being the most important Ah-receptor agonist in marmalade. However, the pure bergapten showed less response in the DR CALUX® assay then the bergapten fraction of the marmalade extract. New experiments show interesting results about the differences in activity between the fractionated marmalade and the pure bergapten pointing at interactions between solvents and target compounds.

L-28

DEVELOPMENT AND OPTIMIZATION OF A MULTITARGET PORTABLE KIT BASED ON THE ELISA REVERSE M&D TO ASSESS MYCOTOXINS AND GMOS IN MAIZE

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The present study deals with the development of innovative analytical methods in line with the EU legislative requirements as Regulation (EC) 1830/2003, concerning the traceability and labelling of food and feed products containing traces of authorised GMOs at or above the fixed 0.9% legal threshold, and to Commission Recommendation (EC) 576/2006, concerning the threshold limits for mycotoxins in cereals and cereal products used for animal feeding. In particular EC 576/2006 recommends to the Member States to test simultaneously the presence of deoxynivalenol, zearalenone, ochratoxin A, fumosinin B1+ B2 in cereals and cereal products in feed, and to collect more data about T-2 and HT-2 toxins.

According to EC Regulation and Recommendation above mentioned, we developed and here described an innovative multitarget portable kit based on the ELISA Reverse m&d (ER) to asses simultaneously the presence of three mycotoxins (deoxynivalenol, zearalenone, ochratoxin) and Cry1Ab GM related protein in maize. The adopted experimental design and the preliminary results are here reported and discussed.

L-29
POTENTIAL OF COACERVATES FOR THE EXTRACTION OF CONTAMINANTS FROM LIQUID FOODS

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Sample preparation is often the bottleneck in food analysis due to the complexity of the matrices. There is a need to minimise the preparation steps prior to determination to reduce both time and sources of error. Furthermore, methods must be sensitive enough to cover the decreasing legislative limits for food contaminants as well as more environmentally friendly, using less solvent and smaller sample sizes. So, solvent free and other emerging isolation technologies are actively being investigated as an alternative to classical liquid-liquid and solid-liquid extractions.

Coacervates constitute a valuable strategy to replace organic solvents in analytical extraction processes. They are colloid-rich liquids that separate from colloidal solutions under the action of a dehydrating agent, namely temperature, pH, electrolyte or a non-solvent for the macromolecule. Coacervates made up of supramolecular assemblies (e.g. micelles, vesicles, etc) have been successfully applied to the extraction of metals and organic pollutants from environmental samples [1-2]. Three main assets of these environmentally friendly liquids for extraction processes are their excellent solvation properties for a lot of organic and inorganic substrates, their potential to reach high preconcentration factors and their suitability for miniaturization.

This study reports for the first time the use of coacervates for the extraction of contaminants from food liquids. Water-induced coacervates made up of reverse micelles of decanoic acid in tetrahydrofuran (THF), recently proposed by our research group, were used for this purpose. The contaminants used as models were bisphenol A (an endocrine disrupter from food packaging material), ochratoxin A (a mycotoxin) and benzo(a)pyrene (a carcinogenic PAH from food processing). The food matrices investigated included wine, vinegar, beer, fruit juices, soda beverages and tea and coffee infusions. Liquid chromatography coupled to fluorescence or mass spectrometry was used for quantitation of the extracted contaminants.

The features of the coacervation process in the liquid food matrices selected were studied in depth. The liquid food/THF volume ratios at which coacervates occurred were similar than those obtained in water samples. The influence of the protein, sugar and polyphenol content on the region encompassed by coacervates in the phase diagrams was elucidated. Parameters affecting extraction efficiency and concentration factors were studied, being the concentration of decanoic acid and THF the most influential ones. The extraction was independent on the pH and the nature and concentration of matrix components, making the procedure particularly robust. Recoveries of the target compounds ranged between 80-95% and the concentration factors varied from 200 to 70 for sample volumes between 9 and 38 ml for liquid foods and 1g for infusions. The detection limits found for the target compounds (1.5-3 ng/L for Ochratoxin A, 0.03 ng/L for Benzo(a)Pyrene, and 0.5 µgL for Bisphenol A) were far below the maximum levels established by EU Directives for these contaminants and covered easily the concentration ranges frequently found in real samples. No clean-up steps were needed, making the methods simple and rapid. The approach developed was applied to the determination of the selected compounds in different food trademarks.

[1] S. Rubio, D. Pérez-Bendito, Trends Anal. Chem. 2003, 22, 470.
[2] R. Carabias-Martinez, E. Rodriguez-Gonzalo, B Moreno-Cordero, J.L. Perez-Pavon, C. Garcia-Pinto, C. E.F. Laespada, J. Chromatogr. A 2000, 902, 251.

L-30

RECENT DEVELOPMENTS IN SPME TECHNOLOGIES AND THEIR APPLICATIONS IN FOOD ANALYSIS

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In this presentation, new developments in SPME, such as cold fiber SPME, thin film SPME, rapid SPME/GC/ToFMS and high throughput 96 well extractions coupled to LC will be summarized and the significance of these new developments to food analysis will be emphasized.

In the first development discussed the internally cooled coated fiber SPME device (cold fiber) was miniaturized. This significantly increased the lifetime of the septum, and consequently allowed automation of this device. The fiber coating was protected with a piece of tubing, and the same coating was reproducibly used for more than 100 injections without any coating failure. The fiber temperature was controlled within 5°C of the preset value by a temperature controller, a solenoid valve, and stainless steel tubing with different inner diameter. The device was mounted and used on the CTC CombiPAL autosampler with minor modifications, and integrated with the autosampler by coupling the temperature control system through a logic circuit built. Up to 10 times increase of extraction efficiencies was observed even under the limited sample temperature. The cold fiber technology facilitates faster extractions and higher sensitivities for both volatile and semivolatile sample components in food matrices. In the second development, SPME samplers were made by using polydimethylsiloxane thin-film (membrane) as the extraction phase. This technique is based on a similar theory as the SPME technique with additional advantage of higher surface to volume ratio facilitating much higher extraction rates and higher sensitivities because of high volume of the extraction phase. More specifically, the development of the thin film sampler involved cutting a section of PDMS thin-film into a specific size and shape, and mounting it onto a stainless steel wire (the handle). Kinetic calibration or equilibrium calibration with the standards in the extraction phase used to introduce an isotopically labeled internal standard. This approach is very useful in high sensitivity multiparameter monitoring food production processes both in situ and in-vivo. In the third development a rapid headspace solid-phase microextraction (HS-SPME) – gas chromatography – time-of-flight mass spectrometry (GC–TOF-MS) method was utilized for the characterization of coffee and icewine aroma profiles. The current study also employs the application of both the traditional univariate optimization methods and the multivariate experimental designs to the optimization of SPME extraction-influencing parameters. The automated SPME procedure was completed by implementing a single DVB/CAR/PDMS metal fiber with excellent durability characteristics that were sufficient to enable the completion of overall sequence of coffee and icewine samples. After the successful finalization of method development, the LECO ChromaTOF automated data processing software was utilized for the data evaluation. The combination of the retention index (RI) system using C8-C40 alkanes and the mass spectral library search resulted in the positive identification of 102 and 201 volatile compounds in coffee and icewine reference samples, respectively. The parameters that were considered for qualitative profiling between different samples are the bean/grape variety, geographical indication and various production-related factors. All these parameters are significant since they influence the consumer acceptance of these two important food commodities. In the forth recent development, the automation of solid-phase microextraction (SPME) coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been accomplished using a 96 multi-well plate format, SPME multi-fibre device and a three arm robotic system This automated configuration is capable of performing all steps necessary to perform the entire SPME procedure in parallel for all samples thus drastically increasing sample throughput compared to other SPME-LC approaches. The optimized automated SPME-LC-MS/MS platform was subsequently fully validated for the high-throughput determinaiton of Ochratoxin A in human urine. The proposed methods allowed the automated analysis of >150-200 samples per day, while achieving excellent accuracy and precision and requiring minimal sample pre-treatment of biological fluids. This represents the highest throughput of any SPME technique proposed to date, thus enabling the use of SPME-LC-MS/MS for food applications requiring high degree of automation and throughput.

L-31

EXTENSIVE LC-MS (QQTOF, ION TRAP, QQQ) ANALYSIS OF PESTICIDE RESIDUES AND THEIR METABOLITES IN FOOD

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Control of food and food products is very important nowadays as well as in the future outlook. This is because of their potential and possible harmfulness for human health when contaminated. Pesticide residues in food and feedstuffs are among the top priorities for human and animal safety. The currently used techniques for their determination, headed by liquid chromatography-mass spectrometry (LC-MS), have different features that must to be exploited for solving the new needs and challenges arising in this field. These analyses are required for (i) verifying that no unexpected residues are occurring, (ii) checking compliance with maximum residue limits (MRLs), and (iii) ensuring that human dietary intakes are at acceptable levels. Consequently, there is an urgent need for accurate, simple and cost-effective methods that can be used as a screening tool for a rapid estimation of any possible pesticide residue in fruits and vegetables. However, this could be self-contradictory –it is tricky to have rapidity, wide scope of compounds and exactitude in the same method.This presentation will examine how the characteristics of different available mass spectrometers offer outstanding possibilities to combine speed with meticulousness. A number of examples for applications in the field of pesticide residue determination –including detection of non-target pesticides and identification of metabolites- will be presented in detail, including the experimental set-up and results. The following aspects will be discussed within the previous bacground:

- Assessment of the structural information obtained by different mass analyzers (triple cuadrupole –QqQ-, ion trap –IT-, or quadruple time of flight -QqTOF) to determine pesticide residues in fruits and vegetables.
- Application of liquid chromatography (LC) and ultra-high performance liquid chromatography (UPLC) in compound separation and degradations products profiling.
- Correctness in the quantification by QqTOF and IT in conjunction with the influence of matrix components for the performance characteristics.
- Evaluation of extraction methods. This is very important, specially, for elucidation of non-target analytes because mass spectrometers can not confirm a substance that is not in the extract.

To conclude, the strategies under which future methods should be developed will be discussed. Furthermore, the influence that these modern methodologies will have for the analyst in view of the demands on food safety will also be an important part of this presentation.

L-32

CAPILLARY ELECTROSEPARATION METHODS WITH MASS SPECTROMETRIC DETECTION: NEW DEVELOPMENTS IN FOOD ANALYSIS

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To meet the steadily increasing requirements on the quality of results in analytical chemistry the use of hyphenated techniques becomes more and more important. This is particularly the case if complex samples like food have to be analyzed. A typical example of such a hyphenated technique is the combination of capillary electrophoresis (CE) with mass spectrometry (MS). In contrast to chromatography where MS detection has already found its way into routine analysis, the coupling of electroseparation methods like CE with MS still can be regarded as a developing technique. Nevertheless substantial improvements have been made over the last few years especially with respect to the applicability of CE-MS for the analysis of real world samples [1]. This development is also reflected in the literature where a distinct trend from papers mainly devoted to basic investigations, towards works describing the adoption of commercially available instrumentation for the analysis of complex sample mixtures originating from various fields of application can be recognized. Although CE-MS is, unfortunately, not yet a prominent tool in food analysis, increasing interest in this topic can be observed.

In this talk new developments in the CE-MS analysis of foods will be presented, whereby the major focus will be set on the problems related to quantitative analysis. Often elaborate sample preparation steps are needed to ensure correct quantitative results, in the case of complex samples. These procedures are necessary to avoid suppression effects in the ion-source mainly originating from co-eluting matrix components. New ionization techniques, such as atmospheric pressure photoionization (APPI) allow new strategies to overcome this problem. Recently APPI has also been introduced to capillary electroseparation techniques [2]. The present talk will provide a comparison of different ion-sources for the CE-MS analysis of food samples. Thereby a strong focus will be set on the susceptibility of these ion-sources to suppression effects and their ability to provide reliable quantitative results even in the case of complex samples without extensive sample preparation.

[1] C.W. Klampfl, Elctrophoresis, 27 (2006) 3.
[2] C.W. Klampfl, W. Buchberger, Anal. Bioanal. Chem., 388 (2007) 533.

L-33
CHARACTERIZATION OF POLYPHENOLIC COMPOUNDS IN HOPS AND BEER USING HPLC/MS/MS

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Polyphenolic compounds play an important role in human diet due to antioxidant properties of these compounds found in different natural products. Hops (Humulus lupulus L.) is a typical example of plant containing polyphenolics with strong antioxidant properties and positive impact on human health, e.g. anticancer effects. Resinous alicyclic phenolic acids found in hops are classified as humulones (alfa-acids) and lupulones (beta-acids), which are converted during the brewing process to isohumulones (isoalfa-acids) and largely contribute to the specific bitter taste of beer. Other polyphenolics in hops are xanthohumol related compounds. We have developed a comprehensive approach for the identification and quantitation of different classes of polyphenolics is hops cones, hops ethanol or carbon dioxide extracts and different branches of beers in relation to the beer quality. Antioxidant properties of beer samples and hops extracts are measured by modified Kaneda method using the reaction of stable DPPH radical monitored spectrophotometrically. Optimized reversed-phase HPLC/UV/MS/MS method is used for the quantitation based on calibration curves of available standards. The combination of ion trap mass analyzer with MSn measurements for studying the fragmentation paths and QqTOF analyzer for exact mass determination is very powerful in the identification of new minor polyphenolic compounds. The right choice of atmospheric pressure ionization technique and polarity mode (positive or negative) is also quite important issue because of different ionization efficiencies and hence sensitivity. Results obtained on a broad range of hops and beer samples will be compared.

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L-34

AUTHENTICATION OF PDO/PGI OLIVE OILS USING NMR AND ISOTOPIC FINGERPRINTING

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Food authenticity and food traceability are of great concern to the consumer, food processor, retailer and regulatory bodies. For instance, one authenticity issue of increasing importance is geographical origin, with some selected products permitted to be marketed using a Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) or Traditional Specialty Guaranteed (TSG) label on the basis of their area of production [Regulation (EEC) No 2081/92]. Extra-virgin olive oil is one of these high value protected agricultural products. The European Commission has already registered in the "Register of protected designations of origin and protected geographical indications" 95 PDO and PGI olive oils, produced in Spain, Italy, Greece, Portugal, France and Slovenia. However, given the financial benefits associated with such labels, it is very likely that economic fraud occurs (e.g. labelling a non-PDO product as a PDO one, adulteration with olive oils that do not fulfil the PDO requirements). Therefore, validated methods to guarantee the authenticity and traceability of PDO and PGI olive oils are necessary to protect both the consumer and the producer from illicit practices in this sector.

Within the European Union, the quality control of olive oils is usually performed applying methods described in the EC Regulation 656/95. The problem of authentication of olive oils with respect to their geographical origin has been studied using various analytical approaches such as NMR (^{1}H, ^{13}C, ^{31}P), NIR spectroscopy, IRMS, LC-MS, and GC-MS [1-4]. However, most of these have considered only a limited number of samples and geographical areas. NMR and isotopic fingerprinting methods seem particularly promising in this context. Indeed, isotopic and NMR methods are used in support of EU policies concerning the origin of agricultural products. These methods can be used to provide objective analytical parameters in order to develop a European system for protecting foodstuffs produced according to certain quality standards such as those of a PDO, PGI or TSG against fraud.

Different approaches are being studied in our laboratory for the determination of the geographical origin of extra-virgin olive oils: *i*) Multivariate analysis of ^{1}H-NMR profiles of olive oils; *ii*) Multivariate analysis of ^{1}H-NMR profiles and isotopic measurements (^{1}H, ^{13}C and ^{18}O) of olive oils; and *iii*) Multivariate analysis of ^{1}H-NMR profiles of the unsaponifiable fraction of olive oils. In the two first proposals, *i*) and *ii*), olive oils are dissolved in deuterated chloroform for NMR analysis. In the third approach, the unsaponifiable fraction of olive oil is obtained by a standard procedure, and then dissolved in deuterated chloroform prior to NMR analysis. NMR spectra are produced by a high throughput NMR method and the isotopic measurements of ^{1}H, ^{13}C and ^{18}O are performed by IRMS. The data provided by these spectroscopic techniques are analysed by unsupervised and supervised pattern recognition techniques in order to achieve classification models for the authentication of olive oils labelled "as of designated origin".

To this aim, MAST in collaboration with other partners (TRACE project (http://www.trace.eu.org), and scientific contacts) has collected a statistically significant number of authentic PDO and PGI extra-virgin olive oils from EU and non EU countries (716 samples) during two seasons, 2005 and 2006. In 2005, we collected olive oils from Italy (226 (63 from Liguria), Spain (72), Greece (43), Turkey (14) and France (9); whereas in 2006 samples were from Italy (252 (79 from Liguria), Spain (38), Greece (46), France (10) and Cyprus (6). Moreover, Stazione Sperimentale dei Oli e Grassi (Milan, Italy) provided us with 94 samples of unsaponifiable fractions of extra-virgin olive oils from Italy, Spain, Greece, Tunisia, Turkey and Syria.

The analytical data of such a large number of samples and from such an extensive range of geographical origins has allowed us to study many aspects of olive oils. In this sense, classification models were developed for the authentication of olive oils of a certain PDO, e.g. "*Riviera Ligure*" (Liguria, Italy) in order to distinguish them from the oils of other protected origins (Objective of the TRACE project supported by the Food and Quality Priority of the EU work VI research programme); the geographical characterization of olive oils at the national level; and the geographical characterization of Italian olive oils at the regional level. The contribution of these results will considerably strengthen our ability to protect both the consumer and the producer in the ongoing fight against fraud in the food sector.

[1] S. Rezzi, D. E. Axelson, K. Héberger, F. Reniero, C. Mariani, C. Guillou. Anal. Chim. Acta 2005, 552: 13-24.
[2] G. Fragaki, A. Spyros, G. Siragakis, E. Salivaras, P. Dais. J. Agric. Food Chem. 2005, 53: 2810-2816.
[3] A. A. Christy, S. Kasemsumran, Y. P. Du, Y. Ozaki. Anal. Sci. (Japan) 2004, 935-940.
[4] N. Ogrinc, I. J. Kosir, J. E. Spangenberg, J. Kidric. Anal. Bioanal. Chem. 2003, 376: 424-430.

L-35

GEOGRAPHICAL ORIGIN OF POLISHED RICE BASED ON TOTAL ORGANIC CARBON, NITROGEN, OXYGEN AND COMPOUND-SPECIFIC HYDROGEN ISOTOPE ANALYSES

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In Japan, polished rice, when packaged, requires labels indicating cultivar, cultivation area, and year of production in accordance with the Japanese Agricultural Standard (JAS) Law. Rice cultivar and cultivation area are important factors in determining the market value of polished rice. However, the geographical location or rice cultivar cannot be distinguished by image analysis, leading to rice authenticity problems such as mislabeling and addition of inferior rice to premium rice. Thus, an analytical method which identifies these factors is required to resolve these rice authenticity problems.

Generally, carbon (C), nitrogen (N) oxygen (O) and hydrogen (H) isotopic compositions of plant materials reflect various factors such as isotopic compositions of source materials (e.g., CO_2, H_2O, NH_4, and NO_2) and their fixation pathways as well as the growth environment. These characteristics of stable isotope compositions have been used to investigate the authenticity of food materials. Recently, compound-specific isotope analysis has also become increasingly important as a useful tool to investigate the source of organic compounds in various studies such as geochemistry, biochemistry, and environmental chemistry. Therefore, in this study, we determined total organic C, N, O and compound-specific H isotopic compositions ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and δD) of polished rice from various cultivated area, in order to discriminate its geographical origin.

As a first approach, we examined a single cultivar: Koshihikari rice (Oryza sativa subsp. japonica) from various cultivation areas such as USA, Australia and Japan. $\delta^{13}C$ values of American rice samples (-25.6 ~ -25.3‰) are higher than Japanese rice samples (-27.7 ~ -26.0‰), which may depend on humidity in cultivation area. δ15N values of rice samples depend mainly on soil nutrition. Organic fertilizers increase ^{15}N content in rice (+3.1 ~ +8.0‰), whereas the utilization of artificial fertilizers decreases rice $\delta^{15}N$ values (+0.3 ~ +3.2‰). $\delta^{18}O$ values of rice samples are ranged from +18.8 to +22.9‰. δD values of fatty acids in rice samples are contributed from. –207 to -164‰.

The geographical origins of these rice samples are clearly distinguished by a radar plot based on the elemental and isotopic compositions. For example, American and Australian rice is characterized by relatively higher oxygen (by ~3‰) and nitrogen (by ~4‰) isotopic compositions than Japanese rice, respectively. Moreover, diagnostic difference is observed within Japanese rice from different locations. Thus, total organic C, N, O and compound-specific hydrogen isotope compositions will become a useful method for discrimination of geographical origin of polished rice.

L-36

ALPHA-LIPOIC ACID IN DIETARY SUPPLEMENTS: DEVELOPMENT AND COMPARISON OF HPLC-CEAD AND HPLC-ESI-MS METHODS

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Alpha lipoic acid is an antioxidant used both in the prevention and treatment of various oxidative stress related diseases. It is an important constituent of some dietary supplements and can also be found in plant and animal sources. A rapid method for the determination of α–lipoic acid in dietary supplements based on high performance liquid chromatography coupled with a coulometric electrode array detector (CEAD) and an electrospray ionization mass spectrometer (ESI-MS) was developed. First, α–lipoic acid was extracted with methanol by sonication, chromatographic separation was then achieved by isocratic elution [acetonitrile/methanol/50 mM potassium dihydrogen phosphate 305:65:630, v/v/v, adapted to pH 3 with phosphoric acid] using an ACE 3-C-18 column at a flow rate of 0.45 ml/min. Alpha lipoic acid was detected by means of a CEAD at +300, +400, +450, +500, +550, +600, +650, +700 mV against palladium reference electrodes. For ESI-MS detection (negative mode), the composition of the mobile phase was changed to 0.1% acetic acid in water/acetonitrile 55:45, v/v applying a flow rate of 0.2 ml/min. The presented methods were utilized to determine the α–lipoic acid content in six dietary supplements. Both chromatographic methods were validated and the results were in good correlation. Rapid and simple sample preparation with short run time makes these methods highly useful for assaying large stocks of samples from different origin. In addition, high sensitivity and selectivity of both methods could be of importance in pharmacological studies where lower concentrations have to be determined.

L-37
ANALYSIS OF DIETARY SUPPLEMENTS FOR HORMONAL ACTIVITY

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The last decade the use of dietary supplements has increased explosively. In particular the Internet has made it possible for consumers to easily obtain dietary supplements for general consumption as well as for the purpose to enhance growth and performance. Often the information on the label of those supplements is misleading or preparations contain banned compounds like anabolic steroids and prohormones.

In the present work we have investigated the hormonal activity of dietary supplements using a sensitive yeast androgen bioassay as a screening tool. This androgen biosensor is based on the constitutive expression of the human androgen receptor (hAR) in combination with an androgen responsive element (ARE) coupled to an enhanced green fluorescent protein (EGFP) reporter system. Prohormones show no or low activity in the androgen bioassay, most likely because yeast cells lack biotransformation capacity. Therefore, in order to test preparations for the presence of prohormonal compounds, sample extracts were incubated with liver S9 enzyme fractions to mimic *in vivo* metabolic activation, prior to application on the androgen biosensor.

We screened 18 supplements which were intercepted at the post office by the Belgian pharmaceutical inspection service and were already tested previously by LC-MS/MS for the presence of 49 anabolic steroids [1]. In addition we screened dietary supplements randomly bought in local shops or ordered via the Internet. Suspect samples were analyzed using a high resolution LC/LCT Premier MS system or UPLC QTOF MS with accurate mass measurement for chemical identification.

In total 15 supplements gave a positive result in the yeast androgen bioassay. After liver S9 incubation 1 additional preparation was screened positive for the prohormone dehydroepiandrosterone (DHEA). The results showed that the androgen bioassay, particularly in combination with a metabolic activation system, has a surplus value for screening preparations for the presence of hormonally active compounds in comparison to screening with LC-MS/MS alone.

[1] C. Van Poucke, C Detavernier, R Van Couwenberghe, C van Peteghem, 2007. Determination of anabolic steroids in dietary supplements by liquid chromatography-tandem mass spectrometry, 586, 35-42.

L-38

SCREENING TECHNOLOGIES TO REVEAL MICROBIAL AND NON-MICROBIAL CONTAMINATED ANIMAL POPULATIONS

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Food scares and the adaptation of food safety laws that followed, have boosted scientific research in the field of detection of microbiological and non-microbiological contaminants. In particular, animal reservoirs of zoonotic agents at population level are targeted at the farm or the abattoir to facilitate adequate and timely measures to control spreading of contaminated animal products in the food-chain. Monitoring of a humoral immune response following an invasive infection is considered a good strategy to identify risk herds or flocks. At this control level, serum may also be targeted to reveal the presence of chemical contaminants, including residues of veterinary drugs. Given prevalence of the contamination, number of samples, low return on products and necessary speed, rapid and cost-effective high-throughput screening methods are required that facilitate multi-analyte and high-throughput analysis [1]. Such technologies are now available and this study presents the results of detecting anti-Salmonella (delivering information on presence of serogroup B, C1, C2, D and/or E), anti-Trichinella spiralis and anti-Toxoplasma gondii antibodies in pig and poultry matrices, including serum, eggs, meat drip and plasma using platforms, such as surface Plasmon resonance biosensors [2,3], chip-based electrochemical detectors and microsphere-based flow cytometry. The microbial assays were combined with a multi-sulphonamide test [4] and enabled rapid detection of microbial and chemical (residue) contaminants in a single run in a single sample. Here, the detection of circulating sulphonamide residues reflects the presence of their counterparts in edible tissues, such as muscle, liver, kidney. The performance of the developed assays was compared to suitable counterparts, such as residue or serum-antibody detection-based ELISA's, showing an improved performance of the new technologies. So here we have analytical instruments with an extensive measuring capability used to detect a vast array of serum components to determine the contamination status of an animal population at the farm or the abattoir to protect community health through secured food safety. This paper will discuss the benefits and disadvantages of each technology and it will show preference for the microsphere-based detection principle.

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[1] Bergwerff AA & F Van Knapen (2006) Surface Plasmon Resonance Biosensors for Detection of Pathogenic Microorganisms: Strategies to Secure Food and Environmental Safety, J AOAC Int 89:826.
[2] Jongerius-Gortemaker BGM, Goverde RLJ, van Knapen F &. Bergwerff AA (2002) Surface plasmon resonance (BIACORE) detection of serum antibodies against Salmonella enteritidis and Salmonella typhimurium, J Immunol Meth 266:33.
[3] Thomas E, Bouma A, van Eerden E, Landman WJM., van Knapen F, Stegeman A &Bergwerff AA (2006) Detection of egg yolk antibodies reflecting Salmonella enteritidis infections using a surface plasmon resonance biosensor,J Immunol. Meth 315:68.
[4] Haasnoot, W., Bienenmann-Ploum, M., Lamminmäki, U., Swanenburg, M., van Rhijn, H. (2005) Application of a multi-sulfonamide biosensor immunoassay for the detection of sulfadiazine and sulfamethoxazole residues in broiler serum and its use as a predictor of the levels in edible tissue, Anal. Chim. Acta 552:87.

L-39

DEVELOPMENT OF IMMUNOSENSORS BASED ON OPTICAL WAVEGUIDE LIGHTMODE SPECTROSCOPY (OWLS) TECHNIQUE FOR DETERMINING MYCOTOXINS IN FOOD

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Mycotoxins are toxic metabolites produced by a number of different fungi, and can be present in a wide range of food and feed commodities. Some mycotoxins are rather rare, others, such as aflatoxins, ochratoxins and zearalenone have been lately quite common. Routinely used methods for the testing of mycotoxins in food-processing industries include TLC, HPLC, ELISA and immunoaffinity columns. Yet none of these methods allow the reliable, specific rapid screening of mycotoxins in real time parallel to the industrial production process.

The Optical Waveguide Lightmode Spectroscopy (OWLS) technique as a label-free immunosensor has been successfully applied for the detection of a number of different compounds in both competitive and in direct assays. In order that the sensitised surfaces to be regenerable, i.e. the sensor can be applied several times, the antigen or the antibody is immobilized on the surface by covalent attachment to silanized surfaces.

When measuring in direct manner the appropriate antibody was immobilized on the sensor surface and the linear measuring range determined. During the competitive measurement the mycotoxin-BSA conjugate was immobilized on the waveguide surface. Standard solutions containing different amount of toxins were mixed with antibodies of appropriate concentration, the mixture was incubated for 1 minute and injected into the OWLS system. Binding of the antibodies in the sample to the coated surface is competed for with free mycotoxin in the sample and only antibodies remained in free form in the mixture bound to immobilized antigen-conjugates. The amount of antibodies bound to the surface of the chip was inversely proportional to the toxin content in the samples. The linear measuring range and the relative substrate specificity of the antibody was studied.

Different type of grain samples and species were spiked with mycotoxins and were analysed using the newly developed methods and a high degree of correlation was observed between the spike level and the detected value using the biosensor.

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L-40

MULTIPLEX DETECTION OF (GM) PLANT DNA TARGETS WITH A PADLOCK-LIGATION METHOD

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Genetically modified (GM) plants are either approved or unapproved for use in the EU. For approved GM lines, a 0.9% labelling threshold level applies, while for unapproved ones a zero tolerance is maintained. With conventional real time PCR, these demands can be met up to a 0.1% level for most targets. While this sensitivity is adequate, the number of different approved (20+) and unapproved (100+) GM plants make detection and especially identification of GM material in food a time-consuming and expensive puzzle in many cases. There is a clear need for a method that can identify as many GM lines as possible within a limited set of experiments at a sensitive level. Such a method would have to include direct detection of event (GM-) specific targets for approved GM lines and unapproved ones for which this information is available, but also GM-element and plant species specific targets. This is necessary as event specific information is not always available for non-approved lines. In these cases, a combination of elements known to be absent from any approved variety can lead to the identification of unapproved GM plant material.

We report on the start-up of such a system. It consists of a combination of ligation detection, PCR amplification and microarray identification, the so-called padlock approach. The padlock approach is based on the principle that a unique DNA sequence is detected by a padlock probe in isolated plant DNA. Only when both ends of the padlock probe hybridize juxtaposed to their specific complementary target sequence, ligation can occur and will result in a circular molecule. After ligation, universal primer sites in the padlock probe will enable amplification of only the circularized padlocks. Each amplified (Cy3-labelled) padlock probe contains a unique ZIP code (a random 20 nucleotide sequence). Only amplified padlock probes will yield a signal when the pool of PCR products is hybridized to a microarray with complementary ZIP codes.

So far we have developed probes for a soy GM line, a maize GM line and 6 plant species. We have detected positive targets in mixtures of up to seven DNA targets. Sensitivity down to 1% was reached for several targets. This clearly indicated that the padlock approach is a very promising one. We are currently expanding the multiplicity and improving the sensitivity of the method. We will report on the latest status of the specificity, sensitivity and multiplicity of the padlock approach.

L-41
ADVANCES IN CHROMATOGRAPHY: TOWARDS MORE COMPLEX AND SOPHISTICATED METHODS

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No doubt, food analysis profits from improved chromatographic technology, such as more selective sampling, faster chromatography, two dimensional separation or improved coupling to mass spectrometry, but these are relatively small contributions to a wealth of existing techniques. The main advances are by better use of these techniques, better combinations and the design of more sophisticated analytical methods.

The analysis of the migration of polyadipate from plasticized PVC into oily foods serves as example. Polyadipates are probably the plasticizers to be used in future, and a rather accurate analysis is required since migration tends to be close to the EU legal limit (30 mg/kg food). Polyadipates are complex mixtures of various compositions and the limit refers to the sum of the components below 1000 Da. The method involves transesterification of the oily food extract and the determination of the resulting dibutyl adipate. Then a conversion factor must be determined to calculate the measured dibutyl adipate into the migrated material of the polyadipate actually used - which is the most demanding step.

The design of the method and its verification system is outlined to show the direction into which advances are possible, but also required by food control. The many techniques available must be integrated into consolidated complex methods. Some thoughts on the consequence of such a trend for food analysis are added, such as the required broad competence of the analysts, the development of modules and the better management of the costs of method development.

Literature on polyadipate analysis
[1] Polyadipates used as plasticizers in food contact: Fraction below 1000 Da determined by size exclusion chromatography with evaporative light scattering detection and segmental response linearization or UV detection. M. Biedermann, K. Grob. J. Separation Science 29 (2006) 114-122.
[2] GC-MS characterization of oligomers in polyadipates used as plasticizers for PVC in food contact. M. Biedermann, K. Grob. Packaging Technology and Science 19 (2006) 159-178.
[3] GC method for determining polyadipate plasticizers in foods: transesterification to dibutyl adipate, conversion to migrating polyadipate. M. Biedermann and K. Grob. Chromatographia 64 (2006) 543-552.

ALLERGEN WORKSHOP

(AL1 – AL7)

LA-1
CHALLENGES IN DETECTING FOOD ALLERGENS – ANALYTICAL METHODS IN THE LEGAL CONTEXT

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Allergen assays have never been used as much as in recent days. This is predominantly due to new legislation having come into force in the USA and Europe. While both, USA and Europe have the 'big eight' allergens, i.e. egg, milk, fish, shellfish, tree nuts, peanuts wheat and soya, Europe in addition requires mustard, celery, sulphite and sesame – and shortly – molluscs and lupine - to be labelled. Currently, allergens are routinely detected by either PCR or ELISA assays, with ELISA being the more established test method.

In order to enforce the regulation, assays for the detection of allergens need to be reliable and robust. A first step is a validation exercise, e.g. AOAC PTM or OMA validation. While this is a very important towards gaining insight into validation-relevant parameters, it is impossible to mimic all possible matrices in such a study. Therefore, the data generated by the routine-user-community will give in-depth information which matrices may not be suited for such a test and which ones are.

The presentation will look at standardisation and validation approaches for allergen detection, discuss the challenges for enforcement and ideas how to handle such issues and problems in the future.

LA-2
HEALTH CANADA`S CURRENT SURVEILLANCE ACTIVITIES FOR UNDECLARED FOOD ALLERGENS

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Surveillance activities can provide important information about the nature of products that are available in the marketplace. The food allergen research program at Health Canada has recently initiated food allergen-related surveillance activities. A survey of chocolate and granola bars was conducted for the presence of selected tree-nuts and peanut. Sampling targeted products with allergen precautionary statements, allergen-free claims and no specific mention of the targeted priority allergen on the product label. Samples were analyzed for peanut, almond, hazelnut and brazilnut, using in-house and commercially available ELISA-based test kits. Survey results, including a small number of samples found to contain undeclared allergens, will be discussed. Preliminary interpretation of the results allowed to ascertain how often, and at what levels, allergens were detected in the samples with precautionary labelling. Beyond specific risk management decisions that were initiated as a result of reports of undeclared allergens, results from this study are being used in support of the on-going policy review for the use of allergen precautionary statements on labels of pre-packaged foods in Canada.

LA-3
PROFICIENCY TESTING (FAPAS®) FOR ALLERGENS IN FOOD

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Proficiency testing in the area of allergens in food presents particular challenges in that the true value of the allergen content in food matrices and the target standard deviation of the methodology are either unknown or at best not well-defined. However, these difficulties rather than precluding the running of proficiency testing, tend to reinforce its importance as a tool, which can be used for aiding and improving analytical measurement. Thus, in these early stages of this type of proficiency testing, it may not always be possible to give definitive scores in terms of laboratory performance (z-scores). The results however provide valuable insights into the degree of difference between approaches to determining allergen content. The results also inform end-users of the limitations of the results currently obtained by labs from these determinations. FAPAS® has distributed test materials covering nuts (peanut and hazelnut) in chocolate, soya and soya protein in milk products, gluten and sesame in infant cereal, and β–lactogluten in infant soya formula. For these rounds participants have tended to use commercial ELISA test kits, which not unexpectedly have given different results as antibodies raised against different proteins are employed by each manufacturer. Proficiency testing for allergens is still in its early days and from experience in other areas with increased participation improved performance can be anticipated.

LA-4
FOOD ALLERGEN TESTING - TODAY

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More than 170 foods have been documented as causing food allergies, but more than 90% of severe reactions are due to a small group of foods or food groups, sometimes referred to as critical allergens. These foods or food groups encompass milk, eggs, fish, crustacea, peanuts, soybeans, (tree) nuts, cereals containing gluten, molluscs, sesame, celery and lupine.

Detection methods able to trace allergens can be classified into three major groups:

Immunological tests allowing the detection of more or less allergenic proteins (ELISA or lateral flow devices)

PCR-based detection methods allowing the detection of marker DNA, which is correlating with the presence of allergenic protein

Alternatives or emerging techniques like LC-MS/MS

The currently mostly used methods, the immunological and PCR-based detection methods, but also few alternative methods will be reviewed. The scope of application, target analyte of the analytical tests, and reporting unit for the results will be discussed. Particular attention will also be given to the limitations of the methods.

The focus will then be drawn on the importance of validation prior to using any method. Finally, sampling will be addressed, explaining when it is required for effective allergen management.

LA-5
QUANTIFICATION OF FOOD ALLERGENS BY MASS SPECTROMETRY

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Food allergies are a serious public health issue. Allergen-adulterated foods can potentially be very harmful to sensitive individuals. Mass spectrometry has the sensitivity and specificity to be used as a confirmatory method for positive ELISA tests, and qualitative confirmatory testing using mass spectrometry has been shown to be a practical technique for allergens. However, quantification is difficult using mass spectrometry since many variables, such as ionization efficiency, can affect the response for the analytical target in question. Methods such as standards addition can be useful, but may not take into account the myriad of matrix-induced variations in signal response. Isotopically labeled internal standards can reduce this problem since the isotopic standard should have the same response as the sample. We will discuss a variety of isotopic labeling methods such as O^{18} labeling, iTRAQ, AQUA that can potentially be used for quantification of food allergens in adulterated foods.

LA-6
NEW SCREENING TECHNOLOGIES FOR FOOD ALLERGENS

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In recent years, different target groups have become interested in screening foods for the presence of trace amounts of food allergens. This has resulted in the demand for custom-made screening methods that can meet the various needs of, for instance, allergic consumers, food manufacturers, and control authorities.

At present, immunoassays are the most widely used methods for allergen detection. Besides the more classical single analyte immunoassays, like ELISA and lateral flow devices, new antibody based screening methods are upcoming. These new methods include various biosensor and flow cytometry based assays, whose speed and option for simultaneous detection of multiple-allergens are their main advantages. However, the relatively expensive equipment and need for trained personnel makes them suitable for larger companies or control authorities only. It is clear that each detection method has its own advantages and disadvantages and, that the required analysis speed, ease-of-use, number of samples, need for multiple allergen screening and available budget will determine the suitability of each method in a specific situation.

In this presentation (preliminary) results for biosensor immunoassays and flow cytometric immunoassays aimed at the detection of multiple allergens are presented. With the high-priced Surface Plasmon Resonance (SPR) biosensors used, Biacore and IBIS (microarray format), 4 or over 100 allergens can in principle be detected simultaneously. With the much cheaper LUMINEX with XMAP^{TM} technology over 100 different compounds can, in theory, be detected at once. These new screening technologies are compared with classical ELISA and lateral flow devices, and the applicability of the different methods for use by different target groups are presented.

LA-7
MOLECULAR PROBES FOR FOOD TRACING AND QUALITY CONTROL OF PLANT PRODUCTS

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Quality control of agricultural products and food material requires that sensitive and reliable methods are available which allow detection and unambiguous identification of contaminants. This can also be extended to the area of food safety/consumer protection where appropriate tests will detect trace amounts of material derived from allergenic plant or animal species. Over the past few years our laboratory has been interested in developing molecular tools that can be used to detect undesired contaminants in agricultural and food products. These tools are based on unique and novel genomic DNA sequences that we identified in target genes (LIM protein genes) following sequence determination and analysis. Results of our analyses of LIM genes in several monocotyledon species including different Triticum species, as well as several species and varieties of Ryegrass (Lolium perenne, L. multiflorum var Italian and var Westerworld) are presented. Primer pairs have been designed that allow amplification of single fragments of different lengths specific for the various species. Primer design was primarily based on sequence differences caused by short deletions/insertions in the target gene. These primer pairs can also be used in Multiplex-PCR for simultaneous detection of several species in a same mix. The work is extended now to allergenic plant species such as various tree nuts. This work is supported by a Research Grant from the Luxembourg National Research Fund as well as by the Luxembourg Ministry of Culture, Higher Education and Research.

POSTER SESSION

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GENERAL FOOD ANALYSIS

(A1 – A37)

A-1
ANALYSIS OF PCBS IN FISH OIL BY GC/MS USING A DEANS SWITCH WITH COLUMN BACKFLUSHING

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Fish oils contain high levels of EPA (eicosapentanoic acid) and DHA (docosahexanoic acid), omega-3 fatty acids that are thought to have beneficial health affects. The American Heart Association recommends that people eat fish twice a week or take a daily fish oil supplement. However, fish high on the aquatic food chain, can bioaccumulate fat-soluble pollutants. Among these are polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). Therefore, fish oil used in supplements undergoes a variety of analyses, including tests for halogenated pollutants. This poster focuses on the analysis of PCBs in fish oil.

Current Methods require that fish oil be cleaned up prior to analysis in order to remove high boiling components that would otherwise lead to contamination, carryover, and retention time shifts. Solvent partitioning, solid phase extraction, and gel permeation chromatography (GPC) are commonly-used techniques. Analysis is by gas chromatography with high resolution mass spectral detection or high resolution MS/MS (GC-HR-MS or GC-HR-MS/MS). The disadvantages of this method is that sample preparation is time consuming and HR-MS systems are very expensive. Long column bakeout times reduce productivity and can shorten the life of GC columns.

In this paper, we propose a much simpler and less costly method for the analysis of PCBs in fish oils. An Agilent 7890A GC, equipped with dual electron capture detectors (ECDs) and a Deans switch, is used to heart cut 7 indicator PCBs (IUPAC congeners 28, 52, 101, 118, 138, 153, and 180) from the primary DB-XLB column on to a DB-200 column for further separation. Fish oil from a supplement capsule is simply diluted 1:10 in isooctane and injected directly. To prevent carryover, contamination, and retention time shifts, the Deans switch is used to backflush the primary column at the end of each run.

A-2
DIRECT DETECTION OF THE DEGRADATION OF FOOD BY A SPECIFIC COLOUR CHANGE

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The detection and identification of food borne pathogens and the detection of microbial food deterioration continue to rely on conventional culturing techniques. These are very elaborate, time-consuming and expensive. The existing test methods are completed in a microbiology laboratory and are not suitable for on-site monitoring.

Therefore we have developed an optical thin film sensor chip able to detect the decay of food through a specific colour change. The design of the sensor relates to the phenomenon of “anomalous absorption”, which can best be described as a thin film enhanced absorption. A metal cluster film positioned at a well defined distance to a smooth metal surface shows that the minimum of spectral reflectivity strongly depends on the thickness of the interlayer: This setup represents a special kind of reflection interference filter. In such a sensor setup we have integrated a biodegradable polymer which is degraded by the same enzymes and at the same rate as food decay will happen. The degradation of the polymer results in reduction of the film thickness and thus in a specific change of the colors.

A-3
SELECTIVE DETERMINATION OF L-GLUTAMATE IN FOODSTUFFS BASED ON AGGREGATION PARAMETER MEASUREMENTS

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L-Glutamate is used worldwide to enhance the flavour of many foodstuffs. This additive is generally recognized as safe at typical ingestion levels, but high doses may result in certain adverse physiological effects. The maximum content of this substance in food products is regulated in most countries (e.g. the limit established by the European Union is 10 g/kg). Because of the complexity of samples, laborious and expensive analytical procedures involving ion-exchange extraction, chromatographic separations or enzymatic reactions are used by food industry laboratories to achieve the required selectivity to determine L-glutamate in foodstuffs.

In this work, a simple, rapid and low-cost analytical method, suitable for the direct routine monitoring of L-glutamate in food products, is presented. It is founded on an aggregation parameter-based methodology recently developed by our research group, namely the surfactant to dye binding degree (SDBD) method [1]. This method is based on the competition established between an ionic dye and the analyte to interact with an ionic surfactant bearing opposite charge to that of the dye. The dye and surfactant used for the determination of L-glutamate were Coomassie Brilliant Blue G (CBBG) and didodecyldimethylammonium bromide (DDABr), respectively. CBBG and DDABr form mixed dye-surfactant aggregates, which are monitored photometrically. In the presence of L-glutamate, the amount of DDABr required to reach a given CBBG-DDABr binding degree (m_s*) increases compared with that needed in its absence (m_s), as a result of the interaction between analyte and DDABr molecules. This interaction occurs through both attractive electrostatic interactions between the γ-carboxylate group in the analyte and the ammonium quaternary group in the cationic surfactant, and hydrophobic interactions between the hydrophobic moieties in both L-glutamate and DDABr molecules. A linear calibration is obtained by plotting m_s*-m_s as a function of the analyte concentration.

The proposed method provides the sensibility (quantitation limit= 0.2 mM) and selectivity required for the direct determination of L-glutamate in foodstuffs. Amino acids, the main interferent compounds in L-glutamate quantitation, do not interfere at the concentrations present in food products. The applicability of this new approach to the determination of L-glutamate in food samples was demonstrated by analyzing liquid and dried soups, paste sauces, mushroom creams and seasonings. Measurements were performed after a minimum sample treatment; dilution of liquid samples and dissolution in water of solid samples, and filtration to remove non-soluble components. Results obtained were consistent with those provided by a commercial enzymatic colorimetric test, the Boehringer Mannheim kit. The precision, expressed as relative standard deviation for the whole analytical process ranged between 1.3 and 1.9 % (n=6).

[1] R. Fabios, D. Sicilia, S. Rubio, D. Pérez-Bendito, Anal. Chem.,75 (2003) 6011.

A-4
CARBONYL VALUE IN MONITORING OF THE QUALITY OF USED FRYING OILS

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In this study, a set of frying oil samples of different compositional properties but passed qualitative and quantitative standards, which were of various vegetable oil sources (individually or as blends), obtained from seven of big oil factories in Iran. Before starting the frying process, all the frying oils had carbonyl values (CV) higher than 2 μmol/g. CV of most frying oils linearly increased until the end of the frying process, whereas for some of them, CV increased and reached a maximum and then decreased to some extent. However, in a set of frying oil samples on average, CV linearly increased as the frying time increased. There was a linear relationship between CV and TPC throughout the frying process with a high determination coefficient (R2 = 0.9747). The values found for carbonyl compounds of the frying oils during frying process ranged from 7.76±0.00 to 123.45±3.70 μmol/g. Assuming that the limit of acceptance for TPC is 24%, this was roughly corresponded to 43.50 μmol/g for CV.

A-5
ANALYTICAL STRATEGY TO ASSESS THE SAFETY OF FOODS

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Food safety assessment is currently based on known individual food components. More and more questions concern the assessment of complex chemical mixtures or matrices with a high percentage of unknown compounds. For these complex food products it is unrealistic to identify and quantify the complete forest of peaks and perform a safety assessment for all peaks observed. A pragmatic protocol for safety assessment of complex food products is currently under development, based on an integrated assessment of exposure, toxicology and chemical analysis.

A cost-effective analytical strategy is being developed aiming to assign substances with structural alerts for genotoxicity in complex mixtures present at toxicological relevant concentrations, rather than time consuming peak-by-peak identification and quantification.

The first step is to determine the numbers and amounts of substances present in samples. The next challenging step is to exclude the presence of substances with structural alerts for genotoxicity. For this purpose the threshold of toxicological concern (TTC) principle is used. The TTC principle was defined assuming threshold values for classes of chemicals based on their chemical structures and known toxicity of chemicals that share similar structural characteristics. The lowest threshold (0.15 microgram/person/day) is applicable for genotoxic substances. TNO is setting up analytical strategies that screen for specific functional groups (with structural alerts) using mass spectrometric data and/or after selective derivatisation. The feasibility of the analytical strategy will be demonstrated for several substances with a structural alert (eg aromatic amines).

A-6

ADOPTION AND IMPACT STUDIES OF HIGH QUALITY BAMBARA FLOUR TECHNOLOGY TRANSFER IN THE NORTHERN REGION OF GHANA

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The Adoption and Impact Studies were undertaken in northern Ghana to investigate the level of adoption of a high quality bambara flour technology transferred to selected beneficiary communities in the area. The impact of adoption on end-users and the determinants for adoption were established. A random sample of 100 women, mainly bambara processors, was selected from the project districts including Gushiegu\karaga, Tolon Kumbugu, Savelugu-Nanton and Tamale districts of the Northern region of Ghana for interview. Statistical Package for Social Scientists (SPSS) and Excel were used for deive data analysis and the Logit model used to investigate the determinants of adoption. The survey findings established an effective utilization level of HQBF at 68%. Variables hypothesized to influence adoption of HQBF from the respondents own assessments included time of awareness, consumer acceptability/quality of products, credit, availability of raw materials and sunshine. However, only time of awareness and consumer acceptability/quality of products were statistically significant using the Logit model. The technology had had economic impact on 28% of the processors interviewed as a result of 12.5% increase in demand for bambara based products.

A-7

APPLICATION OF FACTORIAL DESIGN IN OPTIMIZATION OF ANION EXCHANGE RESIN BASED TRANSESTERIFICATION OF VEGETABLE OIL AND FATS

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A simple, rapid and fairly selective method for the preparation of fatty acid methyl esters (FAMEs) based on anion-exchange resin Amberlite IRA-904 catalyzed transesterification of vegetable oil/fat with iodomethane has been described. The vegetable oil and animal fats used were sunflower oil, palm oil, vanaspati (hydrogenated vegetable oil), tallow and butter. A Plackett–Burman factorail experimental design was used as a multivariate strategy for the evaluation of the effects of varying several variables at once. The effects of five different variables amount of resin, strength of sodium hydroxide, volume of iodomethane, heating time and temperature of thermostatic water bath, on the yield of fatty acid methyl esters (FAMEs) have been investigated. From these studies, certain variable showed up as significant, and they were optimized by a using 23 + star central composite design, which involved 16 experiments. The best conditions for transesterification reaction were as follows: amount of resin 2g, strength of sodium hydroxide 0.25 N, volume of iodomethane 400µl, heating time 2 minutes at 70 0C. A standard IUPAC method was used to prepare FAMEs from vegetable oil/ fats for comparative purpose. Finally samples of oil/ fat obtained from both methods were analysed by Gas liquid chromatography. Analytical results for the FAMEs by resin based proposed method, and conventional IUPAC method showed a good agreement, thus indicating the possibility of using Amberlite IRA-904 based transesterification instead of intensive treatments inherent with the conventional time consuming methods.

Key words: Factorial design, optimization; gas liquid chromatography, transesterification, edible oil and fats

A-8

AMINE OXIDASE-BASED AMPEROMETRIC BIOSENSORS FOR BIOGENIC AMINES DETECTION USING LIQUID CHROMATOGRAPHY

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Due to their importance, the determination of biogenic amines in food represents a new and modern topic in nowadays research. Therefore, this work was focused on the development of a new analytical system for the determination of biogenic amines in fish and meat samples. The novelty consists in using a highly selective bioelectrochemical detector coupled to a weak cation-exchange column specially designed to discriminate between different mono and polyvalent amines.

A bienzymatic approach, based on a recently isolated amine oxidase from grass pea (GPAO) and commercially horseradish peroxidase (HRP), was considered. The bioelectrode design involved the immobilization of both enzymes on solid graphite. The biosensors were operated at a low potential, where biases from interferants are minimal [1]. The separation and identification of six biogenic amines -tyramine (Tyr), putrescine (Put), cadaverine (Cad), histamine (His), agmatine (Agm) and spermidine (Spd)- were investigated. Once the HPLC separation was optimized (see figure 1), quantitative determinations for each amine were performed with a good reproducibility. Biogenic amines from fish muscle (i.e. cod) were extracted using methanesulfonic acid [2] and were analyzed using the new analytical system.

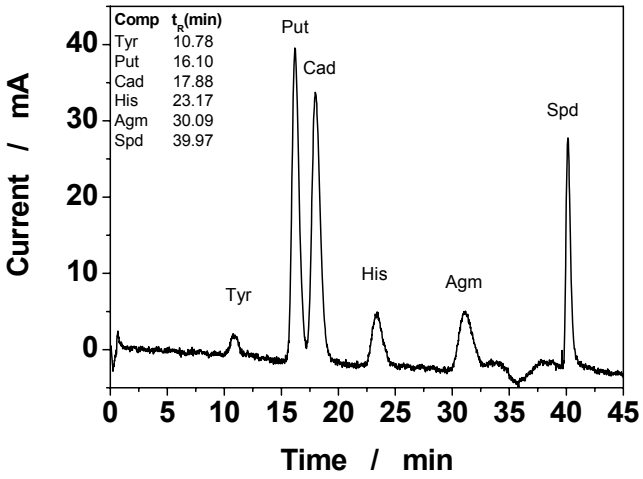

Fig. 1: Chromatogram of a standard solution of six biogenic amines. *Experimental conditions:* applied potential, -50 mV *vs.* Ag/AgCl, KCl_{sat}; flow rate, 0.9 ml/min, substrates concentration, 250 µM in MSA.

[1] Niculescu M., Nistor C., Frébort I., Peč P., Mattiasson B., Csöregi E., Anal. Chem. 72(7) (2000), 1591-1597.
[2] Cinquina A. L., Cali A., Longo F., De Santis L., Severoni A., Abballe F., J. Chromatogr. A 1032 (1-2) (2004), 73-77.

A-9

NMR STUDY OF SOFT CHEESE DEGRADATION FOR THE ASSESMENT OF PACKAGING PERFORMANCES

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The primary task of a packaging is to preserve the quality and safety of a foodstuff in the time. The determination of the package performances in terms of quality preservation of the foodstuff requires the evaluation of several physical and chemical properties in time. The maintaining of the value of some of these parameter in an acceptable range during the degradation process determines the shelf life of the food. In this work we evaluate the quality of two Italian cheese by NMR profiling inside and outside the package. We performed a degradation test on two Italian soft cheese at room (15°C) temperature and in the fridge (4°C), either in the air and in the package. The chemical composition of the cheese as a function of time is compared for the samples inside and outside the packages. The shelf lives of the samples are calculated and compared each other in order to evaluate the package efficiency. The recently emerging application of unilateral NMR (low field) to food assessment suggest us to begin study relaxation time T1 during the degradation process. Some preliminary results on Italian soft cheese are reported.

A-10
PRESSURISED SOLVENT EXTRACTION AND HPLC/DAD-MS/MS ANALYSIS OF ANTHOCYANINS FROM RED CABBAGE

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Red cabbage (*Brassica oleracea* L. var. *capitata f. rubra*) is a native vegetable of the Mediterranean region and south-western Europe that now grows in regions all over the world. Red cabbage belongs to the family of Brassicaceae, and epidemiological studies have stressed the capacity of *Brassica* species to prevent cardiovascular diseases as well as their activity against some types of cancer. Between the substances, that seem to be responsible for those properties, are polyphenols, and red cabbage is a rich source of phenolic compounds, with the anthocyanins being the most abundant class. Anthocyanins are a group of plant pigments that are widely distributed in nature, among flowers, fruits and vegetable, and are responsible for their bright colors such as orange, red and blue. They have also demonstrated to play an important role in the plant physiology and are valuable for food industry as well as in human health. [1,2]

This work reports the use of pressurized fluid for the extraction of anthocyanins from red cabbage; coupled with a monolithic column HPLC/DAD system to accomplish a fast analysis. Pressurized fluid extraction is an alternative, environmental friendly and innovative technique that combines elevated temperature and pressures with liquid solvents to achieve fast and efficient extraction. Monolithic columns represent the most interesting innovation, due to advantages such as shorter analysis times, faster column equilibration, lower backpressures, less contribution with diffusional mass transfer and smaller void volumes in the stationary phase. The best extraction conditions for a majority of the anthocyanin peaks were: 2.5 g of sample; 99°C (at 50 bar); 7 min of extraction and a solvent composition of water/ethanol/formic acid (94:5:1, v/v/v).

Moreover, the HPLC/DAD/MS/MS analyses of 24 anthocyanins, separated by a high resolution liquid chromatography system are reported. In all of the anthocyanins identified in the red cabbage extract, cyanidin was the aglycon, represented as mono- and/or di-glycoside, and some of them acylated, with aromatic and/or aliphatic acids.

[1] Wu, X., and Prior, R.L. Identification and characterization of anthocyanin by High-Performance Liquid Chromatography - Electrospray Ionization-Tandem Mass Spectrometry in common foods in the United States: vegetables, nuts and grains. J Agric Food Chem, 2005, 53, 3101.
[2] G. Mazza and E. Miniati, Anthocyanins in fruit, vegetables, and grains; CRC Press: Boca Raton, FL, 1993, p. 362.

A-11
INNOVATIVE QUALITY AND SAFETY MEASURES TO ENABLE THE DETECTION OF MICROBIAL SPOILAGE THROUGHOUT THE FRUIT JUICE PRODUCTION PROCESS

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Lactic acid bacteria (LAB) are ubiquitous in agricultural products like fruit and cereals. They are used for the production of fermented products but also increasingly reported as promoters of food spoilage, affecting the fruit juice industry and leading to huge financial losses. The presence of LAB often remains unnoticed until several days after the onset of activity, which allows the bacteria to spread and infect huge volumes of juice and parts of the production line. When detected, the organoleptic properties of the juice do not permit a further use for human consumption.

The collective research project QUALI-JUICE aims to develop an easy-to-use biosensing system to provide early warning of the activity of LAB that will allow on-line quality control during juice production.

The project makes use of L-lactate enzyme biosensor technology. This technology enables the determination of changes in the microbial activity by direct measurement of the microbiological fermentation product during the process or in storage tanks.

During the project on- and off-line biosensors are being tested. The project targets at the development of automated sampling systems and hand-held devices suitable for juice production lines and/or companies lacking automated process control facilities. The QUALI-JUICE prototype will be suitable for the on-line analysis of lactate in juice production at the very beginning of the juice spoilage and will be coupled with the process control scheme of the juice production lines. After testing of the biosensors under laboratory conditions, the devices will be used within the installations of the juice production line, making it possible to identify the needs of juice producers and subsequent improvement of the system. Sampling at various points along the production line is carried out enabling inherent 'bacterial breeding grounds' to be pinpointed. The use of this system is calculated to lower the production costs by at least 3%. The decrease of juice spoilage events will decrease by at least 80%.

This project will also help to detect points of improvement on current legislative and other hygiene and food quality/safety regulations. Furthermore, the QUALI-JUICE prototype can be also of great interest to other sectors, like the production of sauces, vegetable products, mayonnaise, dips and creams, where these contamination problems are also known and reported.

QUALI-JUICE is a Research Project approved for funding by the European Commission under the FP6-2005-COLL.

A-12
TRACE CE ANALYSIS OF PROTEINS USING ABSORPTION DETECTION AND ON-LINE CONCENTRATION TECHNIQUES

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Proteins are one of the main constituents of foods and probably also one of the most complex biopolymers. These components can occur at only trace levels (5% of total protein) within a sample matrix (α-lactalbumin and β-lactoglobulin). Therefore, food analysts need access to highly selective and sensitive analytical methods that are also simple and cost-effective. Capillary electrophoresis (CE) can meet these requirements by offering high-resolution separations at a minimal cost in terms of staff time and reagent use. CE has been shown in recent years to be a viable alternative to traditional gel electrophoresis due to its speed, resolution and automation. One of the major problems in CE analyses of proteins is the adsorption of proteins and peptides to the negatively charged fused-silica surface, which leads to distorted peak shapes and poor separation. We attempted the separation of proteins by using a high-ionic strength buffer at high pH (borate buffer at pH about 10.4), to increase the separation efficiency and eliminate the protein adsorption and aggregates. CE inevitably has relatively poor detection limits. To overcome these drawback sensitive detection methods other than UV was applied to protein research. The primary goal of this work is to improve the sensitivity for the trace analysis of protein in CZE using absorption detection. On-line sample preconcentration methods (stacking, sweeping, etc.) represent an effective and versatile way to enhance the UV - concentration sensitivity in CE. Using a model system of bovine serum albumin we have compared the five stacking methods, including field-amplified sample stacking (FASS), head-column field-amplified sample stacking (HCFASS), large volume sample stacking (LVSS) with reversed polarity, stacking with polymer solution and dynamic pH-junction, by sensitivity enhancement factor and reproducibility. A simple CZE-UV method was developed using on-line concentration techniques (LVSS and stacking with polymer PEO solution) to determine the trace amount of albumin “Nanophor 01” (IAI) (UV at 214 nm) with deactivated capillaries. The best results were achieved with the second approach: 120-fold (10 μg/ml) improvement in detection sensitivity and no need to desalting processes.The advantage of the first strategy is that it can be coupled with mass spectrometric detection systems. With this CZE method, theoretical plate numbers in the range of 300 000 – 500 000 were obtained with excellent repeatabilities for migration times and peak areas (RSD 0.085% and 2–4%, respectively).

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A-13
A RAPID SPECTROPHOTOMETRIC METHOD FOR THE QUANTIFICATION OF CONJUGATED FATTY ACIDS

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Conjugated fatty acids (CFA), the general term of positional and geometric isomers of polyunsaturated fatty acids with conjugated double bonds, have attracted considerable attention because of their potentially beneficial biological effects. So far these CFA, such as conjugated linoleic acid (CLA) and conjugated linolenic acid (CLN) isomers, have been usually measured by gas chromatography but this technique requires a time-consuming previous procedure of methylation. It is known that these isomers have the capability of absorbing radiation due to their conjugated double bonds. Related to this, some authors have already optimized the methodology for the quantification of two CLAs: 9c, 11t and 10t, 12c-18:2 which show an absorption maximum at 233 nm. However there are no reports about 9t, 11t-18:2 and CLNs: α-calendic (8t, 10t, 12c-18:3); ß-calendic (8t, 10t, 12t-18:3); eleostearic (9c, 11t, 13t-18:3); jacaric (8c, 10t, 12c-18:3) and punicic (9c, 11t, 13c-18:3) acids, so it would be interesting to determinate the spectrophotometric behaviour of these compounds. Therefore, the objective of this study has been to evaluate a rapid alternative methodology for the quantification of CFA using spectrophotometric techniques.

The UV spectrum of the CLAs and the CLNs was analyzed to establish their absorption maximum, at which the UV standard curve of each isomer was constructed using different concentrations of pure isomer. In addition these isomers were quantified by gas chromatography. In order to verify the suitability of this methodology a comparison between the quantification of the solutions based on the standard UV curve and the gas chromatographic method was established.

As a result, while all the CLA isomers registered an absorption maximum at 233 nm, some differences among the CLN isomers were found. β-calendic acid presented a maximum UV absorption at 268 nm; α-calendic and eleostearic acids at 270 nm and jacaric and punicic acids at 273 nm. Also the CLNs showed narrower absorption bands than the CLAs. The reason for these differences is not clear yet.

None of the isomers studied showed significant differences ($p \geq 0.05$) to the quantification regardless of the method used, demonstrating that the methodology proposed could be a faster and equally accurate alternative to the chromatography for quantifying CFA.

A-14
COUMARIN IN CINNAMON MARKETED IN ITALY: A NATURAL CHEMICAL HAZARD?

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A diet with a high proportion of vegetables and fruits is considered to be healthful and is suggested by nutritionists. However, some plants that are processed into foods often contain natural substances that may be dangerous. One example is coumarin, a flavouring substance which is found in relatively high concentrations in a wide variety of plants. Coumarin is known to cause liver and kidney damage in rats and mice, and there are rare finding of similar hepatotoxicity in humans.

The main source of coumarin in human diet is surely the cinnamon. Besides pepper and vanilla, cinnamon is, in fact, the best-known and most commonly used spice in the world. The name cinnamon is correctly used to refer to Ceylon Cinnamon, also known as “true cinnamon” (from its botanical name Cinnamomum verum). However, other species of the same genus are sometimes sold labelled as cinnamon. This is the case of Cinnamomun aromaticum (Cassia) and Cinnamomum burmanii. Most of the spice sold as cinnamon in the North America (where true cinnamon is still generally unknown) is actually Cassia whereas the more expensive true cinnamon is usually used in the South America and Europe.

In the last years, given its cheaper price, Cassia is replacing true cinnamon also in European food market being largely used in the preparation of some kinds of desserts, biscuits, cakes, chocolate, candies, tea, hot cocoa and liqueurs. For this reason, several European health agencies have recently warned against consuming high amounts of cassia, due to its high content of coumarin. This last is contained at much lower levels in Cinnamomum verum and in Cinnamomum burnanii.

In this study, 35 samples of cinnamon were collected from the Italian market. Moreover, 30 samples of commercial foodstuffs containing cinnamon as ingredient, were also collected. Quantitative determinations of coumarin and cinnamaldehyde were performed by high performance liquid chromatography (HPLC) with UV/Vis detector.

The results have shown that about 51 percent of the samples marketed in Italy and labeled only as cinnamon is actually Cassia, 10 percent is probably a blend of Cassia and true Cinnamon whereas only 31 percent is really true cinnamon. As regards cinnamon-containing foods, the tested samples often exceeded the maximum level fixed in the European Flavourings Directive of 2 mg kg^{-1}. Some foodstuffs contained so much coumarin that the recommended daily dose would already exceed the tolerable daily intake (TDI) established by EFSA and equal to 0.1 mg coumarin kg^{-1} b. w.

A-15
AN OPTICAL FLOW INJECTION ANALYSIS SYSTEM FOR MULTI-NUTRIENT DETECTION IN FOODSTUFF

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In recent years, demand for quality control in food nutrient is increasing. A high performance liquid chromatography and spectroscopy have been developed for measurement of chemical substance. However the equipments are expensive, and need long measurement time and complex operation. A Flow injection analysis (FIA) has been used as the most widely used flow method of chemical analysis. The advantage of FIA is very short time measurement, minute amount of sample requirement and simple to use. In this study, a measurement system for food nutrients with chemiluminescent FIA system was constructed and applied to measure multi-nutrient (glutamic acid, glucose and lactate acid) in foodstuffs. The measurement system consisted of a peristaltic pump, three of enzyme (GLOD: glutamate oxidase, GOD: glucose oxidase and LOD: lactate oxidase) reactors, a photodiode and a personal computer. The GLOD and GOD were immobilized on each polytetrafluoroethylene (PTFE) membranes by crosslinking with a glutaraldehyde, respectively. The LOD was immobilized on the PTFE membrane by photo-crosslinking with a PVA-SbQ. Each enzyme membranes were cut to strip-shaped and crammed to acrylic pipes, respectively. The enzyme reactors were serially concatenated and the each chemiluminescent emission parts in flow line were put on a sensing area of the photodiode. The H2O2 generated by the enzyme reaction oxidized luminol to produce chemiluminescence in the presence of horseradish peroxide. The chemiluminescence was detected by using the photodiode. The measurement system was calibrated with standard glutamic acid, glucose and lactate acid solutions. The calibration range of the system for glutamic acid, glucose and lactic acid were from 2.0 to 60.0 mmol/l, 20.0 to 600 mmol/l and 30.0 to 600 mmol/l, respectively. The glutamic acid, glucose and lactate acid concentrations of food samples such as a lactic acid beverage, vegetable juice, fermented condiment and liquid chemical condiments were measured with the optical FIA system. The vegetable juice was filtered through a qualitative filter paper, and then centrifuged for 10 min at 1000 rpm. The samples were injected 5 µl to carrier stream before the first (GLOD) reactor. The output of the photodiode showed reproducible changes after injections of the each sample. The measurement time for every sample was within 18 min. The glutamic acid, glucose and lactate acid concentrations in food samples could be evaluated with less operation and time.

A-16
EFFECT OF IRRADIATION ON BIOGENIC AMINE FORMATION IN CHEESE DURING STORAGE

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Biogenic amines are a group of biologically active natural compounds, arising mainly from microbial decarboxylation of amino acids. The most important biogenic amines in food are histamine, tyramine, cadaverine, putrescine, agmatine, spermidine and spermine. High amounts of biogenic amines, especially histamine and tyramine, in the human diet may contribute to a wide variety of toxic effects. Biogenic amines in food are of great interest not only for their potential risk to human health but also because they could have a role as chemical indicators of unwanted microbial contamination and processing conditions [1, 2].

The most implicated food group concerning high biogenic amine content is fermented food such as cheese. Cheese represents an ideal environment for biogenic amine production because of the great availability of amino acids and the presence of bacteria.

Nowadays numerous efforts have been made in food science and in the food industry to reduce or to prevent biogenic amines in food. Low dose of gamma irradiation has been considered as one of the useful methods of preservation to extend the shelf life and to reduce microbial population as well as biogenic amine formation in different food.

The aim of this work was to study the effect of low dose irradiation (0, 2, 4, 6 kGy) on biogenic amine formation in Egyptian cheeses during storage (0-90 days) at 10^{o}C. Biogenic amines were extracted with 10% trichloroacetic acid and determined by ion exchange chromatography using an amino acid analyser (AAA 400, Ingos, Czech Republic).

The main amine found in cheese samples was tyramine, followed by putrescine, cadaverine and spermidine. The total amount of biogenic amines in irradiated cheese samples was significantly lower than those of non-irradiated control. The changes of biogenic amines in cheese samples showed significant differences depending on the dose of irradiation. The applied treatments suppressed the formation of histamine and reduced the level of tyramine in the Egyptian cheeses during storage.

Irradiated foods carry a value-added perception of safety among consumers in Egypt, where irradiation is in commercial use. However, in Europe, consumers' acceptance of irradiated foods is being demonstrated slowly. More research is needed to study the application of irradiation technology to cheese in order to determine its effects also on other characteristics.

[1] Beutling DM. Biogene Amine in der Ernaehrung. Springer Verlag Berlin Heidelberg New York 1996.
[2] Halász A, Baráth A, Simon-Sarkadi L, Holzapfel W. (1994): Trends Food Sci Technol 5:42–49

A-17
OPTIMIZATION OF A COPPER-MODIFIED ELECTRODE FOR AMPEROMETRIC DETECTION OF GLUCOSE

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A great number of amperometric bioelectrode have been developed since 1961, when Updicke and Hicks have realized the first glucose biosensor. Last years, metallized enzyme electrodes (e.g. Ru, Rh, Ir, Au nanoparticles incorporated in carbonaceous materials [1] or $Ni(OH)_2$/NiOOH electrodes [2] etc.) exhibiting electrocatalytic properties for H_2O_2 detection, have proved excellent properties for amperometric monitoring of glucose.

By potential cycling between -1 and +0.8 V *vs*. Ag/AgCl,KCl_{sat}, at ambient temperature and in the presence of dissolved oxygen, a Cu electrode contacted with a phosphate buffer solution, gave a Cu/CuO modified electrode. Cyclic voltammetry, performed in different experimental conditions was used in order to characterize and optimize the obtained Cu/CuO electrode. The observed cathodic peaks represent a combination of the reduction of copper oxides and copper phosphate. Using the Cu/CuO electrode as amperometric transducer, glucose selective and sensitive biosensor was constructed. The effect of the enzyme immobilization procedure, enzyme loading and pH of the adjacent solution on the analytical performances of Cu/CuO/GOx biosensor was investigated. Due to the low applied potential (approximately -0.1 to +0.1 V vs. Ag/AgCl,KCl_{sat}), required for the electroreduction of enzymatically generated H_2O_2, the optimized biosensor allow sensitive and selective amperometric biodetection of glucose, even in the presence of various interfering constituents, existing in many food matrix [3].

The above prepared and optimized biosensor was used with good results for amperometric detection of glucose in different fruit juices.

[1] Rodriguez M. C., Rivas G. A., Electroanalysis, 13 (2001) 1179.
[2] Somsundrun M., Kirtikara K., Tarticharoen M., Anal. Chim. Acta, 319 (1996) 59.
[3] Pan D., Chen J., Yao S., Nie L., Xia J., Tao W., Sens. & Actuat. B, 104 (2005) 68.

A-18

COMPARISON OF SEVEN COMMERCIAL HISTAMINE TEST KITS AND HPLC ANALYTICAL METHODS FOR APPLICATION TO SALTED AND FERMENTED FISH PRODUCTS

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Biogenic amines are known to be health concern for food products such as fish. Histamine is determined widely in such products for food safety. The use of practical analytical methods for evaluation of safety risk could aid to control hazards in the products. Therefore, it is important to evaluate currently available commercial practical histamine analysis kits for salted and fermented fish products in comparison with approved analytical methods such as HPLC. In this study, seven commercial histamine test kits (namely, Histamine Food EIA and Hisquick by LND Company, Veratox by Neogen, Histameter and Histaquant by Biomedx; Transia semiquantitative and qualitative by Diffchamb-Raisio) were compared to two different HPLC methods for detecting histamine in several fish products. Different types of fermented and salted fish products from both European countries and Turkey were used in this research.

Results showed that the values of certain types of fish products analysed using some of the commercial test kits significantly agreed with HPLC results although there were significant differences in the results of some of the samples ($p<0.05$) between commercial test kits as well as HPLC methods. The results of two HPLC methods were significantly differed in certain samples that may cause by the presence of salt during the derivatisation step of the methods. Therefore, it was concluded that although some of the test kits gave reliable results in comparison with HPLC methods, it is also necessary to evaluate the reliability of some of the current HPLC methods in terms of the affect of salt or low pH that might affect their method sensitivity. Other types of properties of the methods were also discussed in this study.

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A-19

CAN WE TAKE THE QUALITY OF OUR DRINKING WATER FOR GRANTED? ALUMINIUM DETERMINATION BY ACOUSTIC WAVE SENSORS

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Recently, aluminium content in food and drinks has becoming of great concern due to the knowledge of potential toxic effect of this element. Although there are no reported cases of acute aluminium toxicity in healthy individuals exposed to normal levels of aluminium, several studies have been published which address the possibility of relating aluminium exposure and age-related neurological disorders, such as Alzheimer's disease (AD). Although this linkage between aluminium intake and Alzheimer's disease remain controversial, there are limits for its content in drinking water, and there is no doubt of the severe effects of aluminium intoxication in hemodialysis patients. Encephalopathy, anemia, osteomalacic osteodistrophy and cardiotoxicity are all disorders related to aluminium intoxication in hemodialysis patients.

Aluminium has been also responsible for causing oxidative stress within brain tissue, for having a direct effect on hematopoiesis (the development of blood cells), and for inducing microcytic anemia (resulting of hemoglobin synthesis failure or insufficiency) among other diseases. All these findings cause alarming concern in public health, demanding accurate determination of aluminium.

The analysis of aluminium in water is difficult because of its low concentration. Atomic absorption spectrometry with electrothermal atomization is the technique of choice to determine the low levels of aluminium in hemodialysis solutions and diluting water, although conventional atomic absorption spectrometry (AAS), plasma inductively coupled spectrometry (ICP) and UV/Vis spectrometry can be used in conjunction with preconcentration methods. All these instruments, but specially ICP and AAS, are expensive and new methodologies combining preconcentration methodologies and inexpensive but reliable aluminium sensors based on acoustic wave devices can be attractive alternatives. The aim of this work was to develop a methodology for aluminium determination in waters using an acoustic wave sensor, in order to check whether the aluminium level of water from different sources was below the maximum permissible concentration of aluminium in drinking water in the European Economic Community, fixed at 0.2 mg L^{-1}.

Water samples from domestic network supplies, from public fountains and from private wells, from several places in Portugal, were analysed by the proposed methodology and the results compared with the conventional analytical methodology based on UV-Vis spectrometry. Found aluminium contaminated water samples, with values higher than the guideline values were pointed out and their occurrence briefly discussed.

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A-20

ACCURACY OF QUANTITATION USING EXTERNAL AND INTERNAL CALIBRATION TO ANALYZE DYES IN EXTRACTS OF SPICES

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This paper describes a new and simple method including extraction, HPLC separation and MS/MS parameters for the detection of 13 different azo-dyes (which are of high priority in many European and Asian countries) using Multiple Reaction Monitoring (MRM) on a triple quadrupole mass spectrometer.

The use of Cliquid™ software for Food Testing (easy-to-use software focusing on the typical workflow from LC/MS/MS analysis to automatic report generation) to analyze samples is described. Furthermore a special focus of this work is on ion suppression in various matrices, such as spices and sauces, and how to handle such samples to obtain most accurate quantitative data including a deion of a procedure to determine the ratio of isomeric and co-eluting Sudan IV and Sudan Red B.

A-21

CAN A GENERIC SAMPLE PREPARATION BE USED FOR PREPARING SAMPLES FOR LCMSMS ANALYSIS WHICH MEETS CURRENT EUROPEAN UNION LEGISLATION

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Sulphonamides are a family of broad-spectrum synthetic bacteriostatic antibiotics with activity against most gram-positive and many gram-negative organisms, as well as protozoa. They have been and are used as vetinary medicines and their residues have been found in foods such as eggs, milk, honey and meats. The European Union has set maximum residue limits (MRL) of 100 µg/kg for each parent sulphonamide in food and 10 µg/kg for sulphonamides in baby food. The standard high performance liquid chromatography (HPLC) approach to the analysis of sulphonamides usually involves extensive sample pretreatment in the form of liquid/liquid or solid phase extractions.

This poster presents data acquired on the 3200 Q TRAP® LCMSMS system where a generic approach to sample preparation has been used for the analysis of a set of sulphonamides in several different classes of food including milk, honey, and meat. The approach utilises Dispersive SPE, a technique which has previously been applied as a rapid analytical technique for the extraction of pesticides from fruit and vegetables [1], in combination with an on line solid phase extraction step for sample preparation. This approach offers a simple two stage sample clean-up providing samples, which are suitable for analysis by LCMSMS. Data on recoveries, linearity of response and detection limits for the test set of sulphonamides in different matrices will be presented. Initial results show response to be linear over the tested range and recoveries found to be typically greater than 70%.

A-22

INVESTIGATING SOME QUALITY PARAMETERS OF SALTED AND FERMENTED FISH PRODUCTS OF EU AND TURKISH ORIGIN IN RELATION TO FOOD SAFETY

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In this study, several types of salted and fermented fish products were evaluated in relation to food safety parameters. The products were both provided from commercial companies and household productions. The salt content, pH, aw were determined in all over 60 different samples. Trimethylamine (TMA), thiobarbutiric acid value (TBA), Histamine and nitrosamine content were measured in over 30 samples. Total halophilic and coliform bacteria count as well as presence of some pathogenic bacteria were tested in several products.

The pH value was different from ~ 3.5 to 7.4, aw varied from ~0.81 to 0.98, salt content was found between approximately 3-20%. TMA and TBA values were 4.0 - >40 mg/100 g and 2.0 – 4.0 mg/malonaldehyde/kg, respectively. Histamine values were in the range of < 1.0 – 400 ppm. Nitrosamines were not found in the samples. The values were evaluated in terms of food safety for such products and recommendations were made to control possible health hazard that might originate from such products.

The results also showed that recently produced products that are 1 to 3 months old contained high amount of bacterial counts as well as the presence of several pathogenic bacteria. The products that were stored both at room temperature and cold storage longer between 6- 12 months contained low amount of total bacteria counts as well as coliforms. Only few samples showed the presence of pathogens in these products.

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A-23
PROTEIN PROFILE AND MALT ACTIVITY DURING SORGHUM GERMINATION

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Sorghum is one of the five most important cereals in world food supply. On the semi-arid regions of Africa and Asia, this cereal comprises an important source of energy, proteins, and minerals. However, its poor nutritional quality has deleterious effects on the nutritional conditions of the population.

In developing countries, the beginning of the weaning process has been associated with undernourishment. Sorghum weaning foods have low energy and nutrient densities and are a major cause of malnutrition among 6-24 month old children in sub-Saharan Africa. In order for those children to easily swallow, sorghum porridges are diluted to a flour concentration of 5-10 % to attain viscosities of less than 3000 cP. This concentration provide them an energy density too low to meet their energy requirements [1].

The use of malt in porridge making is referred to as “Power Flour” or “Amylase Rich flour” (ARF) [2]. Addition of small quantities of ARF to thick gruels, liquefy them due to the action of α-amilase. Porridge viscosity is then reduced without lowering their nutrient and energy density. Cereal malts are also used to initiate spontaneous fermentation in African indigenous foods. Addition of malt results in a production of amino acids and peptides required for microorganisms growth.

This study reports changes on sorghum submitted to different times of germination.

SDS-PAGE was used to evaluate changes on prolamins electrophoretic profiles. Fourier Transform Infrared spectroscopy in tandem with multivariate analysis was used to assess modifications on sorghum chemical composition. Sorghum porridge was also incubated with malt proceeding from different days of germination. It was concluded that the use of 5 days sorghum germinated flour in weaning foods preparation, leads to an enhancement on its nutritional properties. This processing method is useful in countries where children intake consist mainly of cereals. This method is inexpensive, suitable for application at home and industrial levels and lead to an easily prepared weaning food based on local and culturally accepted raw materials.

Isabel Correia thanks FCT (Portugal) for PhD grant SFRH/BD/19525/2004

[1] FAO/WHO (1995). In Proceedings of a workshop of fermentation as a household technology to improving food safety in collaboration with the department of health(pp.11-15). Pretoria.
[2] Belton, P.S. and Taylor, J.R.N. (2004). Trends in Food Science and Technology, 15, 94-98.

A-24

FLOW INJECTION-CHEMILUMINESCENT DETERMINATION OF COOPER(II) IN WINES

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Several years ago, our research team has been studied the chemiluminescence (CL) reaction of mercaptoacetic acid (MAA) in the presence of luminol and ferricyanide in alkaline medium, in flow injection analysis (FIA) conditions. Knowing the fact that heavy metal ions are able to form complex combination with MAA, this new reaction was a challenge in the development of a sensitive and fast FIA-CL method for their indirect detection. The analytical information is extracted from the decrease of CL signal, decrease that occurs because of metal ion complexation with MAA and is proportional to the analyte concentration.

Therefore, the aim of this work was to design, optimize and testing a FIA-CL system for heavy metals determination in wine samples. The studied parameters were: the nature and the concentration of the catalyst; reagents concentration; reagents flow rates; injected volume; mixing coil length, heavy metal ions concentration. As it was expected, all the studied heavy metal ions decreased the CL signals and they could be determined over different ranges of concentration. Fortunately, only Cu(II) reacted very fast with MAA and moreover, it could be determined at very low concentration (5 ppb) with respect of the other ions and without any interference from their part.

Taking into account the excellent obtained results, the proposed method was applied to monitorize the total content of Cu(II) in Romanian wine samples and the following analytical performances were achieved: large quantification domain, 0.005 – 0.1 ppm; good sensitivity, LOD of 1 ppb, and reproducibility, RSD = 1.5 – 3.25 %, (n=10); a high sample throughput, 60 samples/hour.

A-25
DEVELOPMENT OF QUARTZ CRYSTAL MICROBALANCE IMMUNOSENSOR DETECTING C-REACTIVE PROTEIN

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The world and Korean domestic market of functional food are increasing rapidly. As a consequence, the importance of functionality evaluation for food has been emphasized. One way to evaluate *in vivo* food functionality rapidly is to determine the rise and fall of specific biomarkers for a disease or metabolic syndrome in the blood plasma of an animal model like rat. Based on this consideration, the final aim of this study is to develop a high-sensitivity quartz crystal microbalance (QCM) immunosensor detecting a cardiovascular biomarker, C-reactive protein (CRP).

A PC-controlled sensor system was constructed with a buffer reservoir, a micro-dispensing pump, an injector, a flow-through cell having the nominal capacity of 150 μL, an oscillator module, a frequency response analyzer and a PC installed with the operating software. The immobilization of biological component was undertaken by chemisorption of the thiolated CRP with sulfosuccinimidyl 6-[3-(2-pyridyldithio)propionamido] hexanoate over one surface of QCM.[1)] The label-free QCM sensor system was operated by a flow mode in indirect-competitive way. System operation was done in the order of baseline stabilization, the competition between the fixed concentration of anti-CRP antibody and the varying concentrations of CRP for the binding site on the sensor chip, and regeneration of the sensor surface with 10 mM NaOH. The reaction buffer for the system was 0.1 M sodium phosphate (pH 7.0) that showed a good compatibility with the sensor chip. When determined in the concentration range of 1.1 pM~212.0 nM of rat CRP, the limit of detection for the analyte was presumed as 1.1 pM.[2)] At higher CRP concentration over 53.0 nM, the antibody binding over the sensor surface seemed to be greatly inhibited. The correlation between analyte concentration and frequency shift was found quite high, with the correlation coefficient (*r*) of 0.9838. From the above finding, it was inferred that the immunosensor of this study might find applicability to the high-sensitivity detection for other cardiovascular biomarkers like low-density lipoprotein.

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[1] Park, I.-S. and Kim, N. 1998. Thiolated Salmonella antibody immobilization onto the gold surface of piezoelectric quartz crystal. Biosens. Bioelectron. 13: 1091-1097.
[2] Vikholm-Lundin, I. and Albers, W.M. 2006. Site-directed immobilization of antibody fragments for detection of C-reactive protein. Biosens. Bioelectron. 21: 1141-1148.

A-26

STUDY OF THE GAS CHROMATOGRAPHIC BEHAVIOUR OF CARBOHYDRATES AS A PREVIOUS STEP TO THEIR ANALYSIS BY COMPREHENSIVE TWO DIMENSIONAL GAS CHROMATOGRAPHY (GCXGC)

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Gas chromatography (GC) has always been considered to be a technique with the high resolution and sensitivity necessaries for the study of volatile and semi-volatile compounds. However, the separation of very complex mixtures of similar compounds is not always affordable with only one dimensional GC, while comprehensive two dimensional gas chromatography (GC×GC) can be a useful tool for this aim: the coupling of two columns with different characteristics through an interface or modulator increases the peak capacity and improves the limit of detection.

GC×GC has been widely applied to a broad range of complex mixtures in the last years. Different methods for the analysis of samples from the environmental field and from the petrochemical industry have been developed. GC×GC has also been applied in the separation of food samples of complex composition. Nevertheless, to the best of our knowledge this technique has not previously been applied to the analysis of carbohydrates.

Carbohydrates appear as simple sugars in many fruits and vegetables and can be easily analysed by GC after their previous derivatization (1, 2). However, other foods such as honey (3), or oligosaccharides synthesised by enzymatic hydrolysis (4) are constituted by complex mixtures of carbohydrates differing in their molecular weight, monosaccharide composition and glycosidic linkage. While disaccharides are readily separated from mono- and trisaccharides by GC, the resolution of compounds within this group can be very difficult.

In this work, we present the gas chromatographic retention behaviour measured at different temperatures for trimethylsilyl oxime disaccharide standards on three different stationary phases (methyl silicone, cyanopropyl silicone and phenylmethyl silicone). Results indicate a high correlation among retention parameters, as was expected for isomers differing only in the position of substituents. However, the effect of temperature on retention has been found to depend on the stationary phase and could be used to enhance selectivity differences.

A Pegasus 4D GC×GC-TOF MS with a four jet modulator from Leco has been used to study the GC×GC separation of mixtures of disaccharide standard compounds and of dissacharides in honey samples. Preliminary assays show that, as expected from GC results, it is difficult to obtain ortogonality with the studied columns, and that an optimization of temperature conditions appears to be necessary in order to attain an optimum separation.

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[1] Sanz ML, Villamiel M, Martinez-Castro I, Food Chem. 87 (2004): 325-328.
[2] Villamiel M, Martinez-Castro I, Olano A, Corzo N Z. Lebens. Unters. Forsch. 206 (1998) 48-51.
[3] de la Fuente E., Sanz M.L., Martinez-Castro I., Sanz J. J. Chromatog. A, 1135 (2006) 212–218.
[4] Sanz ML, Sanz J, Martínez-Castro I. Chromatographia, 2002, 56, 617-622.

A-27

DEVELOPMENT OF A NEW LATERAL FLOW IMMUNOASSAY FOR THE DETERMINATION OF HISTAMINE IN FISH (HISTASURE™)

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A new lateral flow immunoassay for the determination of histamine in fish was developed.

Histamine is a member of a group of compounds known as biogenic amines. Biogenic amines are biologically active compounds normally produced by decarboxylation of free amino acids and are present in a variety of foods: fish, fish products, meat wine, cheese and fermented foods. The presence of biogenic amines in these foods is an indicator of food spoilage. Histamine is produced in fish tissue by the decarboxylation of free histidine by bacteria containing the enzyme histidine decarboxylase. Histamine is used as an indicator for adulterated fish. The FDA sets a limit of 50 ppm in at least two subsamples as indicative of decomposition. To meet the demands of the fish industry to have a fast and accurate test, this fast lateral flow immunoassay was developed. It uses the unique FLORIDA technology (Fluorescent Labelled Optical- Read Immuno Dipstick Assay). FLORIDA is based on a proprietary method of labelling biomolecules (patent pending). The extreme sensitivity of FLORIDA can be achieved by conjugating certain fluorophores in high density to carrier molecules and conjugating subsequently the antibodies to this fluorescent complex. The enhancing effect depends on the very high ratio of fluorophores conjugated to a single antibody. The assay is performed as following: the fish is homogenized with distilled water; 50µl is withdrawn for the acylation step. This acylation step takes only 2 minutes. The sample is then diluted in the running buffer for the lateral flow test. 100µl is withdrawn and applied to the test container filled with lyophilized antibodies. The test strip is placed in the container and after 2 minutes the signal can be read with a simple blue light. To reduce background fluorescence, orange glasses are supplied which produce a brighter image. The cut-off is presently set at 50 ppm, but is adjustable. It is possible to determine histamine with a sensitivity of 5 ppm. Using different pre-treatment schemes the HistaSure™ may be used for measuring histamine in different foods, e.g. fresh fish, canned fish, cheese etc. As to our knowledge this is the first rapid lateral flow immunoassay test to reach sufficient sensitivity to measure histamine in real fish samples.

A-29
HACCP IN BREWING PRODUCTION CHAIN; MYCOTOXINS, NITROSAMINES AND BIOGENIC AMINES

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The Hazard Analysis of Critical Control Point (HACCP) is a system of controls specifically designed to prevent safety problems. It is a tool which identifies and assesses the hazards and risks associated with the processes of manufacture, storage and distribution of foods, and it represents a proactive system of preventive actions rather than a reliance on periodic inspection, end point testing and reactive responses to problems.

Industries are obliged to assurance safety foods, and using the HACCP system they are able to implements the appropriate controls aiming to the elimination or reduction of these hazards at specific points of the production line.

These aims can be attained adopting a systematic and organizational structure, controlling activities procedures and resources according to the standards which constitute the basics for the Total Quality System (TQS).

This work reported the preliminary results obtained in the TRUEFOOD (Traditional United Europe Food) EU integrated project which improving quality and safety, and introduce innovation into traditional European food production systems through research, demonstration, dissemination and training activities. The goal of this research was to identity the Critical Control Points (CCPs) for mycotoxins, N–nitrosamines and biogenic amines in malting and brewing production chain. Mycotoxins are non volatile, relatively low-molecular weight; secondary metabolic substances produced by organisms of the fungus family. Some mycotoxins are carcinogenic, some are vasoactive and someone cause central nervous system damage.

Nitrosamines are formed by reaction of secondary or tertiary amines with a nitrosating agent. N-Nitroso compounds were known almost 40 years ago to be present in food treated with sodium nitrite, which made fish meal hepatotoxic to animals through formation of nitrosodimethylamine (NDMA).

Biogenic amines are commonly found in foods such as cheese, meat and fish products, wine, beer, and other fermented food. High levels of some biogenic amines may cause toxic effects with a wide variety of symptoms.

The CCPs for mycotoxines, N-nitrosamines and biogenic amines were identified. The study highlights the importance of raw materials quality, control, and the hygienic conditions of the brewing plant. After the identification of the CCPs the elaboration of HACCP system was developed.

The proposed HACCP protocol for brewing production chain allows improving the safety and the quality of beer.

A-30

CHARACTERIZATION OF STARCH USING NEAR INFRARED SPECTROSCOPY (NIRS) – A RAPID METHOD FOR THE FOOD INDUSTRY

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Starch is one of the most abundant biopolymers, consisting of the polymers amylose and amylopectin and other minor constituents like water, phosphorus, proteins and lipids. Found as storage polysaccharide in plants it is accumulated as granules in various types of tissues and organs in a range between 65 and 90% of the total dry matter. Potatoes, maize, wheat, rice and tapioca are the sources of commercial interest with a large variety of applications both in food and non food areas, whereas the latter are mainly paper- and textile industries [1].

As a multifunctional and biodegradable ingredient starches are used to improve modern food and beverage processing. The systematic employment of starch plays a decisive role in baked goods, frozen foods, batters and breadings, beverage, emulsions and flavour encapsulation, confectionery, dairy products, fruit preparations, soups and sauces, dressings, meat products and savoury snacks [2].

Starch will always be subject to variations regarding its chemical composition depending first of all on the source but also seasonal and geographical differences are readily identifiable. As the textural benefits of starch depend also on other components like bounded lipids the selection of the matching starch for a complex food formulation and constant quality are very important. Rapid methods of intake control and process control are therefore essential.

Standard methods for the determination of starch lipids are highly time consuming procedures based on acidolysis, hot extraction of the lipid fraction and a subsequent gravimetrical determination of the lipid content. These methods cannot comply with the requirements of modern quality control. Near Infrared Spectroscopy, already applied in many fields in on- and in-line systems, is a promising alternative technique which provides in combination with chemometric methods qualitative and quantitative information. As reference method for the Near Infrared calibrations a new validated method was established, reducing time of analysis and expanding the information content by applying gas chromatography to determine single fatty acids, which are characteristic for different starches.

The measurement of 100 starch samples was accomplished over a range of 4000 – 9500 cm^{-1} in diffuse reflectance mode using a rotating sample cup. Quantitative prediction models, based on a partial least squares (PLS) algorithm for the determination of fat content, the main fatty acids, proteins, water content and the degree of starch modification were developed. All models are characterized by high correlation coefficients (R^2 > 0,98). Principal components analysis (PCA) was applied successfully to distinguish starches regarding their source, provenience and their degree of modification. Both quantitative and qualitative models are cross validated.

The possibility of the determination of many parameters simultaneously by recording a single reflectance spectrum compensates for the time-consuming calibration step.

[1] W. Auhorn, Das Papier, 11 (2003) 40.
[2] P. Taggert, in: A.C. Eliasson (Ed.), Starch in food, Woodhead Publishing, Cambridge, 2004, p. 363

A-31
PROTEIN PROFILE AND MALT ACTIVITY DURING SORGHUM GERMINATION

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Sorghum is one of the five most important cereals in world food supply. On the semi-arid regions of Africa and Asia, this cereal comprises an important source of energy, proteins, and minerals. However, its poor nutritional quality has deleterious effects on the nutritional conditions of the population.

In developing countries, the beginning of the weaning process has been associated with undernourishment. Sorghum weaning foods have low energy and nutrient densities and are a major cause of malnutrition among 6-24 month old children in sub-Saharan Africa. In order for those children to easily swallow, sorghum porridges are diluted to a flour concentration of 5-10 % to attain viscosities of less than 3000 cP. This concentration provide them an energy density too low to meet their energy requirements [1].

The use of malt in porridge making is referred to as “Power Flour” or “Amylase Rich flour” (ARF) [2]. Addition of small quantities of ARF to thick gruels, liquefy them due to the action of α-amilase. Porridge viscosity is then reduced without lowering their nutrient and energy density. Cereal malts are also used to initiate spontaneous fermentation in African indigenous foods. Addition of malt results in a production of amino acids and peptides required for microorganisms growth.

This study reports changes on sorghum submitted to different times of germination.

SDS-PAGE was used to evaluate changes on prolamins electrophoretic profiles. Fourier Transform Infrared spectroscopy in tandem with multivariate analysis was used to assess modifications on sorghum chemical composition. Sorghum porridge was also incubated with malt proceeding from different days of germination. It was concluded that the use of 5 days sorghum germinated flour in weaning foods preparation, leads to an enhancement on its nutritional properties. This processing method is useful in countries where children intake consist mainly of cereals. This method is inexpensive, suitable for application at home and industrial levels and lead to an easily prepared weaning food based on local and culturally accepted raw materials.

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[1] FAO/WHO (1995). In Proceedings of a workshop of fermentation as a household technology to improving food safety in collaboration with the department of health(pp.11-15). Pretoria.
[2] Belton, P.S. and Taylor, J.R.N. (2004). Trends in Food Science and Technology, 15, 94-98.

A-32

MICROFABRICATED FLUIDIC DEVICES FOR RAPID ACCESS TO CHEMICAL AND BIOCHEMICAL INFORMATION

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There has been rapidly growing interest in microfabricated fluidic devices (microchips) over the past decade for use in chemical and biochemical experimentation. The diversity of chemical and biochemical measurement techniques that have been implemented on microchips includes various electrophoretic and chromatographic separations, chemical and enzymatic reactions, noncovalent recognition interactions, sample concentration enhancement, and cellular manipulations. In addition, the types of samples addressed by microchips has been broad in scope, e.g., small ions and molecules, single and double stranded DNA, amino acids, peptides, and proteins. These devices have low cost and small footprints while consuming miniscule quantities of reagents and can rapidly produce precise results. All of these features suggest the possibility to perform chemical and biochemical experimentation on a massive scale at low cost on a bench top, a goal being pursued by many laboratories around the world. This presentation will be an overview of our activities in this area.

A-33

ANALYSIS OF ANTITHYROID DRUGS IN THYROID SAMPLES BY UPLC-MS/MS

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A method for the quantification and confirmation of antithyroid drugs in thyroid samples of bovine, ovine and porcine livestock is presented. The method includes a SPE clean-up and a UPLC separation coupled to a triple quadrupole (MS/MS). It avoids the derivatization step included in most of the published LC-MS methods.

The drugs considered are: Thiouracil, Methylthiouracil, Propylthiouracil, Phenylthiouracil, Methimazole (Tapazole) and Mercaptobenzimidazol. All these substances are included in the EU residues control plans (Directive 96/23/EU) as forbidden substances (Group A), and on May 2006 a general MRPL of 100 µg/Kg was proposed.

The method has been validated in the Laboratori de l'Agència de Salut Pública de Barcelona according to the Decision 2002/657/EU. The following parameters have been established: limit of quantification, CC-α, CC-β, specificity, linearity (from 25 to 250 µg/Kg), precision and recovery. The quantification is based on matrix-matched standards. Dimethylthiouracil is used as internal standard.

The method has been used for the analysis of over 150 samples in 2007. Quality controls include: blank extracts, recovery of spiked samples, linearity, internal standard response and instrumental response.

A-34

DETERMINATION OF BROMATE AS ADDITIVE IN FLOURS BY CAPILLARY ELECTROPHORESIS

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The bread making quality of freshly milled flour tends to deteriorate only after 2 months. The use of flour additives increases the shelf life of flour considerably. Bromate is relied upon as one of the most used flour improvers. In some countries, addition of bromate is allowed to the flovour at the mill or to the dough at bakery or both provided that the total bromate content in these foods is tightly controlled. In some countries, its use is prohibited. In both case, there is urgent need for a fast, robust, and simple monitoring system to detect bromate or to determine bromate content in flour samples.

In the present study, we developed a simple, fast and reliable capillary electrophoretic (CE) analytical method for determination of bromate in flour samples. Determination of bromate in flour samples with the method of CE was performed by direct UV detection at 200 nm as the quantitative. Detection limit (signal/noise:3) is 1.79 µg/mL for bromate. Bromate as 21.4 ppm can be determinated in the flour after the extraction method at the study when it is applied the developed analytical method. Detection limit is decreased as $1.79.10^{-2}$ µg/mL with 100-fold improvement thanks to method of sample stacking that is used so as to decrease of detection limit of method. It can be possible that determined of bromate is 3.58 ppm with sample stacking method.

A-35
GENETIC DETECTION AND IDENTIFICATION OF PATHOGENS IN FOODSTUFFS

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A method for the detection and identification by genetic amplification of pathogen genome fragments in foods by real time PCR (polimerase chain reaction). The detectable pathogens using this method are listed below:

- Escherichia coli 0157:H7
- Listeria monocytogenes
- Staphylococcus aureus
- Salmonella spp.
- Clostridium perfringens

AMPLIFICATION TARGETS

Conserved specific genomic regions from among these pathogens have been selected that enable use of oligonucleotide primers that recognize specifically each one of the above listed microorganisms.
The method described herein is robust, fast and highly reproducible as it includes an internal genetic amplification control that is added to the sample at the start of the process. This protocol enables constant extraction yield follow-up including enzymatic PCR inhibition evaluation.

REAL TIME PCR ASSAY

Conserved genome region amplification from the different pathogens, together with internal control included in, is visualised after automated readings of their corresponding TaqMan and SYBRGreen I fluorescent probe signals.

ASSAY VALIDATION AND SENSITIVITY

The assay validation has been performed using commercially available standards derived from DNA extracted from inoculums of different pathogen strains of known concentrations at 108 or 107 cfu/ml.
Amplification parametres Ct (cycle number at first fluorescent detection) are characteristic for each pathogen concentration detected. These parametres set reproducibility limits measured in Genome/mg. The sensitivity thresholds established is 5 Genomes/mg for *Listeria monocytogenes* and *Staphylococcus aureus,* and of 50 Genomes/mg for *Escherichia coli 0157:H7, Salmonella spp and Clostridium perfringens*.

A-36

THE PIPE BASED BIOREACTORS : AN INNOVATIVE HIGH-THROUGHPUT BIOREACTION PLATFORM WITH A WIDE SPECTRUM OF ANALYTICAL APPLICATIONS

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The fast-growing demand of variable measurement systems for the industrial branch and also for research institutes is the starting point to establish a new easy to use platform accomplishing problems, which are commonly solved with a high technical effort. This system should be adaptive for a large variety of applications, highly efficient, accurate, low-cost and only needs a small amount per assay.

This presentation will introduce the *pipe based bioreactors* platform with examples of applications for different branches or scopes. The Institute for Bioprocessing and Analytical Measurement Techniques (iba) in Heiligenstadt forces the aim to get small fluidic bubbles with a biological or chemical origin within a scale of nL to create bio- or chemical reactors under special conditions. The base of the system is the segmented flow with water soluble samples that can contain cells or chemical compounds. The different solutions will embedded as fluidic bubbles with the help of a special microchip to a carrier fluid of a non-polar organic resolvent.

One big advantage of this bioreaction platform is the statistical reliability. The maintenance of the clean or sterile system can be easily warranted. Additionally, while this system is closed it can protect laboratory employees against potentially harmful samples .The transportable reactors (fluidic bubbles) allow a high flexibility of the system and open possibilties for new applications. Due to the basic composition of the system there is a possibility for low-cost commerzialization for this special high-throughput system. The feasible applications go from medicine (e.g. metabolic disease), environmental techniques (e.g. isolation of oil abolished microorganism) to food technology (e.g. contamination of food). These efforts need a continuative apply of other measuring methods to the *pipe based bioreactors* system.

First results were achieved by using this *pipe based bioreactors* as a high-throughput individual cultivation system in a project called “MINIKULT”. Isolation of rare microorganism from soil samples were successful. The system reached a capacity of 400000 samples per day. The collected experience with this *pipe based bioreactors* system was the background for a new concept which describes a new rapid detection of microorganism. The procedural steps from the biomagnetic separation of cells till the detection and quantification of germs should not excess eight hours at all.

Finally the experience in the area of microsystem applications, adapted measuring techniques to biological environments and in bioprocessing techniques build the best assumption for a successfully introduction of this platform to various analytical efforts in different applications.

A-37
ANALYSIS OF ETHYL ESTERS IN PORTUGUESE MUSCATEL FORTIFIED WINES

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Wines contain various phenolic compounds and some of them are natural antioxidants. Concentration of these compounds in white wines is lower than in red ones due to the wine making process. However an increase in the content on phenolic compounds can be obtained in fortified wines as a result from the pomace contact. The esterification of cinnamic and benzoic acids with ethanol explain the increase in the content of these compounds during the wine making process. Ethanol esters as ethyl caftarate, ethyl caffeate, ethyl coutarate, ethyl fertarate, ethyl cinnamate, diethyl succinate and ethyl gallate were already characterized in Champagne wine and ethyl caffeate has also been quantified in sherry and table wines. Ethyl gallate and caffeate present antioxidant, anti-inflammatory and anti-mutagenic properties.

Liquid chromatography (LC) and mass spectrometry (MS) analytical conditions were optimized in order to identify ethyl esters: ethyl gallate, caffeate and coumarate in samples of sweet fortified Muscatel wines from Setúbal region in Portugal. Diode array, fluorescence mass spectrometry were used for quantitation purposes. Results obtained with the different detection modes were compared in terms of detection and quantitation limits of the methods, linearity and repeatability (n=6) for a wine sample. Comercial wine samples (n=21) were analysed and results obtained ranged from 0,7-9,6 ppm for ethyl gallate and 0,2-2,6 ppm for ethyl caffeate. Peak areas for ethyl coumarate were also compared. Electrochemical detector may be a useful tool in the screening of possible antioxidant components.

The total phenolic content of wine was determined using Folin Ciocalteau method. Identification of phenolic compounds in Moscatel wines using HPLC-APCI-MS has been previously reported [1].

Results obtained show that although wines are all from the same vinification region there must be some differences in the vinification process leading to wines with different chemical composition.

[1] M.N. Bravo, S. Silva, A.V. Coelho, L. Vilas Boas, M.R. Bronze, Analytica Chimica Acta, 563 (2006) 84-92

NOVEL FOODS, GMO, NUTRACEUTICALS, ORGANIC FARMING

(B1 – B8)

B-1
DETECTION OF GENETICALLY MODIFIED ORGANISMS IN FOOD BY PCR TECHNOLOGY

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In recent years, the application of genetically modified organisms (GMOs) in food production has been increased rapidly, that generated growing demand for reliable methods for GM-food analysis. Because of a number of reasons, the detection of food and food ingredients derived from genetically modified organisms (GMOs) is of much current interest. Firstly, the worldwide acting regulatory requirements necessitate GM-food safety assessment and labeling. Secondly, food industry and international trade have claim for science-based accurate information on food composition. Finally, consumers require free choice between GM and non-GM products.

DNA-based polymerase chain reaction (PCR) is the most widespread, effective and precise technology for detection and identification of GMOs. In this study, conventional end-point PCR was used for qualitative detection of GMOs in food samples. The GMO-related important food crops, namely: soybean, maize, wheat, barley, oat and rice as well as soybean and maize GMO standards were analysed. The grains, flour and processed foodstuffs were used as food matrices. The applied analytical procedure included several sequential steps: (1) sample preparation; (2) DNA extraction; (3) PCR analysis. The grains and products were ground to obtain a fine powder. Qiagen DNeasy plant mini kit was used to isolate DNA from food samples. The quality and quantity of the extracted DNA was assessed by spectrophotometer and agarose gel electrophoresis. PCR analysis consisted of three types of reactions, in particular: plant-specific, species-specific and GMO-specific. The presence of plant DNA in food samples was checked by PCR system specific to chloroplast genome conserved sequences and producing amplification product in size of 450-550 bp. The species-specific primers allowed verify the existence of suitable species DNA in food samples. The primers appropriate to GMO regulatory elements, such as: Cauliflower Mosaic virus 35S promoter and Agrobacterium tumefaciens NOS terminator were applied for screening of genetically modified organisms. The PCR products were examined using agarose gel electrophoresis and photograph under ultra-violet light after ethidium-bromide staining. Testing of negative controls and GMO reference materials as positive controls confirmed high specificity and sensitivity of the described analytical procedure for monitoring of GMOs in food. The presence of GMOs in foodstuffs was evaluated after results comparison and interpretation. The results obtained indicate that PCR-based technology described in this study might be used successfully for GMO detection at international level.

B-2
HOW TO DEAL WITH GM FOOD ANALYSES IN THE MIRROR OF EU REGULATIONS

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Food produced from Genetically Modified Organisms (GMOs) has become a part of the food chain. GM food has appeared as a results emerging scientific and technological development in biology within the past decades reflecting demands of the market in some parts of the world. GM food is not widely accepted in EU and precautionary principle applies to the issue. Several EC regulations have been accepted concerning identification, labelling, traceability and transboundary movement. Due to asynchronous authorisation of various GM namely between USA and EC built up the problem of approved and unapproved GMOs identification in EC enforcement laboratories.

ISO guidelines and methods displayed by CRL (Community Reference Laboratory) in JRC Ispra validated through ENGL (European Network of GMO Laboratories) are available. As well reference material either provided by IMMR or control material available cloned in plasmid can be utilize by control laboratories. However, only approved GMO can be identified in the food using published and validated PCR, real-time PCR methods. In case of unknown GMO and multiple stacked genes more advanced, high throughput inexpensive methods are needed. DNA arrays based on pre-amplification step and coupled with silver-staining represent such option. The equipment and procedure available at the moment need still to be fully optimised for the requirement of the market. PCR coupled with ligation and subsequent analysis of the products is also under development.

We have tested the throughput of *in house* developed DNA arrays based on control amplicons. PCR products were spotted on a glass support and hybridised against plasmid DNA, PCR products, total genomic DNA and treated DNA. Several hybridisation conditions (stringency), washing procedures were tested to elaborate optimised protocol. Laser scanner representing an expensive option in DNA array analyses was used to read the results. The results will be discussed along with possible application of the technology.

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B-3

DETECTION OF GENETICALLY MODIFIED ORGANISMS IN RAW AND HIGHLY PROCESSED SOYBEAN AND MAIZE FOODSTUFFS BY POLYMERASE CHAIN REACTION

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The soybean and maize are the most important genetically modified crops, totalising 83.8 millions of planted hectares, which correspond to 57% and 25% of global biotechnological planted area, respectively (James 2006). Since the genetically modified organisms (GMO) entered the food chain, a scientific and public debate concerning their safety and the need for labelling information came up especially in Europe. For this reason, the EU has dedicated special attention to consumer information by requiring a compulsory labelling for food products containing more than 0.9% of GM material.

The need to monitor and to verify the presence and amounts of biotechnology derived material in food products demands analytical methods able to detect, to identify and to quantify either the introduced DNA or the expressed protein(s). The DNA based methods, namely PCR techniques, are the methods of choice due to their high sensitivity and specificity, allowing the detection of very small amounts of DNA in raw materials and processed. In the present work, the isolation of DNA from foods was carried by using the CTAB reagent and the Wizard clean up columns. Yield and purity of DNA extracts were assessed by spectrophotometry, while amplifiability was evaluated by targeting the lectin and invertase genes for soybean and maize, respectively. Two types of PCR assays were developed and used: conventional PCR assays for detection of RR soybean and screening GM maize and real-time PCR assays for quantitative purposes. The screening of GM maize products was carried out targeting the 35S promoter. The samples included soybean and maize processed foodstuffs such as, protein isolates, beverages, tofu, snacks, precooked foods, and desserts etc., obtained from the local supermarkets. The results showed that contamination of foods by GMO is present in products commercialised in Portugal

[1] James C. 2006. Global Status of Commercialized Biotech/GM Crops: 2006. ISAAA Brief No. 35. New York, Ithaca: ISAAA

B-4

BABYFOOD: INVESTIGATIONS WITH REGARD TO BETTER NUTRITIONAL ADVICE AND QUALITY OF THE EUROPEAN COMMERCIAL INFANT FOOD

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The main objective of the BABYFOOD project is to provide the European Union with basic information about the impact of commercial infant food on nuclear receptor based modulation. The generation of this information aims at non-breastfed children during their early development between zero and nine months. The focus is to interpret the scientific results with regard to nutritional advice and infant food quality.

In many parts of Europe mothers are increasingly reluctant to breastfeed their babies. In addition commercial infant food is supplied by a small number of companies present in the European market. BABYFOOD focuses specifically on infants because they are suspected to be more susceptible to (at least some) nutritional exposures. A nutritional factor may affect growing individuals and tissues differently than exposure of a mature individual in whom the growth process is complete.

The market share data of 2007 for infant formula and solid baby foods were purchased from international food and drink consultancy. The 22 countries included were taken to represent the whole EU market share. Each category of products of the brands that altogether constitute over 80% of the market were identified. Products from the main brands were sampled and pooled so that their weight in the sample will be proportional to their market share at European level. The geographical variability in concentration of NR modulators in the formula and solid food basket will be assessed through chemical analysis of selected substances, e.g. cadmium in four countries.

In order to design the monthly average food baskets elaboration of a detailed nutritional plan was developed. Most babies receive a unique infant formula from 0 to 4 months and then a unique follow-on formula from 4 to 9 months. Samples of infant formula, honey, herbal tea and solid foods will be collected and analysed for BPA, PCDD/F, PCB, genistein and the pesticides vinclozolin, procimidone and iprodione separately. It will be possible to make different hypothesis by considering the introduction of solid foods at 4 months, 5 months or later and to assess potential exposure to NR modulators through calculation in the different scenarios. Thus, it will be possible either to test in vitro and in vivo solid foods and infant formula separately or to make pooled samples of solid food and infant formula that would represent the whole diet of a baby and to test it.

B-5
DETECTION OF GENETICALLY MODIFIED FLAX AND POTATO

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The detection of genetically modified organisms (GMOs) by the polymerase chain reaction (PCR) constitutes of three main steps: DNA extraction and purification, PCR reaction and detection or quantification of amplified products, and data evaluation. For the isolation of DNA from potato (leaf, sprout, cortex of tubers, parenchyma tissue, total tuber - parenchyma plus cortex,) and flax (seed and leaf) the extraction method with the ionic detergent cetyltrimethylammonium bromide (CTAB) was successfully used.

Primers complementary to the region of the chloroplast DNA in tested plants were used for PCR verification of the possibility to amplify the isolated DNA. Species specific reactions with primers complementary to the 3´end of the flax L gene and to the potato light-inducible tissue-specific ST-LS1 gene encoding the light-inducible leaf/stem specific protein of *Solanum tuberosum* (StSL protein) followed. The specific products were obtained from DNA isolates from different flax and potato varieties, whereas no products were detected from other tested plant species such as soybean, maize, potato, flax, oat, barley, wheat, sunflower, pea, feterite, rape, tobacco and cotton.

For the quantification of the potato DNA the probe supplementary to authentic primers mentioned above were designed. Function of the quantitative system was verified by real on time PCR with potato varieties L. cv. Desireé, Arielle, etc. The plasmid DNA containing the end of 35S promoter, the CTP peptide and the beginning of the *katE* gene was used for quantification as standard.

The presence of the GM screening sequences (35S promoter, T-nos terminator of tranion or *nptII* selection marker) determining the inserted transgenic DNA in analysed samples was examinated. In case of any presence of these sequences, the samples were analysed subsequently by the PCR with specific primers complementary to the studied GMO (*bar* gene or *katE* catalase gene from *Escherichia coli*).

The results confirmed that methods based on PCR can be successfully used for the detection of genetically modified flax and potato.

We are very grateful to Prof. Patrick du Jardin for providing the genetically modified (GM) potato lines (FUSAGx, Belgium), to Potato Research Institute Ltd. (Czech Republic) for providing the non-GM lines of potato and to Agritec Ltd. (Czech Republic) for providing the flax seeds.

B-6

PRODUCTION OF TOMATOES BY TWO DIFFERENTS AGRICULTURAL METHODS IN BRAZIL: TOMATEC PROJECT

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To increase the productivity of their tomato crops, farmers frequently make use of large amounts of soluble fertilizers and pesticides. Thus, the contamination of soil and surface waters at the vicinities of the tomato plantations as well as the occurrence of very serious public health problems is frequently seen.In this opportunity the production of tomatoes by two differente agricultural methods was studied. In the first method the tomatoes were produced in a conventional way (Traditional Tomatoes). In the second method the tomatoes were produced according a new agricultural technology, which intended to minimize the amount of pesticides used in the agricultural production as well as to maximize the productivity and the quality of the fruits (Ecological Tomatoes).The project was carried out in the district of S.José do Ubá, in the State of Rio de Janeiro in two different segments: In the first segment (prior to 2006) two farmers that produced the Ecological Tomatoes and five that produced Traditional Tomatoes participated. In the year of 2006 and aftewards, three producers of the Ecological Tomatoes and one of Traditional Tomatoes, participated.At fist, only production technologies aiming to protect the agricultural soil and to promote a better usage of irrigation water as well as integrates pest management was used. Starting in 2006, in addition, immediately after sprouting the tomatoes were enclosed in wax paper bags until they were collected. In which it concerns to pesticide residues, prior to 2006 the Ecological and Conventional Tomatoes were analyzed only for dithiocarbamates. No residues of that class of pesticides were found in the Ecological Tomatoes.In contrast, residues of Dithiocarbamates in concentration above the allowed limit (ANVISA/2007, 2,0 mg/kg CS_2) were found in the traditional Tomatoes. Starting 2006, besides Dithiocarbamates, the tomatoes were analyzed quantitatively (GC-ECD/FPD) for 28 halogenated an 18 organophosphate pesticides, as well as qualitatively (GC-MS) for 100 pesticides residues. As before, no residues were found in the Ecological Tomatoes. Finaly, it should be mentioned that another advantage of the methodology used in the production of Ecological Tomatoes was the insignificant loss of fruit observed: the Ecological Tomatoes farmers lost less than 1 % of the fruits.

B-7
MINERAL TREATMENT, NONPOLLUTING FOR SOIL AND WATER, USED FOR THE IMPROVEMENT OF THE SWEET POTATO'S QUALITY

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Mineral treatments with zinc induce an acceleration of the biosynthesis of the active compounds in plants. Treatments with mineral nutritive solutions with zinc are necessary both for the development of plants and for its contribution in the human diet, but its have to not exceed the maximum level allowed in soil and water. In plants, zinc stimulates the biosynthesis of the proteins and vitamins P and increases auxine's activity. Also, zinc is cofactor and activator for many enzymes and influences the biosynthesis of the cytochrom C. In human organism, zinc is constitutive part of over 70 metallenzymes (dehydrogenases, peptidases and proteases), therefore many diseases are caused by the low enzymatic activity of some enzymes that contain zinc.

The present paper is the results of the researches performed on the tuberised roots of the sweet potato in order to find an optimal concentration for the nutritive solution with zinc so that this element not to be a polluting factor for the environment. Also, is necessary that the amount of zinc in the mineral solution to be adequate for a favourable impact on the plant growing and on the human health.

Because of the great importance of zinc in biological systems, the mineral treatments have to take into account the needs of plant and of the human diet for this chemical element, but only below the safe limit for the ecosystems.

B-8
MONITORING OF GENETICALLY MODIFIED ORGANISM IN FOODS

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The construction of the analysis management system requires the scientific post-market surveillance of genetically modified foods since the practice of the enforcement of labeling in 2003. We monitored total 302 samples in the 5 regions that the whole country is divided into in terms of physical distribution. In order to investigate the actual state of genetically modified(GM) foods in Korea, we tested total 302 samples of soybean, maize and processed foods. 302 samples were consisted of 212 samples containing soybean, 80 samples containing maize, and 10 samples containing both soybean and maize. 39 samples(12.9%) contained GM ingredients by qualitative PCR analysis in the total 302 samples. The monitoring result showed that 24 samples(11.3%), 13 samples(16.3%), and 2 samples(20.0%) included genetically modified soy, maize, and both soy and maize, respectively.

The analysis of the food types showed that the percentage of GM detection was 23.8% in the meat processed product, 22.7% in the beverage, 17.5% in others foods, and 13.1% in the standard exceptional foods, and etc. The monitoring results in the distribution region showed the regional difference of Kwangju.Chunra province(1.6%) and Daejeon.Chungchong province(26.3%) in the rate of GM detection. But it was resulted from the difference of the collected samples than the region. In order to acquire the exact result, the samples will have to be classified and be analyzed according to the criterion of the regions and the food types. In comparison with the monitoring result in the last three years, the percentage to be detected in foods containing genetically modified soybean, maize, and both soybean and maize, respectively showed that the remarkable differences were not in the some food types. However, the annual percentage of total GM detection showed the definite decrease from 27.4% in 2003 to 12.9% in 2006. The result of the postmarket surveilance showed that 36 samples among 39 samples had identity preserved handling certificates or government certificates but 3 samples did not. 2 samples violates the GMO labelling and 1 sample did the food labelling.

FOOD CONTAMINANTS (ENVIRONMENTAL), NANOPARTICLES

(C1 – C29)

C-1

CHARACTERIZATION OF OCPs AND PCBs: ANALYTICAL METHOD AND MONITORING SYSTEM ANALYSIS

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Pesticides are substances that are widely used in an environment and can have a negative impact on human's organism. The aim of our work is determination of organochlorine pesticides (OCP) in traditional meat products that contain several 10 % of fat and monitoring their proliferaion along the production chain. Therefore we have modified standard procedure (SIST EN 1528-4: 1998) for the measurements of organochlorine compounds in food products. The standard procedure was slightly modified and validated. The main modifications were made in the preconcentration step, where Soxhlet extraction was used instead of a cold extraction technique. The modifications were needed to improve recovery of the extraction procedure due to high fat content in our samples. Clean-up and sample concentration steps were also optimized so that the amount of used organic solvents was minimized while maintaining good analytical information. For clean-up step the use of smaller columns was proposed and for sample concentration step Kuderna-Danish concentrators were used instead of a rotary evaporator/nitrogen purging. Parameters (such as temperature program and flow rates) for determination of OCPs with gas chromatography in conjunction with an electron capture detector were also optimized. At the end our optimized method was used to perform the determination of OCPs in a lean stag meat. Recovery values for all analyzed pesticides were over 50 %.

C-2

THE OCCURRENCE OF BENZENE IN SOFT DRINKS: A SURVEY OF THE SITUATION ON THE BELGIAN MARKET

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The daily intake of benzene, classified by the International Agency for Research on Cancer (IARC) as human carcinogenic compound (group 1), may vary significantly and may originate from natural sources (e.g. forest fires) or from human activities such as smoking or exhaust fumes. Other sources of benzene are drinking water and food, both through contamination from the environment.

Already in 1993 Gardner *et al.* (*1*) described that the combination of sodium benzoate and ascorbic acid in food could lead to the formation of benzene, especially in acidic beverages such as soft drinks. Since that moment several surveys on benzene contents in food (*2*) and soft drinks (3-*5*) have been published. At this moment no legal limit for benzene in soft drinks exists, consequently the limit in drinking water is mostly used as reference value. However several different limits for benzene in drinking water exist making a uniform evaluation of the benzene content difficult.

In order to sketch an image of the benzene content in soft drinks on the Belgian market a gas chromatographic – mass spectrometric method using headspace injection was developed and validated in-house and a survey in co-operation with the Belgian Federal Agency for the Safety of the Food Chain (FAVV) was commenced. Over 100 samples have been analyzed. In about 70 percent of these samples benzene was detected and in 20 percent the detected concentration was above the quantification limit of 0.3 µg L^{-1}. Several samples exceeded the guideline of the European Commission, who has fixed the limit for benzene in drinking water at 1 µg L^{-1}, and one sample was even above the World Health Organization (WHO) limit for benzene in drinking water of 10 µg L^{-1}.

The evaluation of these data indicate that although the presence of the preservative sodium benzoate and ascorbic acid and the combination of benzoic acid and an acidity regulator (e.g. citric acid, phosphoric acid, …) have a significant effect on the detected benzene concentrations, other factors may as well play an important role in the benzene formation.

[1] Gardner, L. K.; Lawrence, G. D., Benzene Production from Decarboxylation of Benzoic-Acid in the Presence of Ascorbic-Acid and a Transition-Metal Catalyst. Journal of Agricultural and Food Chemistry 1993, 41, (5), 693-695.
[2] McNeal, T. P.; Nyman, P. J.; Diachenko, G. W.; Hollifield, H. C., Survey of Benzene in Foods by Using Headspace Concentration Techniques and Capillary Gas-Chromatography. J. AOAC Int. 1993, 76, (6), 1213-1219.
[3] Fabietti, F.; Delise, M.; Bocca, A. P., Investigation into the benzene and toluene content of soft drinks. Food Control 2001, 12, (8), 505-509.
[4] SURVEY OF BENZENE IN SOFT DRINKS. http://www.food.gov.uk/multimedia/pdfs/fsis0606.pdf (15 February 2007),
[5] Data on benzene in soft drinks and other beverages. http://www.cfsan.fda.gov/~dms/benzdata.html (15 February 2007),

C-3
CHEMICAL CONTAMINANTS IN FOOD FROM ROMANIA, 2006

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Romanian Food Contamination Monitoring programme was designed according to UNEP/FAO/WHO food contamination monitoring principles (GEMS/Food) and has been launched in 2001. The national Food Contamination Monitoring comprises basic foodstuffs and mostly popular contaminants for Romania including the groups of heavy metals, nitrates, residues of pesticides and aflatoxin.
The study presents the results obtained in 2006.
Lead and Cadmium were monitored in potato,carrot, spanish, lettuce, apple, wheat flour, maize flour, juice and daily diets.
Nitrates were monitored in spanish and lettuce.
Pesticide residues (DDT, HCH, lindane) were monitored in daily diets
Aflatoxin total(B1,B2,G1,G2) were monitored in wheat flour, rye flour, nuts and peanuts.
3088 foodstuff samples were analysed during the 2006 period. In 3% of samples analysed the contaminants were nonconformity EU limits.
There were no statistically reliable changes found in nitrate content in vegetables, the mean contents of nitrates were 308.3 mg/kg.
Lead and Cadmium presence are observed in some foodstuffs but in the EU limits.
Residues of pesticide were detected in solitary cases. The detected levels of lindane have no exceeding of official limits.
Aflatoxin total were detected in nuts and maize flour
Analysed contaminants consumption data (by 24 hours food consumption recall method) shoved that none of the consumed contaminants exceeded ADI-value
Determinations of these chemical contaminants in food are important in environmental monitoring for the prevention, control and reduction of pollution as well as for occupational health and epidemiological studies

C-4

DETERMINATION OF TRACE ELEMENTS IN BLACK AND FRUIT TEAS BY GFAAS AND ICP-OES

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Tea is one of the most popular beverages in the world and its importance and consumption is rising due to the elevated ecological awareness nowadays. Besides the organic components the trace elements present in tea and fruit infusions (fruit tea) play a crucial role regarding human health.

The determination of trace elements in teas, dried fruits and their infusions is important because of the metabolic role of some metals. On the one hand there is knowledge of the food's nutritional value which refers to major or minor-elements, and on the other hand there is a concern to verify that food does not contain minerals in toxic quantities. These elements might be dangerous for health, regardless whether their presence is naturally occurring or is due to contamination.

Highly accurate analytical methods are needed for the determination of trace and ultratrace elements in infusions as well as in digest solutions of tea powder. Both, graphite furnace atomic absorption spectrometry (GFAAS) and inductively coupled plasma – optical emission spectrometry (ICP-OES), offer low limits of detections. The former requires less sample volume per analysis and the latter allows simultaneous determination of all elements of interest.

In order to estimate the possible effects of trace elements in teas on human health their concentrations should not only be determined in prepared infusions, but also in the leaves or the powder used for preparation of the beverage.

This study covered the determination of 27 elements, namely Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Sc, Se, Si, Sm, Sn, Sr, Tl, and Zn. Variuos kinds of commercially available teas, such as fruit tea and black tea, both from classical as well as from organic agriculture, were analyzed. Furthermore fruit tea with mineral forticiation were investigated and compared with non-fortified products.

C-5

EFFECTIVE STRATEGIES FOR THE SCREENING OF CONTAMINANTS IN FOOD USING TOF/MS AND NOVEL DATA MINING TOOLS

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Identification and monitoring of contaminants and residues is of major importance in food safety with the number of compounds under consideration ever expanding. Sensitive and accurate screening places stringent demands upon both analytical instrumentation and associated software and successful screening strategies must address both issues.

Multi-class screening is beneficial in terms of productivity but, by definition, tends to use sample preparation techniques with low selectivity resulting in complex sample extracts. Clearly, efficient separation techniques facilitate screening by offering resolution of contaminants from matrix components. Capillary GC, and now the emergence of Ultra Performance Liquid Chromatography offer the potential for high efficiency separations of broad ranges of compound classes.

Historically for targeted screening, techniques such as SIR or MRM have dominated to maximise sensitivity but are limited by the number of ions and transitions that can be monitored. For non-targeted screening, scanning techniques have been used and the instrumental requirement is for sensitive full spectrum acquisition and hence the development of ToF/MS solutions is of great interest.

Here we describe GC- and UPLC-ToF/MS solutions based on two approaches. The first allows sensitive pre-targeted screening, detecting specific ions and calculating ion ratios. Post-target screening is also possible by adding compounds (ions) to the method after analysis extending the number of residues monitored without compromising sensitivity. A second approach for non-target screening relies upon automatic peak detection, spectral deconvolution, and searching against experimental or theoretical spectral libraries with further confirmation of identity by accurate mass scoring and isotope pattern fitting.

C-6
STRATEGIES FOR USING THE DR CALUX® ASSAY IN DIOXIN AND DL-PCB ANALYSIS

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The DR CALUX® assay has proven to be a valuable screening tool for dioxins and dioxin-like (dl) PCBs in food and feed. The assay uses modified rat hepatoma cells that produce luciferase in a dose-related manner upon exposure to compounds binding to the Ah-receptor. A selective clean-up of samples, based on acid silica columns increases the specificity for dioxins and dioxin-like PCBs. Until November 2006, only dioxins were regulated in the EU by a set of action and tolerance limits. Since then, the test should be able to detect samples that exceed the action limits for dioxins and dl-PCBs (total TEQ), and in particular the tolerance limits for dioxins and the sum of dioxins and dl-PCBs.

RIKILT uses the so-called screening method, based on the comparison of the response of an extract of the test sample with that of a set of control samples with levels around the action and tolerance limit. Samples are declared negative of suspected, in the latter case requiring confirmation by HRGC/HRMS. Thus far the test focused on the action limits for dioxins only, although also dl-PCBs would end up in the extract and thus contribute to the response. The difference between the background levels for total TEQ in the Netherlands and the action limits for dioxins allowed this approach without an unacceptable amount of false-positives results. It is proposed to continue along this line, now that the action limits for dl-PCBs have been introduced. Although for some commodities the action limits for dl-PCBs are lower than for dioxins, this should not result in unacceptable levels of false-negative results. Alternatively, a split method may be used based on activated carbon or florisil in order to receive a dioxin and dl-PCB fraction. This will however complicate the extraction of samples and thus incriminate the screening nature of the assay.

C-7

THE DETECTION AND IDENTIFICATION OF UNKNOWN CONTAMINANTS IN FOOD AND FEED BY LC/QTOF-MS AND STATISTICAL TOOLS

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The recent occurrence of animal illness and death in the United States caused by the apparent deliberate adulteration of feed ingredients with melamine and cyanuric acid makes relevant the need to be able to detect and identify unknown contaminants. When a significant toxicological event occurs in a population, the ability to make the identification of the cause, determine the source, and apply remedial action can be crucial. Fortunately, in the above event no human life was at risk. However, it is clear that the analytical community needs to be prepared. The problem of detection and identification extends to the fact that the cause may be organic, inorganic, polar, non-polar, or an ionic chemical or mixture of chemicals. The importance of multiple sample preparation procedures and the use of various analytical tools such as GC/MS for less-polar compounds, LC/MS for more polar compounds (or IC and IC/MS), and ICP/MS for metal analysis exemplifies the challenge of detecting and identifying a true unknown. This presentation will describe the sample prep requirements and statistical tools to interrogate raw LC/MS data used to determine differences between samples or sets of samples. In addition, the use of accurate mass measurement by LC/QTOF-MS to identify detected unknown components will be described. The statistical tools, Mass Profiler and Genespring will be used to demonstrate how significant differences in samples can be determined directly from raw data. From those results targeted accurate mass measurement MS/MS will show how the analyst can identify those components that have been determined to be significantly different.

C-8

TRUENESS IN PAHS ANALYSIS: TOWARDS A NEW GENERATION OF CRMS

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants, which are highly toxic and carcinogenic. For non-smokers and non-occupational activities, air inhalation and food ingestion are the main ways of exposure. In order to minimise the health risk from the dietary PAHs exposure, the European Union (EU), following the recommendations of the European Scientific Committee on Food (SCF), adopted in 2005 a new Regulation regarding the levels of benzo[*a*]pyrene (BaP) in certain foodstuffs. Although at present BaP is used as a marker for the occurrence of the carcinogenic PAHs, the SCF recommended a list of 15 additional carcinogenic PAHs to be monitored in food commodities (*i.e.* benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*a*,*h*]anthracene, dibenzo[*a*,*e*]pyrene, dibenzo[*a*,*h*]pyrene, dibenzo[*a*,*i*]pyrene, dibenzo[*a*,*l*]pyrene, indeno[1,2,3-*cd*]pyrene, 5-methylchrysene). This will serve to obtain a better knowledge of the PAH profile and to evaluate the suitability of maintaining BaP as a marker, as laid down in Commission Recommendation 2005/108/EC.

To support the implementation of the new EU legislation, the Institute for Reference Materials and Measurements (IRMM) has responded by developing and optimising chromatographic methodology for the determination of the 15+1 EU priority PAHs. Furthermore IRMM is enlarging the list of PAHs certified reference materials (CRMs) already available with new materials dedicated to food analysis. CRMs are an essential tool to assure analytical quality: they are used for the calibration of instruments, for the validation of new analytical procedures, and to allow comparable results based on their metrological traceability. Main findings on the analytical methodology optimisation and validation as well as the steps followed for the production of CRMs including the EU priority PAHs in food matrices will be presented.

C-9

DEVELOPMENT OF A RAPID AND SENSITIVE METHOD FOR THE SIMULTANEOUS DETERMINATION OF 1,2-DIBROMOETHANE, 1,4-DICHLOROBENZENE AND NAPHTHALENE RESIDUES IN HONEY USING HS-SPME COUPLED WITH GC-MS

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In this study a new method for the simultaneous determination of 1,2-dibromoethane (1,2 DBE) 1,4-dichlorobenzene (p-DCB) and naphthalene residues in honey has been developed. Analysis was carried out using gas chromatography-mass spectrometry (GC/MS) in selected ion monitoring mode (SIM), after extraction and pre-concentration of target analytes by head space solid phase microextraction (HS-SPME), with a 100 μm film thickness polydimethylsiloxane (PDMS) fiber.

Several parameters that are affecting the extension of the adsorption process (i.,e., addition of salt, adsorption time, adsorption temperature) were studied. The optimal conditions for the determination of these analytes were established and calibration curves were constructed.

The proposed HS-SPME method showed good sensitivity, without carryover between the samples. Linearity was held over a 1000-fold concentration range from micrograms per kilogram to milligram per kilogram level with coefficients of determination (r^2) ranging from 0,9901 to 0,9999. The repeatability showed a relative standard deviation below RSD<4% within the above mentioned concentration range, while the detection limits were found to be 3 μg/kg, 2 μg/kg, 0.1 μg /kg honey for 1,2- DBE, p-DCB and naphthalene respectively. The percentage recoveries that were evaluated with the proposed HS-SPME method using standard addition calibration technique gave values among 72.8 and 103% for individual measurements of each analyte as well as measurements of each analyte inside a mixture of the other two.

The above method was applied for the analysis of unknown honey samples. The results proved the excellent applicability of the proposed method for the determination of the target compounds in honey samples.

C-10

EXTRACTION OF ENDOCRINE DISRUPTING COMPOUNDS AND THEIR METABOLITES FROM VARIOUS FOOD MATRICES USING AN OPTIMIZED MATRIX SOLID-PHASE DISPERSION METHOD

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A low-cost sample preparation technique that considerably simplifies sample pretreatment is proposed for the determination of phenylurea herbicide residues; diuron and linuron suspected for endocrine disrupting activity as well as their main metabolites in carrots, potatoes, apples and orange juices. The presence of their residues in food commodities can irreversibly affect the consumer's health. Therefore, an urgent demand appears for the development of analytical methods to monitor Endocrine Disrupting Compounds (EDCs) in food so that regulatory limits may be enforced.

Matrix Solid-Phase Dispersion (MSPD) has been evaluated for the extraction of these herbicides and metabolites in food samples. MSPD is a relatively recent extraction and clean up technique used for the simultaneous determination of various pollutants from semi-solid and solid samples. This procedure combines the use of mechanical forces generating from the grinding of samples with irregular shaped particles (silica or polymer based solid supports) with an adsorption capacity of a support-bound polymer (octadecylsilyl or others) to produce a sample/column material from which dispersed sample matrix components can be selectively isolated [1]. The main objective of this study was the effect estimation of different parameters, such as the extraction solvent, the adsorbent and the sample size on the method extraction yield, in order to find the optimum conditions for the method application to real samples.

Food samples (1g) were ground in a mortar with Florisil sorbent and the homogenized mixture was packed into a SPE cartridge and subsequently eluted from the MSPD cartridge using dichloromethane. A clear eluate was obtained, which was evaporated, redissolved, and analyzed by high-performance liquid chromatography coupled to UV-diode array detector. MSPD procedure was shown to be linear over a wide range of concentration, exhibited satisfactory repeatability, and reached limits of detection usually in the low ng/g range. Recoveries, at spiked concentrations below the maximum residue levels established by the European Union, were good in the range of 62% to 96% for all analytes. The proposed method was successfully applied to the analysis of different food commodities sold in Greek supermarkets taken in the course of a year.

[1] Barker Steven, Matrix solid-phase dispersion, J. Chromatogr. A, 885, (2000), 115-127.

C-11

OPTIMISATION AND VALIDATION OF AN HPLC-SPECTROFLUORIMETRIC METHOD FOR THE DETERMINATION OF 16 PRIORITY EU-PAHS IN VEGETABLE OILS

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Polycyclic Aromatic Hydrocarbons (PAHs) are a large class of organic compounds containing two or more aromatic fused rings. It has been demonstrated that the main source of PAHs exposure for human beings is through food, particularly fats and oils, due to the lipophilic nature of PAHs.

Investigations on PAHs throughout the world have generally focused on benzo[a]pyrene (BaP) (considered the most hazardous) or on all the 16 PAHs highlighted by the US Environmental Protection Agency (EPA) in the 1970s. In early 2005, the European Union (EU) introduced a new legislation with the aim of avoiding disparities in the limits of PAHs in foods among different European Member States, and in response to food contamination problems, as highlighted by the Opinion of the Scientific Committee on Food (SCF) expressed in 2002. The SCF (today EFSA) identified 15 PAHs as both carcinogenic and genotoxic and concluded that BaP could be used as a marker. EU also recommended that all the Member States investigated the levels of the 15 PAHs pointed out by the SCF and the one PAH (benzo[c]fluorene-BcF-) highlighted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2005 in order to review the limits already set. The review should have been done by the 1 of April 2007, but until today nothing has changed. Very few works dealing with EU-PAH determination in food matrices have been published up to date.

The aim of this study was to set up an HPLC spectrofluorometric method for rapid determination of the 16 EU-PAHs in vegetable oils. At this purpose a Solid Phase Extraction (SPE) method, previously proposed for the determination of the EPA-PAHs in vegetable oils (involving pre-separation of PAHs from triglycerides on a 5 g silica cartridge), was modified in order to allow the elution of the heavier benzopyrenes from the cartridge. Chromatographic conditions (gradient, flow rate, column temperature) and detection parameters were also optimized in order to achieve, in about 30 minutes, an optimal separation and quantification of the 16 EU-PAHs on a reversed phase HPLC column (250 mm x 3 mm id). A longer gradient elution program (about 50 min) was proposed for simultaneous separation of all the priority pollutant EPA-PAHs and the new introduced EU-PAHs. A particular attention has been focused on the effect of temperature on chromatographic separation of dibenzo(a,e)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)pyrene.

The proposed method was validated and showed good performances.

C-12

RESOLUTION AND MASS ACCURACY CAPABILITIES OF HYPERBOLIC TRIPLE QUADRUPOLES FOR THE ANALYSIS OF SMALL MOLECULES IN FOOD SAMPLES

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Triple-quadrupole mass spectrometers coupled to liquid chromatography have become the most popular mass analyzers for quantitative determinations. In particular, for the analysis of small molecules in complex matrices such as food samples triple-quadrupole mass spectrometers, operating in selected reaction monitoring (SRM) mode, are widely used because of the high selectivity provided by the transition monitored for both quantitation and confirmation purposes. However, standard quadrupoles operate at unit mass resolution and they are not able to provide accurate mass measurements.

Nowadays, fast chromatography demands high speed mass analyzers to monitor very narrow peaks. Moreover, high resolution and mass accuracy measurements show an increasing demand for confirmation purposes. Although these goals can be achieved using time-of-flight mass analyzers, sensitivity must be sacrificed.

Recently, technological advances in new quadrupole mass spectrometers have been produced. The new hyperbolic quads provide unrivalled performance in resolution, high scanning speed and mass accuracy measurement possibilities without scarifying sensitivity.

In this work, some examples have been selected to illustrate the performance of an hyperbolic triple-quadrupole mass spectrometer coupled to a liquid chromatography system. High-resolution MS/MS and mass accuracy measurements have been used to analyse small molecules such as quaternary ammonium herbicides, heterocyclic amines, bisphenol A and related compounds and the results have been compared with those obtained with Q-TOF instruments. Moreover, some advanced scanning functions such as reversed energy ramp (RER) and quantitation enhanced data-dependent (QED) have been applied to improve simultaneously sensitivity and structural information. Finally, the RER scan has been tested in order to generate product ion libraries for different applications.

C-13
DETECTION OF PERFLUORINATED COMPOUNDS IN 18 RAW AND COOKED FISH & SHELLFISH SPECIES

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Dietary intake is a major route of exposure for perfluorinated compounds. Although fish and seafood contribute significantly to the total dietary exposure of these compounds, few studies have reported concentrations in marine muscle tissue. Furthermore, concentrations are often reported in raw samples; a paucity of data exists for cooked fish and seafood samples. Eighteen fish species purchased from markets in Toronto, Mississauga, and Ottawa, Canada were analyzed for perfluorooctanesulfonamide-based fluorochemicals (PFOSAs) and perfluorinated acids (PFAs) in raw and cooked (baked, boiled, fried) samples. Of seventeen analytes, PFOS was detected most frequently; concentrations ranged from 0.21 to 1.68 ng/g ww. Total concentrations of PFOSAs and PFAs ranged from 19.6 pg/g ww to 0.11 ng/g ww and 0.21 to 9.21 ng/g ww, respectively, consistent with previous studies. All cooking methods reduced PFA concentrations. Baking was the most effective method; after baking samples for 15min at 325C, PFAs were not detected in any of the samples. The margin of exposure (MOE) between toxicological points of reference (LOEL, $BMDL_{10}$) and the magnitude of dietary intake of perfluorinated compounds was substantial, even for a high fish consumption scenario.

C-14

DETERMINATION OF SELECTED BIOCHEMICAL MARKERS IN CHUB (LEUCISCUS CEPHALUS) AS BIOINDICATORS OF THE CONTAMINATION OF THE SVITAVA AND THE SVRATKA RIVER

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Substances which are contaminating water environment can affect both integrity of ecosystems and physiological functions of aquatic animals. A lot of these substances, so-called endocrine disruptors, are able to modulate or disrupt endocrine system of animals. Cytochrome P450 and an enzyme related to it - ethoxyresorufin-O-deethylase - are the most frequently monitored biomarkers which are important in the first phase of detoxification of xenogenic substances. The conjugation enzymes (as gluthatione S-tranferase) are important in second phase of detoxification. Vitellogenin and 11-ketotestosterone are used as biomarkers of endocrine disruption.

The aim of our study was to determine these biomarkers in blood plasma and liver samples of male chub (*Leucisus cephalus*) which were collected on seven localities on the Svratka and the Svitava River. Vitelogenin and 11-ketotestosterone were measured in blood plasma by ELISA (Enzyme linked immuno sorbent assay) commercial kits. The activity of cytochrome P450 and gluthatione S-transferase were measured spectophotometrically after the homogenization of liver samples. The determination of ethoxyresorufin-O-deethylase in chub liver samples was done on spectrofluorimeter. The results showed that the locality Svratka under the Brno city is the site most contaminated by xenoestrogenic and xenoandrogenic substances in comparison with other analysed localities.

C-15

TARGET SCREENING SOFTWARE FOR GCxGC-TOF-MS: COMPARISON OF AN IN-HOUSE DEVELOPED SOFTWARE MODULE BASED ON METALIGN WITH LECO CHROMA-TOF

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Comprehensive two dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOF-MS) is a very powerful technique for comprehensive screening and determination of residues and contaminants in the food chain. Recently, the qualitative and quantitative performance of a generic method based on GCxGC-TOF-MS was evaluated for determination of 106 pesticide residues and environmental contaminants in a complex cereal based matrix [1]. The method was successfully validated. LOQs were in the 1-20 µg/kg range.

At present, for multi-target compound analysis, sample pre-treatment, instrumentation and conditions for GCxGC-TOF-MS analysis are well established. However, the data files generated with this technique are very large (typically >400 MB per injection) and data handling using the system's software (Leco Chroma-ToF in this case) was found to be very time consuming. This is even more true when higher numbers of target analytes and extended target libraries are involved. In other words, while information on potential presence of thousands of food contaminants is available in the raw data, the lack of adequate software for data handling within acceptable time seriously limits current use of GCxGC-TOF-MS for routine comprehensive target compound analysis.

Within our institute MetAlign software [2,3] has been developed for preprocessing of GC- and LC-MS data for application in the field of metabolomics. In this work the software has been modified and extended to enable automatic multi-target screening of raw data files obtained after GCxGC-TOF-MS analysis.

The software performes the following steps, batch wise and fully automated, after conversion of raw data files into common data format (.cdf):

1. Splitting of .cdf data into second dimension sections (modulations).

2. Pre-processing of each section through MetAlign. This includes noise estimation, smoothing, baseline correction and peak-picking (only peak maxima are retained) for each m/z measured. This results in compression of the data by a factor of 50-100. No deconvolution is performed.

3. Re-combination of modulations pre-processed by MetAlign into one compressed data file.

4. Identification of peaks from the pre-processed data.

For this a newly developed software module with adjustable criteria is used to search for and identify compounds in the compressed data file using any NIST compatible library. Within a narrow moving time window (< peak width) m/z signals are combined into a partial spectrum. Only the m/z's in the partial spectrum are matched to the corresponding m/z's in library spectra. The match factor obtained is used as one of the decision criteria for identification. Other criteria are the number of m/z in the partial spectra, the TIC value of a partial spectrum, the percentage of the library spectrum represented by the partial spectrum and the empirical ability to explain the absence of m/z's by simulating neighboring peaks. An additional feature is a penalty factor for m/z's which are present in the sample spectrum but not in the library spectrum and vice versa. Furthermore, it is possible to include retention time data to aid in the identification and reduce false positives (not possible with qualitative data evaluation using Chroma-ToF software). All output from the module is compatible with Excel.

The performance of the MetAlign based software module with respect to automatic identification of target compounds was evaluated and compared with the most recent version of the Leco Chroma-ToF software. For this purpose data generated by GCxGC-TOF-MS analysis of extracts spiked with 106 pesticides and environmental contaminants in the range of 1-100 µg/kg were used. At various concentrations, the numbers of false negatives was verified. Furthermore, the effect thresholds of identification criteria on number of false negative and false positive findings were evaluated. The current version of the developed software module showed equal or better performance with respect to identification of target compounds and was found to be more flexible and faster. Further improvement of the software module is in progress and will increase attractiveness of GCxGC-TOF-MS for comprehensive contaminant screening.

[1] M. K. van der Lee, G. van der Weg, W.A. Traag, H.G.J. Mol, Determination of pesticides and contaminants in animal feed using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry, submitted to J. Chromatogr. A (2007)
[2] http://www.metalign.nl/UK/ (download and publications)
[3] A. Lommen, G. van der Weg, M. C. van Engelen, G. Bor, L.A.P. Hoogenboom, M.W.F. Nielen, Analytica Chimica Acta 584 (2007) 43–49. An untargeted metabolomics approach to contaminant analysis: Pinpointing potential unknown compounds

C-16
PERFLUORINATED COMPOUNDS IN POTATO AND POTATO PRODUCTS – A PILOT STUDY

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Perfluorooctanoic acid (PFOA) and perfluorooctansulfonic acid (PFOS), both toxic, were often used as indicator substances for Perfluorinated Compounds (PFC), a substance group of increasing concern. They are found worldwide in the environment. Due to persistency, mobility and bioaccumulation they are also found by ingestion in wildlife and humans.

Almost all investigations concentrate onto the determination of PFOA and PFOS, because of their public importance and known analytical procedures. Furthermore most scientific publications concentrate on their occurrence in the environment and not in food.

In this investigation also additional PFCs individuals were analysed in potatoes and potato products of diverse origin. Due to the different content of fats and proteins in raw potato and its products, method development was required for nearly each type of product. Validation was performed for nine perfluorocarboxylic acids (C4 through C12), three perfluorosulfonic acids (C4, C6, C8) and perfluorooctane sulfonylamide (PFOSA). LC-MS/MS using four 13C-labelled reference materials was employed.

The results show that PFOA and PFOS are “not alone”. The other PFC compounds, which might have a toxic importance, can contribute significantly to the total load on PFCs. Altogether they showed different distribution in raw potato and in potato products which allows the assumption of a contamination during processing.

C-17
WHY DO GC- AND LC-MS RESULTS FOR HBCD IN FISH DIFFER SO MUCH?

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Hexabromocyclododecane (HBCD) is a frequently used brominated flame retardant and is added at relatively high concentrations to various materials [1]. HBCD is persistent and lipophilic and was detected in breast milk and human blood [1] showing the exposure of humans to HBCD. The human dietary exposure to HBCD was estimated at 141 ng/day (median) in Sweden [2] and 8.3 ng/day in the Netherlands (medium bound; fish only) [3]. The European Food Safety Authority (EFSA) recommends monitoring of BFRs, including HBCD, in food and feed using GC-based techniques (www.efsa.europa.eu). HBCD consists of three diastereomers, α-, β- and γ-HBCD. These diastereomers can be quantified individually by LC/ESI-MS/MS, whereas total-HBCD (sum of the diastereomers) can be measured by GC/ECNI-MS. The additional diastereomer profile information is beneficial when assessing the fate and behaviour of HBCD.

Forty-four marine and freshwater fish samples were analysed and α-HBCD was found as the predominant diastereoisomer. The samples were also analysed by GC-ECNI-MS/MS and these results are a factor of 4.4 higher than the LC based results (based on the regression analysis). There could be several reasons for this large difference. As regards GC/MS, the thermally labile HBCD diastereomers can rearrange above oven temperatures of 160°C [1] and this may considerably effect the results, as the diastereomers have different response factors (relative response factors of α-, β- and γ-HBCD were 100, 71 and 73%, respectively). This is also relevant as the diastereomer profile in biological samples (in which α-HBCD predominates) is different from the profile in the used standard solution (equal concentrations of α-, β- and γ-HBCD). As a result, the GC/ECNI-MS results may have been altered by 10-20%, causing biased results. More important than this will be the correction of the internal standard. In case the difference between the concentration of the internal standard and that of HBCD in the sample becomes (much) too large, more substantial errors can occur. As regards LC, the issue of different response factors is not relevant because the diastereomers are separated chromatographically. However, signal suppression may occur in the electrospray ionisation due to co-eluting matrix constituents, but ^{13}C-labeled HBCD internal standards should correct for that . Also, the samples have been submitted to a very thorough clean-up, which strongly reduces the chance of matrix effects in the MS. Haug *et al.* [4] reported on a comparison of GC/MS and LC/MS results from an interlaboratory study and found only small differences between both methods (GC results were 1.1-1.3 times higher). A better ratio between internal standard and HBCD concentration in the sample may explain this smaller difference. The better sensitivity of new LC/MS instruments is another good reason to use this technique instead of GC/MS for the determination of HBCD.

[1] A. Covaci, A.C. Gerecke, R.J. Law, S. Voorspoels, M. Kohler, N.V. Heeb, H. Leslie, C.R. Allchin, J. de Boer, Environmental Science & Technology 40 (2006) 3679.
[2] Lind.Y., M. Aune, S. Atuma, W. Becker, R. Bjerselius, A. Glynn, P.O. Darnerud, Organohalogen Compounds 58 (2002) 181.
[3] S.P.J. van Leeuwen, J. de Boer, Molecular Nutrition & Food Research (2007) *Accepted.*
[4] L.S. Haug, C. Thomsen, G. Becher, Organohalogen Compounds 68 (2006) 89.

C-18
PRIONS "SURVIVE" IN SOIL OVER YEARS – CONSEQUENCES FOR THE FOOD CHAIN?

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The EU integrated approach to food safety aims to assure a high level of food safety, animal health, animal welfare and plant health within the European Union. By publishing the farm to fork approach, the food quality and safety should be controlled during the entire food chain, from primary production to product delivery. Therefore, also soil as a starting point for agriculture production should be controlled. With regard to pesticides, heavy metals etc. this is implemented in practice. However, with regard to infective bio molecules in soil there are only slight efforts or possibilities to analyze such molecules. This holds particularly true with respect to prions, the causative agents of transmissible spongiform encephalopathies (TSEs) such as BSE, scrapie or chronic wasting disease (CWD). The entry of the TSE pathogen into the environment can take place due to several sources, e. g. infectious placenta or amniotic fluid of sheep. Furthermore, environmental contamination by excrements, non-sterilized agricultural organic fertilizer or saliva is conceivable. Natural transmission of scrapie in the field seems to occur via the alimentary tract in the majority of cases.

With outdoor experiments, we simulated a contamination of standard soil with hamster-adapted 263K scrapie prions and analyzed the presence and biological activity of the soil-associated PrPSc. Our results showed that the scrapie agent can persist in soil at least over two years. Strikingly, by conducting bioassays we could demonstrate that the contaminated soil taken from the long-time incubation experiments retained high levels of infectivity as evidenced by oral administration to Syrian hamsters. Also by feeding aqueous soil extracts, it is possible to induce disease in the reporter animals. These results indicated that prion-contaminated soil may lead to contaminated ground or pore water. Therefore, it is possible that prion-contaminated soil acts as a source for further TSE-infections in animals over the oral route. An effective farm to fork approach will raise the question if these results lead to consequences of the entire food chain.

C-19

CHRONOPOTENTIOMETRIC FLOW INJECTION ANALYSIS OF MERCURY IN FISH SAMPLE BY USING GOLD ELECTRODES FROM RECORDABLE CDS

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Mercury determination in fish sample was possible by using chronopotentiometric flow injection analysis. A special flow cell containing a gold working electrode (prepared from a recordable CD) with a surface of 25 mm^2 was developed. The flow cell volume was of about 5 µL. It was used a Ag/AgCl reference electrode and an auxiliary electrode made of a tube of stainless steel with an inner diameter of 1.4 mm and a surface of 132 mm^2.

Various parameters have been optimized to yield low detection limit (0.25 ng/mL for 10 minutes deposition at 0.3 V). The optimum flow rate was established to be 1.0 mL/min. The precision was very good (RSD = 0.55 % for 10 repetitive measurements using a 20 ng/mL concentration of mercury). Calibration curve was found to be linear over the range 5 – 100 ng/mL of mercury for a deposition time of 3 min.

The flow cell developed was used for analysis of mercury in cod fish samples. For this type of samples it was observed that the same surface could be used for many determinations. The results obtained with the developed system were in good agreement with those obtained by atomic absorption spectrometry.

C-20

DIRECT DETERMINATION OF ESTROGENIC COMPOUNDS IN BEER BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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The present work describes the simultaneous determination of four potent estrogenic compounds in beer, the phytoestrogen 8-prenylnaringenin, the mycotoxin zearalenone (ZON) and two of its metabolites α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL). The flavanone 8-PN has been identified as a potent phytoestrogen coming from the use of hops [1]. The female flowers of the hop plant are used as a preservative and as a flavoring agent in beer. In addition it has been shown that 8-PN has an activity greater than other established plant estrogens (daidzein, genistein, coumestrol) [1]. ZON is a nonsteroidal estrogenic mycotoxin with a phenolic resorcyclic acid lactone structure. It is produced by Fusarium species, which colonize several grains like maize, oat, barley, wheat and sorghum under prolonged cool and wet weather conditions in temperate and warm regions. It exhibits distinct estrogenic and anabolic properties in several animal species resulting in severe effects on the reproductive system. ZON and its derivatives, α-ZOL and β-ZOL, may be transmitted from contaminated grains into beer during the brewing process. It has also been shown that the two aforementioned metabolites of zearalenone are formed during fermentation of wort for beer production [2].

A direct and confirmatory analytical method, based on liquid chromatography coupled with electrospray ionization mass spectrometry (LC-ESI-MS) was developed for the determination of 8-prenylnaringenin, zearalenone and its derivatives α-zearalenol and β-zearalenol in beer. Experimental design was applied to assess the effects of the LC-ESI-MS parameters (mobile phase flow rate, drying gas flow, nebuliser pressure, capillary potential and fragmentor voltage) on the obtained signal and to optimize the values in order to provide maximum sensitivity and detectability. The proposed method is simple consisting only of degassing the beer and diluting with water (1:1 v/v) before injection. The method was validated using spiked samples of beer and zearalanone (ZAN) was used as internal standard. The recoveries % of the compounds from beer were: 78.6 for 8PN, 118 for ZON, 93.2 for α-ZOL and 66.0 for β-ZOL. The intra-day precision (RSD %) of the method ranged from 2.7% for 8-PN to 8.0% for ZON (n = 4), while the inter-day precision ranged from 5.0% for β-ZOL to 10.3% for α-ZOL (n = 8). The limit of detection was 1.1, 1.3, 1.4 and 1.0 ng/ml of beer for 8-PN, ZON, α-ZOL and β-ZOL, respectively. Commercial beer samples were tested and low concentrations of 8-PN were detected close to the limit of detection.

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[1] Milligan S., Kalita J., Heyerick A., Rong H., De Cooman L. and De Keukeleire D., The Journal of Clinical Endocrinology & Metabolism, **1999**, 83, 2249-2252.
[2] Scott M., Kanhere R. Daley F., and Farber M., Mycotoxin Research, **1992**, 8, 58-66.

C-21
MONITORING OF LEAD AND CADMIUM IN KIMCHI AND PICKLE BY ICP-MS

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The objective of this study is the precision measurement of heavy metal contents in frequently consumed foods in Kimchi and pickle for the risk assessment and evaluation of pre-established standards of heavy metals contents in frequently consumed foods. Target heavy metals are lead (Pb) and cadmium (Cd). For analysis, the Kimchi and pickle were purchased from the modern and traditional markets in Seoul, Busan, Inchoen, Daejeon, Kwangju, Jeonju, Daegu, Ulsan and Kangwon. The samples included Kimchi (Chinese cabbage Kimchi; CK), Radish Kimchi (RK), Pickled Cucumber (PC), Pickled Perilla leaf (PP) and Pickled Radish (PR). About three-hundred samples were digested microwave and subjected to measurement with Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The values of heavy metals in Kimchi (CK) [minimum-maximum (Mean±SD), unit: mg/kg] are 0.010-0.064 (0.026±0.018), 0.005-0.012 (0.007±0.002) for lead and cadmium, respectively. The values of heavy metals in Radish Kimchi (RK) [minimum-maximum (Mean±SD), unit: mg/kg] are 0.008-0.109 (0.038±0.033), 0.004-0.007 (0.005±0.001) for lead and cadmium, respectively. The values of heavy metals in Pickled Cucumber (PC) [minimum-maximum (Mean±SD), unit: mg/kg] are 0.027-0.076 (0.043±0.024), 0.001-0.002 (0.001) for lead and cadmium, respectively. The values of heavy metals in Pickled Perilla leaf (PP) [minimum-maximum (Mean±SD), unit: mg/kg] are 0.050-0.124 (0.078±0.026), 0.001-0.007 (0.004±0.002) for lead and cadmium, respectively. The values of heavy metals in Pickled Radish (PR) [minimum-maximum (Mean±SD), unit: mg/kg] are 0.023-0.105 (0.038±0.032), 0.001-0.008 (0.004±0.003) for lead and cadmium, respectively.

C-22

VALIDATION OF A PRESSURISED LIQUID EXTRACTION (PLE) MULTI-COLUMNS LOW PRESSURE LC COUPLED TO GC-HRMS FOR PCDD/FS AND DIOXIN-LIKE PCBS IN GUAR GUM

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During the last decade a number of dioxin related incidents occurred in the food and feed chain. As a result, monitoring programs in European countries were intensified. The most recent incident occurred during the last summer period with contaminated guar gum. The contamination with polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and pentachlorophenol (PCP) was discovered in Switzerland in additive samples with levels far above the maximum EU limits. Guar gum, produced only in India and Pakistan, is extracted from the green vegetable guar bean. It is used as an additive in a wide range of foods as well as dairy products. Its role is that of an emulsifier, thickener and stabilizer. The present paper describes the validation of an automated parallel pressurized liquid extraction (PLE) coupled to preparative multi-column low pressure liquid chromatography for sample preparation. This new approach matches the high sample throughput demand of routine laboratories while reducing human input at affordable cost. The optimisation of the extraction parameters is toluene/ethanol 90:10, temperature 150°C, pressure 1500 psi, extraction time 20 min and minimum 2 cycles. The extract was further cleaned and fractionated with a Power-Prep system using acid silica, alumina and carbon columns. PCDD/Fs fraction was injected into a gas chromatography - high resolution mass spectrometer (GC-HRMS, autospec ultima, Waters, UK). All details regarding the analytical procedure for Power-Prep and GC-HRMS can be found elsewhere[1]. The limit of quantification (LOQ) for most of the toxic congeners is 0.01 ng/kg. The precision assessed by repeatability tests provided relative standard deviation (RSDs) between 1 to 20% for PCDDs, PCDFs and dioxin-like PCBs. Within-laboratory reproducibility study provided RSDs between 3 to 17% for the same congeners. Recoveries are between 54 to 93% and the bias was calculated with spiked samples. The bias met the EU directive[2] requirement (i.e. ± 20%) for results expressed in toxic equivalent units.

[1] J.-F. Focant, G. Eppe, C. Pirard, E. De Pauw, J. Chromatogr.A 925 (2001) 207.
[2] Commission Regulation 1883/2006 Official journal of the European Union L364/32-43

C-23

A SCREENING ASSAY FOR DIOXIN-LIKE PCBS IN RETAIL FISH USING A SURFACE PLASMON RESONANCE SENSOR

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Dioxin-like polychlorinated biphenyls (PCBs) are classified as dioxins, and often make up the majority of the toxic equivalent (TEQ) contribution of dioxins in fish samples. It is therefore important to develop screening methods for determining TEQ concentrations of dioxin-like PCBs in retail fish to be able to carry out risk assessments.

We have developed a rapid immunoassay for dioxin-like PCBs using a surface plasmon resonance sensor (Biacore3000). Samples mixed with a monoclonal antibody (Mab) specific to PCB 118 interacted with PCB analog-bovine serum albumin conjugate immobilized on a CM5 sensor chip. After competitive reaction, the Mab that had bound to the conjugate was detected using surface plasmon resonance. The total run time for each cycle was about 12 min. The fish extracts were first cleaned up on a multi-layer silica gel column followed by an alumina column, then subjected to the assay. The quantitative limit of the assay was 100 ng PCB 118/mL in the standard curve, corresponding to 1 ng PCB 118/g in the tested sample. Dilution and recovery tests using purified fish extracts suggested that the matrix effect was minimized in the assay by diluting the analyzed samples. The reproducibility of the assay was excellent (coefficient of variation <10%). The assay results for retail fish samples (n = 7) showed a good agreement with the results obtained by ELISA using the same Mab. ELISA has been already validated for determining dioxin-like PCBs in fish samples, suggesting that our assay performed well in the analysis of dioxin-like PCBs in fish samples. Finally, the results for retail fish samples (n = 10) showed a good correlation between this method and high-resolution gas chromatography coupled to high-resolution mass spectrometry for the determination of the TEQ concentrations of dioxin-like PCBs (r = 0.89). These data indicate that our method is useful for screening retail fish to determine the TEQ concentrations of dioxin-like PCBs.

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C-24

DETERMINATION OF BIO-ACCESSIBLE ARSENIC SPECIES IN SEAFOOD BY COMBINATION OF FAST CONTINUOUS LEACHING AND IEC/ICP-MS SPECIATION

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The toxicity of arsenic (As) is dependent on its speciation i.e. chemical forms (species). Yet, at the time being, government agencies assess the safety of food items based solely on the concentration of total As. Foods that contain a level higher than the total concentration guidelines regulation are declared unsafe, irrespective of the form of the element, which may be poorly toxic. Moreover, the respectivebio-accessibility of As species which is defined as their solubility in artificial gastrointestinal fluids, is not considered either. Seafood are considered as contributor of interest regarding general human exposure to As. The majority of methods developed to determine bio-accessibility of elements induces static methods that do not always represent well human physiology and are often very complex to perform and time-consuming.

The goal of this project is to develop a rapid method to determine bio-accessibility of As species from seafood. First, the process involves continuously leaching of solid samples with, successively, artificial saliva, gastric and intestinal fluids. The each reagent is sequentially pumped through a mini-column of sample (maintained at 37 °C in a thermostated water bath) that is connected to the nebulizer of an inductively coupled plasma mass spectrometry (ICP-MS) instrument. The high sensitivity of ICP-MS allows the continuous detection in real time of the progressive release of total arsenic from samples by each reagent. Then, speciation of arsenic in each leach is determined by a relatively rapid HPLC/ICP-MS method optimised previously, involving the baseline separation, within 11 min in a single chromatographic run, of 7 As species on an Dionex IonPac AS 7 anion-exchange column with a nitric acid gradient eluent.

The bio-accessibility of total arsenic and each 7 As species from seafood Certified Reference Materials (CRM) had been determined with this quicker and simpler approach. The results show that the majority of bio-accessible As species in tested samples is released by the artificial saliva in less than 5 min. These results were compared with that of the static batch method.

C-25

DEVELOPMENT OF A HPLC/UV-FLD METHOD TO DETECT THE 15 (+ 1) EU PRIORITY POLYAROMATIC HYDROCARBONS (PAH) IN FOOD SUPPLEMENTS

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Polycyclic aromatic hydrocarbons (PAHs) are a large group of over 200 different chemicals formed during the incomplete burning of organic substances and widely present in the environment as a result of industrial activities but also cooking processes, such as drying or smoking.

In the past, the US Environmental Protecting Agency (EPA) identified the most frequent PAHs in environmental samples ("the 16 EPA PAHs"). Some of them are known as carcinogenic or mutagenic and give rise to serious health concern. In the European Union, the Scientific Committee on Food published another list of 15 priority PAHs for controlling PAHs contamination in food, including some of the US EPA list and by adding new genotoxic compounds (SCF/CS/CNTM/PAF/29/Final, 2002). Moreover, the Joint FAO/WHO Experts Committee on Food Additives appended to the list a 16th compound also considered as genotoxic (JECFA/64/SC, 2005).

Food supplements are plant or animal-derived products (dried plants, plant oils, seaweeds, fish oils, bee products…) which may contain large contaminations with PAHs (1). Maximum tolerable levels for benzo(a)pyrene (BaP) exist for some foodstuffs (Commission Regulation (EC) No 1881/2006) but there is currently no legislation in place controlling the maximum levels of BaP or other PAHs in food supplements. In the mean time, maximum levels for BaP in oils and fats are applied.

The final aim of this study is to obtain an indication of the Belgian consumer exposure to PAHs through food supplements consumption, and try to make categories of contamination levels within the different classes of food supplements.

This research describes the development of a HPLC/UV-FLD method to detect and quantify the 16 EU priority PAHs in food supplements covering the categories of dried plants, plant, oils and "exotic" products (out of Europe). The HPLC/UV-FLD parameters were previously described (2). Extraction of oil matrix was adapted from existing methods (3) and a clean-up procedure was set up for dried plants (solid-liquid extraction followed by purification on a hand-made silica column). Short validation was made in agreement with quality criteria described in the Commission Regulation (EC) N° 333/2007.

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[1] Food Standards Agency. PAHs in dietary supplements, 2005.
Available online at http://www.foodstandards.gov.uk/science/surveillance/fsis2005/fsis8605.
[2] Brasseur C., Brose F., Pirlot A., Douny C., Eppe G., Maghuin-Rogister G. and Scippo M.-L., *Accred. Qual. Assur.*, 2007, DOI 10.1007/s00769-007-0295-0.
[3] Simon R., Palme S., Anklam E., *J. Chromatogr. A*, 2006, 1103:307-313.

C-26

OCCURRENCE OF PERFLUORINATED COMPOUNDS IN CZECH FISH AND FISH PRODUCTS IN CZECH MARKET

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Perfluoroalkylated substances (PFAS) are a large group of chemicals. Their physico-chemical properties are unique in that they have high chemical stability, thermostability, hydrophobicity and oleophobicity. Due to these properties they are widely used for many applications e.g. as surface treatment agents, polymerization aids and in fire-fighting foams. Because of their persistence in the environment, their potential to bioaccumulate and/or biomagnificate in the food chains they are of scientific concern.

The aim of presented study was to monitor the pollution extension in the Czech aquatic environment and possible sources of human's exposure by this group of organohalogen contaminants. The study was focused on analyses of perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonamide (FOSA), the most widely studied PFAS, in two different types of samples. The first one was livers of chub species (*Leuciscus cephalus*) caught from May to June 2007 in 15 sampling sites in various rivers in the Czech Republic in cooperation with the Research Institute of Fish Culture and Hydrobiology, Vodňany, the another one was canned fish products (sardines, herrings, cod livers, mackerels, tune fishes) bought in the Czech retail market. The target analytes were extracted using a fast method based on an application of activated charcoal clean-up step, formerly optimized in our laboratory. Separation, identification and detection were performed by high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometric detector (MS/MS). Measured data showed the good agreement with published method.

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C-27

MONITORING OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) AND PESTICIDE RESIDUES OCCURRING IN OILS AND FISH PRODUCTS

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Various chemical contaminants may occur in olive oil and polycyclic aromatic hydrocarbons (PAHs) - as well as pesticide residues - are the most common. In recent years, several RASFF notifications were released on PAHs and benzo[*a*]pyrene (B[*a*]P) in many food products. These carcinogenic compounds are formed either by smoking or heating processes that allow combustion products to come into direct contact with food. The contamination has been reported in the past in vegetable oils and particularly in olive – pomace oil. The risk associated with dietary PAHs has also been recognized by Scientific Committee on Food (Commission, 19 December 2006). The maximum limit recently set by the Regulation No. 1881/2006/EC on benzo[*a*]pyrene (B[*a*]P) for the most of smoked food products is 5 μg/kg and for oil 2 μg/kg.

The growing public concern about health and the potential risks posed by the presence of pesticides residues in the human diet has substantially altered crop protection strategies. Pesticide usage is subjected to strict regulation, especially as regards residual levels in food and crops.

In this study, 12 EPA-PAHs and 112 pesticides in olive oils (19 extra virgin olive oils, 3 olive-pomace oils and 3 olive oils) and canned fish (13 various samples of smoked canned fish products, 14 samples of non-smoked canned fish products) samples were examined.

The analysis of PAHs was routinely carried out using high performance liquid chromatography coupled with fluorescence detection (HPLC/FLD). In addition GC/MS was also employed for comparison and for PAHs not amenable to fluorimetric detection. Separation, identification and quantification of pesticides was performed by capillary gas chromatography coupled with mass spectrometry (GC/MS). For cleaning of crude extracts a gel permeation chromatography (GPC) was used. The effect of clean up by (GPC) followed by SPE technique was demonstrated by GC x GC / TOF - MS.

The sum of 12 EPA-PAHs ranged from 15.9 to 67.4 μg/kg in virgine olive oil samples, from 9.9 to 21.8 μg/kg in olive oil samples, and from 20.8 to 229 μg/kg in olive pomace oil samples. Nevertheless, all samples complied with the legislation limit set by the Reg. 1881/2006/EC for benzo[*a*]pyrene. On the other hand, 6 samples of canned fish exceeded the maximum limit for B[*a*]P and the sum of PAHs ranged from 0.5 to 1 192 μg/kg.

Residues of pesticides were found in almost all analysed samples of canned fish. Regarding virgin olive oils, endosulfan sulphate was the most frequently detected pesticide among all the others. Contrary to virgin oils pesticide residues were not detected in refined olive oils.

The study was carried out within the project MSM 6046137305 supported by the Ministry of Education, Youth and Sports of the Czech Republic.

C-28

BROMINATED FLAME RETARDANTS AND ORGANOCHLORINE POLLUTANTS IN HUMAN ADIPOSE TISSUE SAMPLES FROM THE CZECH REPUBLIC

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Brominated flame retardants (BFRs) – polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) and other persistent organohalogen pollutants, such as polychlorinated biphenyls (PCBs) and selected organochlorine pesticides (OCPs) are persistent and lipophilic xenobiotics bioaccumulating in the fatty tissues of biota. The presence of these compounds in human tissues is of particular concern because of their toxicological potential to interfere with neurodevelopment and endocrine system.

As for the most countries, the levels of BFRs have been previously investigated in bioindicator matrices such as freshwater fish and human breast milk 1-3 also in the Czech Republic but this is the first study, which determined the BFRs in human fat of the population living in the Czech Republic.

The isolation of target compounds from adipose tissue was performed by Soxhlet extraction with hexane-dichloromethane followed by gel permeation chromatography (GPC) purification step. Identification and quantification of PBDEs and HBCD was carried out by GC–MS operated in negative chemical ionization (NCI). GC/2ECD method was used for determination of organochlorine compounds.

Levels of BFRs selected organochlorine pollutants were measured in adipose tissue samples (n=100) of humans living in the Czech Republic. Samples were collected from January to August 2007 at the Institute of Plastic Surgery in Prague on occasion of liposuction procedure. Mean levels of sum PBDEs and sum of seven indicator PCBs were 4.3 and 121.9 ng/g lipids, respectively. Among PBDEs, congeners 153, 47, 99 and 100 were the most frequent and abundant and together constitute 96% of the total amount of PBDEs in adipose tissue. Among PCB congeners, PCB 153 and 180 presented the highest concentrations and contributed 75% of all PCBs. The levels of PCBs were similar to those described on other European countries. Concentrations of PBDEs (sum of 10 congeners) were in the lower end of reported concentration in European countries.

No age dependency was found for PBDEs (r=0.211); whereas PCBs showed higher correlation coefficients with age (r=0.657). There was no relationship between PBDE and PCB levels (r=0.312) what documents different exposure sources.

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[1] Hajšlová J., Pulkrabová J., Poustka J., Čajka T., Randák T.: Brominated flame retardants and related chlorinated persistent organic pollutants in fish from river Elbe and its main tributary Vltava. Chemosphere 2007, 69, 1195-1203.
[2] Pulkrabová J., Hajšlová J., Poustka J., Kazda R.: Fish as Biomonitor of Polybrominated Diphenyl Ethers (PBDEs) and Hexabromocyclododecane (HBCD) in Aquatic Environment: Pollution of Elbe River-Basin. Environ. Health Perspect., 2007, in press, doi10.1289/ehp.9354.
[3] Kazda R., Hajšlová J., Poustka J., Čajka T.: Determination of polybrominated diphenyl ethers in human milk samples in the Czech Republic - Comparative study of negative chemical ionisation mass spectrometry and time-of-flight high-resolution mass spectrometry. Anal. Chim. Acta 2004, 520, 237–243.

C-29

PREPARATION OF OLIVE OIL REFERENCE MATERIAL FOR DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS

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PAH can contaminate foods during smoking or drying processes that allow combustion products to come into direct contact with food. In addition, environmental pollution may also cause contamination with PAH. The popularity of consumption of olive oils increases however the occurrence of increased levels of polycyclic aromatic hydrocarbons (PAHs) is of a health concern.

In order to protect public health, the Regulation No 1881/2006/EC was introduced last year as a response to food-contamination problems and maximum levels for benzo[*a*]pyrene, a main representative of PAHs, in certain foods including oils were set. In addition to benzo[*a*]pyrene, Scientific Committee on Food identified and recommended to monitor all 15 PAHs that possess both genotoxic and carcinogenic properties.

For the enforcement of legislation and risk assessment, reliable analytical methods are necessary. Their performance criteria should comply with requirements set by the new Regulation No 333/2007/EC and cover a large range of concentrations of all compounds of interest.

Certified reference materials (CRMs) play a vital role in verifying the accuracy of analytical methods and in establishing traceability of analytical measurements. In that respect, matrix CRM representing real samples is especially important in trace organic analysis of complex matrices and it is also highly desirable for the determination of PAHs in relevant food samples.

In this study the preparation of the olive oil reference material appropriate for the determination of 15 EU PAHs is described. In fact, 2 candidate CRMs containing different PAH levels were prepared: (i) “low level oil“ purchased on the market and (ii) “spiked oil” – the identical material, where the PAH levels were increased by spiking to achieve PAH levels similar to a highly contaminated olive oils.

Selection of material and analytical - as well as statistical - methods used for candidate CRM testing is described. Preparation of materials, their packaging, handling and testing was performed in compliance with BCR guidelines for Feasibility Studies on Certified Reference Materials (EUR 20574 EN, 2002) and relevant ISO documents (especially ISO Guide 35).

Testing of homogeneity and stability is described.

For cleaning of crude extracts a gel permeation chromatography (GPC) and solid phase extraction (SPE) was used. For the final identification and quantification, high performance liquid chromatography coupled with fluorescence detection (HPLC/FLD). In addition GC/MS was also employed for comparison and for PAHs not amenable to fluorimetric detection. As the certified values are not expected to deviate from the true value “significantly”, the traceability and uncertainty of temporary assigned values and their uncertainties are demonstrated.

Candidate CRM will be subjected to the certification process through collaborative trial during 2008.

This study was undertaken within the project MSM 6046137305 supported by the Ministry of Education, Youth and Sports of the Czech Republic.

RESIDUES – PESTICIDES

(D1 – D32)

D-1
PESTICIDE RESIDUES IN FOOD MATRICES: RESULTS OF THE MONITORING PROGRAMME OF ROMANIA, 2001 – 2006

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The objectives of the pesticide monitoring projects in Romania were to check compliance with the maximum residue levels laid down by EU and by national authorities and to monitor the residue levels in foods to enable an evaluation of the exposure of the Romania population to pesticides.

The authors present the research results obtained in 2001 -2006 period of some chemical pollutants with cancer risk (organochlorine pesticides residues) in diets from Romania area.

In 2001 - 2006 period were analyzed the organochlorine pesticide residues in total diets from Romania area. Organochlorine pesticide residues: DDT total [(op+pp') DDE, DDD, DDT] and HCH total (alpha, gamma, beta, delta) were analyzed used gas-chromatographic method. A HP/ GC-ECD were equipped with column (680 HP). Injector and detector temperatures were 240ºC and 325ºC respectively. Column temperature was 200ºC. One µl was injected. Carrier gas was nitrogen (pressure in column = 1.8 at).

Organochlorine pesticides residues were found present in all analyzed samples. Generally, a wide variation between individual samples was observed. The results were examined in relation to differences in living conditions with regard to agricultural activities, dietary habits and reported use of pesticides in the various sampling areas.

The mean residues levels of HCH-total varied between 44.2 µg/kg (2001) and 24.3 µg/kg (2006). DDT-total varied between 80.2 µg/kg (2001) and 59, 7 µg/kg (2006).

The determinations of the chemical pollutants in food are important in environmental monitoring for the prevention, control and reduction of pollution as well as for occupational health, legal decisions and epidemiological studies

D-2
QUALITY CONTROL FOR RISK EVALUATION OF CHLORDECONE IN FOOD FROM GUADELOUPE

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A previous work related the results from the validation of two analytical methods of chlordecone in food, and the quality control test proposed in this study to shed light on the true variability achievable by intra-laboratory and inter-laboratory analysis[1]. This previous study was set on the analysis of food from Martinique (French Antilles). The current study complete this fist work. It presents the challenging of quality control achieved in the course of a network of laboratories implicated in the analysis of food from the Guadeloupe.

Chlordecone was used until 1993 as a pesticide in the banana plantation of Martinique and Guadeloupe against the root borer. This organochlorine pesticide was lipophilic, remnant and toxic for human beings with both acute and chronic effects. It was strongly absorbed and stored in soil. Survey conducted in 2001 revealed its presence in soil, rivers and domestic food products. The contamination of food by chlordecone was confirmed during the first study in Martinique.

The French National Reference Laboratory for pesticides carried out and validated two analytical methods, one for food of animal origin and an other for food of fruit and vegetable origin. About 900 samples from Martinique where sent to a French network of 13 laboratories for analysis of chlordecone. A quality control program was set in the same time in order to get the best confidence in analytical results. The first study revealed the limits of this control and the limits of the confidence expected from a network of laboratories. Based on this first experience a new program was established for the Guadeloupe study.

The quality control organized for the second study was based on the analysis of about 20% of the 800 samples from Guadeloupe. Test samples were chosen among samples of the series on pre-determined rules by the National Reference Laboratory. Performance parameters were requested to achieve by analytical laboratories: the limit of quantification must not be greater than 1 ng/g in food from animal origin and 5 ng/g in fruit and vegetable. Analytical results were compared between expert laboratories to check the results. Positive and negative false were looked for. The results are discussed here and we explain the objectives, the parameters chosen to achieve a best quality in analytical results and the grounds to get the good quality results. We analysed some of the reasons why results were not always reliable and why a simple proficiency test can be sometimes not enough to get confident in analytical results.

[1] Intern. J. Environ. Anal. Chem., 2007, 1-14, accepted for publication

D-3

PESTICIDE MULTIRESIDUES IN FOODSTUFF AFTER EXTRACTION WITH THE QUECHERS METHOD AND ANALYSIS WITH GC/MS AND HPLC/MS: PROBLEMS AND LIMITS OF THE METHOD

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Pesticides in foodstuff are becoming a major issue due to their intensive use in agriculture. Thus an appropriate control of their residues in food samples has to be operated. In this study we aimed at validating the analysis of 105 pesticides with gas chromatography coupled to single quadropole mass spectrometry (GC/SQ-MS) and 46 pesticides with liquid chromatography coupled to mass spectrometry (HPLC/IT-MS) after extraction with the QuECheRS method in 4 matrices (grape, lemon, onion and tomato). The analyses were carried out in simultaneous full scan and SIM modes for the GC/MS and in MS mode for the HPLC/MS. 9 pesticides (carbaryl, cyromazine, dimethoate, imazalil, monocrotophos, omethoate, paclobutrazol, propamocarb and thiabendazole) were analysed with both GC/MS and HPLC/MS due to their physico-chemical properties.

For GC-amenable substances, the LOD and LOQ in SIM mode ranged from 0.4 to 48.2 µg/kg and from 1.2 to161 µg/kg, respectively. For HPLC-amenable substances, they varied in the MS mode from 1.0 to 115 µg/kg and from 3.3 to 382 µg/kg, respectively. 52 % (grape) to 84 % (tomato) of the GC-amenable substances and 63 % (lemon) to 72 % (grape and tomato) of the HPLC-amenable substances presented LODs lower than 10 µg/kg, residue limit set for the organic farming foodstuff. It could be determinated that around 75 % of the GC-amenable substances and 67 % of the HPLC-amenable substances were satisfying or almost satisfying this organic farming threshold in all matrices; 16 % of the GC-amenable substances and 24 % of the HPLC-amenable substances were not respecting it in more than 2 matrices; finally 9 % of the GC- and HPLC-amenable substances were showing LODs far higher than the allowed 10 µg/kg. For the 9 substances analysed with both GC/MS and HPLC/MS, it was shown that carbaryl, cyromazine, dimethoate and monocrotophos presented similar LOD/LOQ on both devices, omethoate presented far better LOD and LOQ with GC/MS and the remaining imazalil, paclobutrazol, propamocarb and thiabendazole had lower LOD/LOQ with HPLC/MS. The substances presenting the more trouble for analysis were cyfluthrin, cypermethrin, fenhexamid, methamidophos, methidathion, monocrotophos, propargite, thiabendazole and tolylfluanid for the GC analysis and avermectin B1a, monocrotophos, omethoate and thiamethoxam as far as the HPLC analysis was concerned. Most of these substances are known to be highly polar and/or thermolabile, making their analysis tedious.

The recovery, the repeatability and the accuracy of the method were satisfying for almost all the substances and the matrices (grape, lemon, onion and tomato) at the LOQ and 10 times the LOQ fortification levels. With GC/MS, 61 to 82 % of the substances showed a recovery in the range of 70-110 % and 6 to 30 % presented a recovery higher than 110 % at the 500 µg/kg fortification level. With HPLC/MS, 87 to 93 % of the substances presented recoveries in the range of 70-110 % at the 500 µg/kg fortification level compared to 78 to 85 % at the 50 µg/kg fortification level. The method was proved to be repeatable with RSD lower than 20 % at 500 µg/kg and lower than 15 % at 50 µg/kg with both devices.

Lemon and onion showed principally poor recoveries but are known to be difficult matrices due to their high acid and high sulfur content, respectively.

Roughly speaking, 59 % of the GC-amenable substances presented recovery between 65 and 115 % in all matrices, 27 % recovery higher than 115 % in more than 2 matrices (azoxystrobin, bitertanol, bromopropylate, chlorothalonil, cyfluthrin, cypermethrin, deltamethrin, difenoconazole, dimethoate, dimethomorph, fenbuconazole, fenhexamid, fenitrothion, fenoxycarb, fenvalerate, fluvalinate-tau, lindane, methidation, monocrotophos, ofurace, omethoate, parathion-methyl, prochloraz, propargite, propiconazole, pyriproxyfen, tetradifon, thiabendazole and triazophos), 10 % recovery lower than 65 % in more than 2 matrices (biphenyl, carbaryl, carboxin, cyromazine, imazalil, malathion, mecarbam, propamocarb, quintozene, tecnazene and tolylfluanid) and 4 % of the GC-amenable substances presented recovery higher than 115 % or lower than 65 % depending on the matrix (iprodione, methamidophos, methiocarb and quinoxyfen).

The recoveries obtained for the substances analysed with both GC/MS and HPLC/MS fitted together. Cyromazine and propamocarb showed low recoveries with both devices, paclobutrazol recoveries between 70 and 110%. Dimethoate, omethoate and thiabendazole presented higher recoveries with GC/MS than with HPLC/MS, carbaryl and imazalil lower recoveries. For monocrotophos it was depending on the matrix. Dimethoate, monocrotophos and omethoate were preferably analysed with GC/MS, imazalil, propamocarb and thiabendazole with HPLC/MS due to their peak form, which was in accordance with the better LOD/LOQ obtained either with GC/MS or with HPLC/MS. Because of its very low retention on the HPLC/MS column, cyromazine was more easily analysed with GC/MS. The quantification of carbaryl and paclobutrazol was equally achieved with GC/MS and HPLC/MS.

Due to the limited clean-up step of the QuECheRS method on the one hand, only a small portion of the matrix components can be removed from the extract, allowing matrix interferences and thus leading to higher recoveries. On the other hand, most of the substances that were not satisfying the recovery range of 70-110 % were already reported as hardly analysed in the literature.

The method was proved to be accurate with the analysis of proficiency tests that showed for more than 80 % of the analytes a good estimation of the concentration. For some substances, the results were either over- or underestimated, mainly because of the low concentration in the test or the degradation of the substances in the standards.

The QuECheRS method for the analysis of apolar, middle polar and polar pesticides in conventional farming foodstuff was proved to be adapted for almost all of the 140 analysed substances. It presents the advantage of being quick, easy and less polluting than other methods like the well-known ethyl acetate extraction. But, this method must still be optimised for organic farming samples since many LODs remain higher than the allowed residue concentration (10 µg/kg) for this kind of products. Moreover, an additional clean-up step should be implemented to remove the residual matrix interferences.

D-4

PESTICIDE RESIDUES IN CONVENTIONAL FARMING FOODSTUFF: CONTAMINATION OF THE SAMPLES ON THE AUSTRIAN MARKET FROM 2003 TO 2006

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More and more pesticides are used nowadays in the agriculture, which leads to pesticide residues in foodstuff. With the introduction of synthetic plant protection chemicals, people started to deliberately supply and guarantee the existence of unwanted residues in their alimentation. Since each pesticide produces residues, the question is to know how toxic these substances are on human beings by determining the amount of pesticide residues that will remain in the crop, which depends on the agricultural method, the climate and the state of the used technology.

The goal of this study was the analysis of 4,300 conventional farming fruit and vegetable samples over a period of 4 years (from 2003 to 2006) in Austria after extraction with the QuECheRS method, the DFG method S19 and a dithiocarbamate-selective method and analysis with gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to mass spectrometry (LC-MS). The idea was to pinpoint the ratio of samples contaminated with pesticide residues, the amount of samples with residue values higher than the authorised Maximum Residue Limits (MRLs) fixed by the European Authorities and the acute toxicity of the pesticides analysed in some food samples on the Austrian market in comparison with the reports of the European Community.

Over these 4 years it was shown that 46% of the samples presented residues lower than limit of detection (LOD), 49% of the samples presented residues up to MRLs and 5.7% of the samples contained pesticide residues with concentrations higher than MRLs. Between 2004 and 2006, the amount of conventional farming foodstuff samples without residues (i.e. lower than LOD) increased from 39% to 52% and the amount of samples with residues higher than MRLs decreased from 7.1 to 4.6%. In the same time period, the amount of samples with more than one pesticide residue also decreased from 35% to 28%, which could be a consequence of a monitoring system that took place from 2004 on in Austria. The amount of samples with residue concentrations over MRL was significantly low (2.5%) in 2003 due to the late analysis period from July to December, which usually present lower pesticide contamination.

The most common found pesticides were procymidone, cyprodinil, chlorpyrifos, iprodione, fludioxonil, metalaxyl, pyrimethanil and azoxystrobin. 8 of the 10 most common pesticides were fungicides, 2 were insecticides. Procymidone is an organochlorine pesticide, chlorpyrifos an organophosphorus pesticide, cypermethrin belongs to the pyrethtroids and the remaining 7 substances are organonitrogen pesticides. The 10 most common pesticides were mainly found in grapes. Since grapes have a long vegetation period of around 6 months, they are threatened by many fungal diseases and quite strongly treated with pesticides to avoid a high crop loss.

The pesticides with concentration exceeding MRLs were pyridaben, dichloran, iprodione, etofenprox and chlorpropham. Apart from iprodione, the other quoted pesticides are prohibited in Austria, although allowed in other countries like Italy, Spain or Turkey for instances.

When compared to the situation in the rest of Europe (reports from the Commission of the European Community, available until 2004), the samples on the Austrian market in 2004 had a higher pesticide contamination than the samples on the European market with 39% of the samples without pesticide residues (i.e. lower than LOD) and 7.1% of samples with pesticide residues over MRLs compared to 53% of the samples without measurable residue and 5.0% of the samples with residues above MRL. An explanation is that 20% of the samples analysed in our laboratory were grapes and mixed peppers, which are well-known foodstuff samples for high pesticide contamination. Furthermore, contrary to the Austrian samples, the European samples included no follow-enforcement-samples, which are usually highly loaded. Moreover, in 2004, 35% of the samples on the Austrian market presented multiresidues against 23% for the European market. An explanation is again the higher fraction of grapes samples (10% in 2004) in our laboratory, since 75% of the grape samples presented multiresidues. Additionally the European Union included a higher proportion of cereals (4.9%), that are commonly less contaminated.

Concerning the acute toxicity, 15 pesticides in 30 foodstuff products led to an exceed of the 100% Acute Reference Dose (ARfD) exhaustion. These exceeds ranged up to 2300% of the ARfD exhaustion of a 2 to 5 years-old child, which means 1 to 23-times worth the ARfD-value. Moreover, 12 of the 15 pesticides exceeding the 100% ARfD exhaustion presented pesticide residues lower than the dedicated MRLs. This clearly showed that MRLs have to become suitable for children and that lower ARfDs should be included.

D-5

SELECTED APPLICATIONS FOR THE DETERMINATION OF PESTICIDE RESIDUES USING THE LC-MS/MS TECHNIQUE IN A HIGH-THROUGHPUT LABORATORY

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The determination of pesticide residues in food and food products is of great importance to food processors as well as to consumers. Global trade and ever faster turnarounds especially for fresh products are the reasons why establishing modern analytical methods and techniques is vital today. Over the last years more and more laboratories have successfully implemented the technique of LC-MS/MS for the determination of pesticides. Due to its selectivity, sensitivity and robustness, LC-MS/MS has been proven to be a very powerful detection system, especially in combination with multi residue methods. With modern LC-MS/MS systems hundreds of pesticides can be determined simultaneously.

The time for sample preparation can be reduced significantly by applying fast and simple multi residue methods. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) multi residue method [1] plays a very important role in monitoring pesticide residues in different matrices of plant origin. In addition to multi residue methods, the need for specific single methods is still given because not all pesticides can be detected with multi residue methods. For example, a specific single method is particularly necessary for the determination of polar or ionic components such as diquat and paraquat in various fruit and vegetable samples. Special aspects of the method development will be discussed.

Using multi residue methods, the time and man power spent on each sample is more and more shifting from the sample preparation towards the evaluation of the huge amount of analytical data, which nowadays turns out to be the bottleneck of the whole analytical process. In order to fulfill customer expectations regarding fast turn-around times, high quality results and flexibility of target choice, suitable solutions have to be found to handle the whole process of analysis (including the data evaluation) in a practical, safe and effective way. Therefore a data base based software was developed by Specht laboratories. This software (eQuant) links the Lab Information Management System (LIMS) with the analytical software. All the data necessary for performance and plausibility check (e.g. calibration parameters, mass transitions ratios, retention times) are displayed in a simple way that gives the operator all information “at a glance”.

[1] Anastassiades, M., S. J. Lehotay, D. Stajnbaher and F. J. Schenck; Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce; 2003; Journal of AOAC International 86(2); 412-431
[2] Application Note (Waters); M.S. Young, K.M. Jenkins; Oasis WCX: A Novel Mixed-Mode SPE Sorbent for LC-MS Determination of Paraquat and Other Quaternary Ammonium Compounds; 2004
[3] M.M. Ariffin, R.A. Anderson; LC/MS/MS analysis of quaternary ammonium drugs and herbicides in whole blood; 2006; J. Chromatogr. B; 842; 91-97

D-6

ANALYSIS OF POLAR PESTICIDE RESIDUES IN FRUITS WITH HIGH WATER CONTENT BY HOT WATER EXTRACTION AND CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY

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Fruits are essential to a nutritionally adequate diet. Research shows that eating five or more servings each day can decrease the incidence of some cancers. Among the healthy dietary patterns, the traditional Mediterranean diet is highlighted. This diet is characterized by the high fruit intake, especially during summer time. However, there is a public concern about the presence of harmful levels of pesticides in these products. Screening and monitoring of these hazardous residues in food can increase the confidence of consumer's in the safety and benefits of consuming fresh fruits.

In this work, a procedure based in capillary electrophoresis-mass spectrometry (CE-MS) is developed for the analysis of seven pesticides (flutolanil, simazine, haloxyfop, acifluorfen, dinoseb, picloram and ioxynil) in four Mediterranean summer fruits with high water content (peaches, melon, water melon and apricot). Several conditions were studied for the optimization of both, the separation (buffer composition, concentration and pH, as well as temperature and voltage) and the electrospray (ESI) connection (sheath liquid composition and flow rate, fragmentor voltage and polarity). The best results were obtained using 35 mM ammonium formiate (pH 9.7) as separation buffer, 20ºC as capillary temperature and 23 kV as applied voltage in an uncoated fused silica capillary with 75 cm total length, 50 cm thermostated length (in an special external detector adapter cartridge), 25 cm at room tempetarure length, connected to an MS detector by an ESI sprayer kit. The appropriate sheath liquid was ammonium formiate with 10% of formic acid at 5 µL/min. Conditions were optimized for the seven pesticides under study, monitoring simultaneously one characteristic mass for each analyte. Separation time for all pesticides was achieved in 12 min.

Extraction was carried out by pressurized liquid extraction (PLE) in an ASE200 system from Dionex using hot water at 160ºC and 1500 psi, followed by a solid-phase extraction (SPE) clean-up step using X-strata cartridges. This procedure allows to remove as many interfering compounds as possible, at the same time that improve the limits of detection (LODs) and quantification (LOQs) at levels below the maximum residue limits (MRLs). The recoveries of the analytes ranged from 58 to 88% and the relative standard deviations were from 9 to 19%. LODs and LOQs were lower than 0.01 and 0.05 mg/kg, respectively. The potential of the method was demonstrated by analyzing different samples taken from agricultural cooperatives.

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D-7

DEVELOPMENT OF A UPLC-TOF-MS METHOD FOR COMBINED DETERMINATION OF PESTICIDES, MYCOTOXINS AND PLANT TOXINS IN FOOD AND FEED

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Cereals and other products of plant origin may contain residues of pesticides and natural toxins which is a concern with respect to food safety. For pesticides and mycotoxins, legal limits have been established [1,2], while plant toxins were only recently recognized as a potential issue [3]. For efficient monitoring and control of pesticides, multi-residue methods are routinely used [4]. More recently, a similar development can be observed in the field of mycotoxins [5], while for plant toxins the number of analytical methods is limited and usually restricted to a small number of compounds only. Many of the methods available are based on LC-MS.

This work is part of an extended program for development of generic methods for multi compound screening and determination through chemical analysis. This contribution focuses on the development of one LC-MS based method which allows simultaneous detection of pesticides and natural toxins. With an ever increasing demand for faster analysis in mind, the work was conducted with LC columns packed with small-particle stationary phases (UPLC). For detection, full scan mass spectrometry, in this case time-of-flight (ToF) MS with enhanced resolution, was chosen for two reasons. Firstly because the number of target analytes is too high for targeted acquisition as is done with MS/MS. Secondly, because of the ability to detect compounds for which no reference standards are yet available, as is the case with many plant toxins. An additional advantage is that full scan acquisition allows re-analysis of sample measured earlier through retrospective analysis of data previously acquired.

An important aspect during method development was to establish a uniform and overall optimum eluent composition, both with respect to chromatographic separation and MS sensitivity. Different eluents were evaluated using a mix of 178 compounds. The mix included pesticides (basic, neutral, acidic), mycotoxins and plant toxins (pyrrolizidine alkaloids and their N-oxides; ergot alkaloids, glyco alkaloids). With respect to MS detection, selection of the cone voltage, optimal scan speed and the possibility for continuous positive/negative ion detection were studied. With the final method developed, sensitivity, selectivity and linearity obtained with ToF-MS detection was evaluated for standard solutions and generic extracts of wheat, other cereals based matrices and soya. A comparison of ToF-MS with triple quadrupole MS/MS detection (the current 'golden standard' in residue analysis) was also made, using the same UPLC conditions.

The presented work shows that combined analysis of multiple groups of residues and contaminants by UPLC-TOF-MS is a very promising approach for more generic and efficient analysis of samples from the food chain.

[1] Regulation (EC) no 396/2005 of the European parliament and of the council on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC, Official Journal of the European Union, L70/1, 16.3.2005 (2005)
[2] Commission regulation (EC) No. 1881/2006 setting maximum levels of certain contaminants in foodstuff, Official Journal of the European Communities L 364/5, 20.12.2006
[3] Opinion of the scientific panel on contaminants in the food chain on a request from the European Commission related to pyrrolizidine alkaloids as undesirable substances in animal feed, The EFSA Journal (2007) 447, 1-51
[4] H.G.J. Mol, A. Rooseboom, R.van Dam, M. Roding, K. Arondeus, and S. Sunarto, J. Anal. Bioanal. Chem (in press 2007) (DOI 10.1007/s00216-007-1357-1)
[5] M. Sulyok, F. Berthiller, R. Krska, R. Schuhmacher, Rapid Commun. Mass Spectrom. 2006; 20: 2649–2659

D-8

AN ASSESSMENT OF PHOSPHAMIDON RESIDUES ON MUSTARD CROP IN AN AGRICULTURAL FIELD IN BIKANER, RAJASTHAN (INDIA)

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Mustard / Rapeseed cultivation is done widely throughout India. India produces around 7 million tones of rape/mustard annually and occupies 3rd position in the list of rapeseed / mustard producing countries contributing about 11% of the world's total production. It is produced in states of Rajasthan, Uttar Pradesh, Haryana, Punjab, Gujarat, Madhya Pradesh, Jammu and Kashmir, West Bengal, Punjab, Assam, Bihar, Himachal Pradesh & Orissa. Mustard is a major cash crop in India, accounting for almost 65% of the total Rabi produce. Rajasthan and Uttar Pradesh account for majority share, contributing to over 50% of the total Indian produce that stands at an average of 5 million tons. These areas also have maximum area under cultivation for this crop.It is basically a winter crop, and requires a temperate climate to prosper. The planting season or the sowing period in India is the Rabi season i.e. October to November. The harvesting period is from February to March. It needs the right proportion of rainfall that is provided by the monsoon during the sowing seasons of the crop. The rapeseed/mustard crop acts as a very good cover of soil in winters.The greens including leaves and stem of mustard are used as vegetables, edible oil from the seeds is extracted and seed cake is used as animal food.

As all other crops this crop is also attacked by a number of insect pests. Being a cash crop, to protect it against pest infestation, insecticides are sprayed on it, one of them being Phosphamidon. The present study was undertaken to evaluate the persistence of phosphamidon on mustard crop (foliage and siliqua) and soil in an agricultural field in Bikaner (28°N latitude and 73°18'E longitudes), Rajasthan, India.

The crop was sprayed with 0.05% phosphamidon @ 700 litre/ha before flowering and 15 days after flowering. For the study samples of soil were taken on 0,5,10.1,20,27 days, while, for foliage the samples were collected on 0, 1, 5,10,12,15 days after first day and, siliqua fruits at same intervals as that of foliage after second spray. The analysis was done following the method given by Getz and Walt (1964) as modified by Jain et al. (1974).

The phosphamidon residues in soil obtained at different time intervals were noted. The initial deposit was found to be 0.60 mg/Kg of soil just after the spray, which gradually decreased with time and became non-detectable on the 27th day after spraying. The analytical results pertaining to residues on foliage and siliqua were found to be 10.83 mg/Kg and 8.53 mg/Kg respectively after first and second spray and which declined to 0.25 0.31 mg/Kg respectively after 15 days of spraying. On the basis of tolerance limit of 0.5mg/Kg for phosphamidon as assigned by FAO/WHO (1975) the results show that the residues which persist till the 12th day after application are more than the prescribed limits rendering it unfit for human consumption as it could be harmful. The results are further analyzed in the light of literature available.

[1] Getz, M. E. & Watt, R. R. 1964.Application of 4-p-nitrobenzyl pyridine as a rapid quantitative reagent for organophosphate pesticides. Journal of Association Agricultural Chemists, 47: 1094-1096.
[2] Jain, H. K., Pandey, S. Y., Agnihotri, N. P. and Dewan, R. S. 1974. Rapid estimation of organophosphorous insecticides. Indian Journal of Entomology, 36: 145-148.
[3] Food and Agriculture Organization/World Health Organization 1975. Report of the eighth session of the codex committee on pesticide residues, Codex Alimetarius commission. Food and Agriculture Organization/World Health Organization, Geneva.

D-9
STRATEGIES TO EVALUATE ILLEGAL AND MISUSED LC AMENABLE PESTICIDES IN FOOD

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International trade of fresh fruits and vegetables is a billion dollar business that has significantly increased in the last two decades. This trade is important for many countries and may account for an important share of their hard currency earnings. In the EU, in the last years, international markets have rejected exports of fruits and vegetables containing non-authorized pesticides. The use of a large number of well known and frequently applied pesticides has been banned from the European Union as a consequence of the Directive 91/414/EEC.

Recent rapid sanitary information or alerts reported by European countries have pointed out a serious problem related with presence of illegal or misused pesticides in various crops, particularly in peppers. This presence of non-authorized pesticides in the European Union can be motivated by (i) a lack of authorized insecticides to avoid some persistent pests that has become resistant against common insecticides, (ii) the confusion provoked by the presence of more than one legislation (European and individual EU country) that in some cases can coexist until the current Regulation EC 396/2005 will not be fully adopted, (iii) “internet market” that allow to buy easily commercial pesticides all over the world when important differences in pesticide authorizations can appear from different EU and non EU countries and finally (iv) the presence of an important “black market” of pesticides.

In this work, we describe for the first time analytical strategies for the determination of these important non authorized or misused substances in vegetables at concentrations in the low μg Kg^{-1} range. The proposed methods consist of a sample treatment step based on QuEChERS extraction method followed by quantitative analyses by LC-TOFMS, LC-MS/MS and LC-MS/MS using an hybrid triple quadrupole linear ion trap mass analyzer. The sensitivity, linearity, repeatability and LODs obtained with the different techniques studied, have been evaluated. Finally, the proposed method has been successfully applied to the determination of these insecticides in vegetable samples

D-10
MULTI-RESIDUE ANALYSIS OF 301 PESTICIDES IN FOOD SAMPLES BY LC/TRIPLE QUADRUPOLE MASS SPECTROMETRY

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An analytical methodology for screening and confirming the presence of 301 pesticides in vegetable samples was developed using a triple quadrupole mass spectrometer (QqQ). The pesticides studied belonged to different chemical families of herbicides, insecticides and fungicides; some degradation products were included as well. A thoroughly optimization was performed for each analyte to achieve individual optimum fragmentor and collision energy voltages. A narrow particle size C_{18} column (1.8 µm) was used for the chromatographic separation of the mixture, providing very narrow peaks and allowing an excellent separation of all the analytes in a 30 minute period. We found that, of the 301 compounds, 90% could be identified using this high-throughput procedure with a limit of detection (LOD) in vegetable matrices of 0.01 mg/kg (ppm), which is the level for baby food and banned substances and is typically the most sensitive MRLs used by the European Union. These levels were reached in a single analysis using positive ion electrospray with 100 transitions per segment and a quantifying and confirming ion for each compound. The analytical performance of the method was evaluated for different types of vegetables: tomato, green pepper and orange, showing little or no matrix effects. Linearity of response over 2 orders of magnitude was demonstrated ($r^2 > 0.99$). This study is a valuable indicator of the potential of the QqQ for routine quantitative multi-residue analysis of pesticides in vegetables.

D-11
STROBILURINS RESIDUE LEVELS IN PEPPERS IN THE FIELD AND UNDER REFRIGERATION

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The dissipation of two fungicides (azoxystrobin and kresoxim-methyl) in peppers was evaluated in a study carried out in the field and under cold storage conditions. Pepper samples were collected during a five weeks period in which two successive treatments of these pesticides were performed. Gas chromatography (GC) with nitrogen-phosphorus detection (NPD) and electron-capture detector (ECD) were used to study the disappearance of these compounds in peppers. At the preharvest interval, the residue levels were below the legal limit established in Spain. The disappearance rates of azoxystrobin and kresoxim-methyl on peppers were described as pseudo-first-order kinetics (r between 0.950-and 0.992) and half-life in the range of 10.28 and 15.21 days. These fungicides show similar behaviours to peppers grown in greenhouse because they are structurally similar. After twenty one days under cold and darkness storage conditions, dissipation of azoxystrobin and kresoxim-methyl were not observed.

KEYWORDS: Axoxystrobin; kresoxim-methyl; peppers; residues; fungicides; disappearance.

D-12

PROCYMIDONE AND AZOXYSTROBIN RESIDUE LEVELS IN LETTUCE IN THE FIELD AND UNDER REFRIGERATION

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The dissipation of two fungicides (procymidone and azoxystrobin) in lettuces was evaluated in a study carried out in the field and under cold storage conditions. Lettuce samples were collected in an experimental greenhouse during 5 week period, in which two successive treatments of these pesticides were performed. Gas chromatography (GC) with nitrogen-phosphorus detection (NPD) was used to study the disappearance of these compounds in lettuce. The disappearance rates of these compounds on lettuces in field after two applications were described as pseudo-first-order kinetics with strong correlation between residue and time (r was in all cases higher than 0.983). The half-lives for first and second treatments were of 5.31 and 4.65 days for procymidone and 6.23 and 4.87 days for azoxystrobin, respectively. When procymidone and azoxystrobin were applied two times during cultivation, at maximum recommended dose, the residues of both pesticides were below LMRs after the recommended preharvest intervals. After twenty one days under cold and darkness storage conditions, dissipation of procymidone and azoxystrobin were not observed.

KEYWORDS: Procymidone; azoxystrobin; lettuces; residues; fungicides; disappearance; refrigeration.

D-13

APPLICABILITY OF GAS CHROMATOGRAPHY/TRIPLE QUADRUPOLE TANDEM MASS SPECTROMETRY TO THE ANALYSIS OF PESTICIDE RESIDUES IN CROPS

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The demand of modern pesticide residue analysis is to detect pesticides significantly and sensitively at low ppb levels. But, a great number of chemicals registered for the use on a diversity of plant cultivars makes pesticide residue analysis an on-going challenge for scientists. Their work is made even more difficult by the fact that traces of pesticides have to be determined in complex matrices, such as cereals, feedingstuffs, herbs, spices, babyfoods, which are often burdened with large quantities of other components after extraction. Hence, the use of highly sensitive and specific techniques, such as tandem mass spectrometry (MS/MS)) is desirable to provide an unambiguous evidence for the presence of pesticide residues in crop samples in accordance with recent guidelines (SANCO/10232/2006, 2002/657/EC). The aim of this presentation is to demonstrate the potential of gas chromatography/triple quadrupole tandem mass spectrometry (GC/MS/MS) for pesticide residues analysis, based on validation data and multiple examples of analyses of real-samples carried out in our laboratory. The developed GC/MS/MS method is capable of performing a screening analysis for more than 100 pesticides under one set of experimental conditions using two multiple reaction monitoring (MRM) transitions for each of them to enable quantification and confirmation of detected residues, simultaneously. In this presentation, the MS/MS technique will be compared with previously used selected ion monitoring (SIM) and full scan techniques, and a comparison of triple quadrupole MS/MS with ion trap MS/MS will be made. Unquestionably, the MS/MS gives much higher degree of certainty in analyte identification than any single stage mass spectrometry technique, because isobaric interferences are avoided and multiple-component spectra can be resolved. Furthermore, triple quadrupoles avoid the drawbacks of ion traps, such as vulnerability to space charge effects. In consequence, more compounds can be spectrometrically resolved in a reduced analysis time, which also reduces the need for clean-up. Up to now, more than 1000 real-samples, such as fruits, vegetables, cereals, animal feeds, herbs, water and soil, have been analysed using the developed triple quadrupole GC/MS/MS conditions. The method performance was also verified through participation in proficiency tests.

D-14

VALIDATION OF A GC/MS/MS MULTI-RESIDUE METHOD FOR THE SIMULTANEOUS ANALYSIS OF 122 PESTICIDES IN CEREALS AND DRY ANIMAL FEED

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A multi-residue screening method for the simultaneous analysis of 122 gas chromatography amenable pesticides in dry matrices such as cereal grain and certain feedingstuffs is presented. The method entails a simple extraction of re-hydrated sample with acetonitrile followed by a dispersive solid phase extraction (SPE) clean-up step prior to the final determination by gas chromatography/triple quadrupole tandem mass spectrometry (GC/MS/MS). In the developed GC/MS/MS acquisition method, two multiple reaction monitoring (MRM) transitions were set for each pesticide to eliminate the need for re-analysis of potentially positive samples, and provide unequivocal identification of detected pesticides in accordance with recent guidelines, in a single analytical run. Method validation experiments at three spiking levels (0.01, 0.02 and 0.05 mg kg-1) yielded average recoveries in the range 73 – 129% with relative standard deviations (RSD) in the range 1 – 29%, for the majority of pesticides. Limits of detection less or equal to 0.01 mg kg^{-1} were obtained for approximately 68% of pesticides. The applicability of the proposed method to detect and quantify pesticide residues has been demonstrated in the analysis of 136 real samples. Additionally, the method was favorably compared with an acetone extraction method (accepted as a reference method by some European and U.S. authorities) in the analysis of real samples known to contain pesticide residues.

D-15

DETERMINATION OF QUATERNARY AMMONIUM PESTICIDES (QUATS) RESIDUES BY HYDROFILIC INTERACTION CROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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Quaternary ammonium pesticides (quats) are used in agriculture as herbicides (parquat, diquat and difenzoquat) and growth regulators (mepiquat and chlormequat). Institutions are concerned about environmental pollution (drinking water) and food safety (fruit and cereal base products). Current methods for the determination of residues of these compounds in food use ion chromatography (1) or ion pair reversed-phase liquid chromatography with an reagent (2). Hydrophilic interaction chromatography (HILIC) is a useful alternative to reversed-phase chromatography for applications involving polar compounds. In the HILIC chromatography, aqueous–organic mobile phases are combined with polar stationary phases to provide normal-phase retention behaviour. Silica and amino columns with aqueous buffer–acetonitrile mobile phases offer potential for their use in the HILIC mode (3).

In this study a new HILIC liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been developed to determine chlormequat in food samples. Parameters such as pH, ionic strength and the percentage of the organic modifier have been optimized. A mobile phase composed by acetonitrile-formic acid/ammonium formate buffer has provided the best results. Heated electrospray ionization (H-ESI) in positive mode has been used as ionization source for LC-MS and due to the inherent charge of these compounds the molecular ion $[M]^+$ was observed as base peak in the single MS spectra. These molecular ions were used as parent ion for tandem mass spectrometry (MS/MS) experiments. Two transitions have been selected for each compound, the most intense for quantitative analysis and the second most selective to confirm the identity of the compound identified. Isotope dilution was used to quantify chlormequat, paraquat and diquat, while mepiquat and difenzoquat were determined using chlormequat-d_4 as internal standard. For food analysis a simple and fast sample treatment with SPE clean-up has been carried out and quality parameters of the method have been evaluated. Good results in linearity, precision and limit of detection at low ppb levels were obtained. The method has been applied to the analysis of a wide variety of food samples: fruits, coffee, beer, tomatoes, cereal bread, etc.

[1] Peeters, M.-C.; Defloor, I.; Coosemans, J.; Delcour, J. A.; Ooms, L.; Deliever, R.; De Vos, D. Journal of Chromatography, A (2001), 920(1-2), 255.0
[2] Castro, Rocio; Moyano, Encarnacion; Galceran, Maria Teresa. Journal of AOAC International (2001), 84(6), 1903.
[3] Schlichtherle-Cerny, Hedwig; Affolter, Michael; Cerny, Christoph. Analytical Chemistry (2003), 75(10), 2349

D-16

IMPROVED ANALYSIS OF PYRETHROID AND ORGANOCHLORINE PESTICIDES IN MILK. PART I: DEVELOPMENT OF A DIRECT SPME STRATEGY OPTIMISATION

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A simple and rapid method based on solid-phase microextraction (SPME) technique followed by gas chromatography with micro electron capture detection (GC-μECD) was developed for the simultaneous determination of more than 30 pesticides (pyrethroids and organochlorines among others) in milk. Negative matrix effects due to the complexity and lipophility of the studied matrix were reduced by diluting the sample with distilled water. A 2^{5-1} fractional factorial design was performed to assess the influence of several factors on the SPME procedure and to determine the optimal extraction conditions. The main factors and second-order interactions could be estimated with this design. On the basis of the literature and preliminary experiments, five variables were selected to define the experimental field: type of fiber coating (A), sampling mode (B), stirring (C), extraction temperature (D), and addition of an inert salt such as sodium chloride (E). The results showed that the sampling mode, stirring and temperature were the most significant variables affecting extraction efficiency. Several interactions (BD, DE and BC) were also found to be significant for most of the target pesticides. Therefore, after optimization of all the significant variables and interactions, the recommended procedure was established as follows: DSPME (using a PDMS/DVB coating) of 1 mL of milk sample (0% NaCl) diluted with Milli-Q water (1:10 dilution ratio), at 100ºC, under stirring for 30 min.

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D-17

IMPROVED ANALYSIS OF PYRETHROID AND ORGANOCHLORINE PESTICIDES IN MILK. PART II: VALIDATION OF A SPME GC-MICROECD METHOD AND APPLICATION TO DIFFERENT TYPES OF MILK SAMPLES

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Analysis of spiked full-fat milk samples (containing known levels of pesticides) were carried out in order to assess the performance of the SPME GC-μECD optimized procedure. The linearity of the method was checked (R>0.99) with linear calibrations in the range of 0.5-100 ng/mL for most of the studied compounds. Limits of detection (LOD) at the sub-ppb level were reached and the technique was found to be reliable with relative standard deviations (RSD) lower than 12%. The method was applied to liquid milk samples covering the whole commercial range. Firstly, four different brands of full-fat milk (three liquids and one powder) were investigated with an observed variability between them below 12.1% (RSD). The efficiency of the extraction process was studied at several concentration levels obtaining quantitative recoveries for all types of full-fat milks. Skimmed and half-skimmed milk samples were also considered finding that, for most of the target pesticides, the chromatographic response was fat percentage dependent. The method was validated with a powder milk certified reference material (BCR 188) which was quantified using external calibration and standard addition protocols. Finally, the DSPME-GC-μECD methodology was applied to the analysis of milk samples collected in farms of dairy cattle from NW Spain.

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D-18
DISAPPEARANCE OF FLUQUINCONAZOLE AND TRIFLOXYSTROBIN RESIDUES DURING WINE-MAKING PROCESS

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Recently, several Spanish regions have allowed new substances for pest control in vineyards. The fungicides fluquinconazole and trifloxystrobin are within them. Fluquinconazole, [3-(2,4-dichlorophenyl)-6-fluoro-2-(1H-1,2,4-triazol-1-yl)quinazolin-4(3H)-one], is a steroid demethylation (ergosterol biosynthesis) inhibitor. Trifloxystrobin, [methyl(E)-methoxyimino-{(E)-α-[1-(α,α,α-trifluoro-m-tolyl)Ethylideneaaminooxy]-0-tolyl}acetate], belongs to a Quinone outside Inhibitors (QoI) group, one of the most important groups used in viticulture and also allowed by Integrated Pest Management.

The objective of this work was the determination of the influence that different wine-making steps, which are carried out during wine-making process, had on the disappearance of fluquinconazole and trifloxystrobin residues. Two field treatments were carried out to study the disappearance of the residues. One was carried out obeying the pre-harvest interval (PHI) and good agricultural practices (GAP). The other was carried out at the harvesting simulating critical agricultural practices (CAP). Micro wine-making processes (15 kg) were carried out for both assays in triplicate. Once the harvest was introduced into the fermentators, it was macerated 8 days at 24-28ºC with diary agitation. After the pressing and once alcoholic fermentation had finished, the wine was racked and subjected to the clarification and filtration processes. Samples were taken to determine its disappearance in all cases. The quantification of both fungicides was carried out by GC-ECD with a capillary column HP-5 in grape, must, wine and their by-products (pomace and lees). After this, samples were confirmed by GC-MSD with the same column. The extraction was carried out with acetone and ethyl acetate:hexane (1:1). This method was validated under European ISO 17025 norms and SANCO Guidelines. The linear interval was 0.05-0.2 µg/ml with an r^2 of 0.985 for both standards. The coefficients of variation in precision were lower than 13% for both fungicides. The mean recoveries were 74.4%, 76.5% for trifloxystrobin and 72.9%, 76.0% for fluquinconazole, at 0.05 µg/ml and 0.20 µg/ml respectively. Both limits of quantification (LOQs) were 0.05 µg/ml.

No initial residues over the LOQ were found for the trifloxystrobin assay carried out with GAP. Residues were found in the case of fluquinconazole but they were eliminated completly during the pressing since they were eliminated linked to the pomace. For the assays with CAP, both fungicides were totally eliminated during the wine-making process with no residues in the racked wines. In both products the disappearance is mainly due to the elimination of the residues with the solid parts (pomace and lees) in the pressing and racking steps; and only a small amount is degradated by the microorganisms responsible for the fermentation and also by the hydrolisis produced in a aqueous medium.

D-19

EFFECTS OF NEW FUNGICIDE RESIDUES ON THE COLOUR OF RED WINE. PHENOLIC COMPOUNDS OF LOW MOLECULAR WEIGHT AND ANTHOCYANINS

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Factors that influence the colour of wines have viticultural and oenologycal character. Firstly, we can highlight the pedoclimatic characteristics, cultivation techniques, variety of grapes and pesticide residues. Secondly, the duration of the maceration and the elaboration techniques in cellar are remarkable. In this paper, the influence that several fungicide residues (famoxadone, fenhexamid, fluquinconazole, kresoxim-methyl, quinoxyfen, trifloxystrobin) have on phenolic compounds of low molecular weight and anthocyanin content in red wine has been studied.

Two treatments were carried out with authorized formulates at the manufacturer doses. The first one was carried out under good agricultural practices (GAP), obeying the preharvest interval, and the second one under critical agricultural practices (CAP), applying at the day of harvesting. The trials were carried out in triplicate. The wines obtained in the thirteen assays (one control, six with treated grapes obeying the preharvest interval and six treated at the day of harvesting or at most unfavourable conditions) were analysed by HPLC-DAD. For the extraction of phenolic compounds of low molecular weight an on-line micro method was used. Samples were shaken with isooctane:toluene (1:1) and diethyl ether:ethyl acetate (1:2). The organic phases were concentrated and dissolved in methanol:water (1:1). For anthocyanins, Johnston and Morris' method (1997) was used.

The target compunds were anthocyanins (delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3glucoside, peonidin-3-glucoside and malvidin-3-glucoside) and phenolic compounds of low molecular weight (hidroxicinnamic acids, flavonols and trans-resveratrol). Data obtained in this experience was processed statistically by the comparison of the untreated wine samples against the other ones, each one elaborated in presence of a fungicide, in order to test if the average values of the studied parameters were significantly different. For these, we did a variance analysis (ANOVA of one factor) by the application of the standardised minor difference (SMD). The processing was done using the computer application SPSS 13.0.

In the total amount of anthocyanins it is observed that no significant differences exist in relation to the control sample except for the assay treated with fluquinconazole where the final results were lower than the control one for both treatments (control 625.8±53.6 mg/l against 499.2±31 for GAP assay and 525.9±21.2 for CAP assay). For the phenolic compounds of low molecular weight no significant differences exist between the treated assays and the control assay. In some case the absolute values were higher for the treated assays. For these compounds, most of the assays done with critical agricultural practices had lower content of these compounds than the ones done with GAP conditions.

D-20
DEVELOPMENT OF A GC/MS CONFIRMATION METHOD FOR THE ANALYSIS OF EIGHT PYRETHROIDS IN BOVINE FAT

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Pyrethroids are insecticides and are widely used in veterinary practice as ear-tags, pour-on formulations, sprays and dips. Persistent residues of various pyrethroids have been reported in animal fat in monitoring programs in other countries.

A method previously developed in our laboratory for the analysis of eight pyrethroids in bovine fat used GC-ECD for detection and although the combination of isomers for each compound meant that there was very little ambiguity in the results, except for co-eluting isomers of cypermethrin and flucythrinate, a mass spectral confirmatory method was still required. One of the problems encountered in the mass spectral analysis of pyrethroids is the similar chemical structure of all the compounds, leading to common fragments for all compounds. Development of the method included assessment of electron impact and positive and negative chemical ionization using methane, ammonia or isobutane as reagent gas.

Pyrethroids were found to be most amenable to GC-MS analysis by EI or NCI with methane gas.

Once the method was successfully characterised, it was validated and applied to a pilot study and the results compared with those obtained by GC-ECD.

This poster will show a comparison of EI and NCI results for a selected number of isomers and discuss the merits of each technique for pyrethroid analysis.

D-21
PESTICIDE RESIDUES MONITORING IN FOODSTUFFS OF PLANT ORIGIN, IN HUNGARY, 2002-2006

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In Hungary the use of plant protection products is defined in the registration documents issued by the Ministry of Agriculture and Rural Development. There are around one thousand plant protection products on the Hungarian market, so it is an increased demand for the control of the residues of these products in the foodstuffs. After joining the European Union, the number of imported foodstuffs on the market has increased significantly so the control of these products was also necessary.

Pesticide residues are analysed by the analytical laboratories of the Hungarian National Plant Protection Organisation. Samples are taken from the domestic products (internal market, from fields at harvest, from exported commodities) and from imported lots. The national sampling programme is focused on commodities requiring intensive plant protection.

The analysis of pesticide residues in foodstuffs is carried out by the authorized laboratories, under the control and guide of a working group specialized in this field. There are six laboratories in the Hungarian pesticide residue analysis network; all are accredited according to OECD-GLP system.

For the determinations, multi residue methods and pesticide group selective methods are used. The pesticide residue analyses are carried out with standardised methods using GC, HPLC, GC-MS and HPLC-MS-MS techniques. In the examined period the extraction of the samples was carried out mainly using ethyl-acetate as extraction solvent. Since the beginning of 2007 we are using the QUECHERS method.

In the checked period there were analysed yearly 2800-3700 samples. The most frequently analysed products were:

Vegetables: lettuce, cucumber, tomato, green pepper, radish, cabbage, green peas, potato, carrot, parsley.

Fruits: apple, grape, sour cherry, apricot, peach, strawberry, raspberry, orange, lemon, banana, mango, mandarin, grapefruit, kiwi

The number of checked active ingredient is increasing year by year, now is above 210.

0.8-2.2 % of the checked samples contained residue levels above the MRL values. In 57-63% of the analysed samples pesticide residues were not detected, in 27-38% of the samples there were detectable amount of pesticides, below the MRL values. The above results shows, that the pesticide residues levels meet the Hungarian MRL-s criteria in most cases.

Each year the Hungarian laboratories take part in the coordinated Community monitoring programme and in the European proficiency test in fruit and vegetables.

The regular control of pesticide residues and the use of up-to-date plant protection technologies provide the environment-friendly plant protection activity with the smallest possible impact on the consumers.

D-22

MULTIRESIDUE LOW LEVEL QUANTIFICATION OF PESTICIDES IN FOOD USING HIGHLY RESOLVED SELECTED REACTION MONITORING ON A TRIPLE QUADRUPOLE GC/MS SYSTEM

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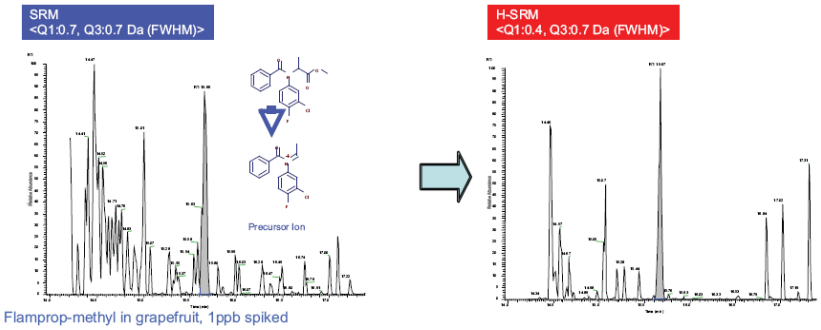
Food safety concerns are on the rise among consumers worldwide. In 2006, sweeping changes were made to the Food Hygiene Law in Japan regarding residual agricultural chemicals, including pesticides, in foods. The presented method covers the simultaneously low level analysis of pesticides with a selected reaction monitoring triple quadrupole method. For the first time in GCMSMS a higher resolved precursor selection at a peak width of only 0.4 Da was used for the significant increase in analytical selectivity. Additionally, the simultaneous structural confirmation of positive analytes was achieved by MS/MS spectra.

The SRM transitions and the optimum collision energy for each of the compounds are given. The highly selective reaction monitoring could eliminate chemical noise and allowed for lower LOQs, and thus reduced the likelihood of false positive results.

The structural confirmation in the run was achieved by data dependant scanning. For a signal above a selectable threshold an MS/MS scan delivered an information rich mass spectrum that was used to confirm the existence of compounds by an in-built MS/MS library while they were being quantified.

Simultaneous analysis for a total of 103 pesticide residue compounds in food products were performed with linear calibrations in the range of 0.1 to 100 ppb. Results obtained indicated excellent reproducibility (10% at 5 ppb) and linearity (R^2 > 0.995). Interferences from the sample matrix background were substantially reduced.

[1] Okihashi, M.; Kitagawa, Y.; Akutsu, K.; Obana, H.; Tanaka, Y. “Rapidmethod for the determination of 180 pesticide residues in foods by gaschromatography/mass spectrometry and flame photometric detection”; J Pestic Sci **2005**, 30(4), 368-77.
[2] The Japanese Ministry of Health, Labour, and Welfare:
a. http://www.mhlw.go.jp/english/topics/foodsafety/positivelist060228/index.html(English)
b. http://www.mhlw.go.jp/topics/bukyoku/iyaku/syoku-anzen/zanryu2/index.html(Japanese)



D-23

THE DETERMINATION OF SULFITE AND FUNGICIDES MIGRATED INTO FOOD SIMULANTS FROM WOODEN CHOPSTICKS

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Sulfite is a widely used as additive for food preservation. And, fungicides such as o-phenylphenol(OPP), biphenyl(BP), thiabendazole(TBZ) and imazalil(IMZ), are used as posthavest treatment to preserve fruits, wood and so on. In this study, we determined the migration levels of sulfite and four fungicides into food simulants from wooden chopsticks.Sulfite was monitored by using ion chromatography(IC), which was validated with 0.2 ppm of limit of detection(LOD). Four fungicides were determined by using high performance liquid chromatography(HPLC) with UV detector. Simulataneously analytical method was validated with 0.1 - 0.2 ppm of LOD and R^2>0.999 of linearity for each fungicides. As a result, the migration levels of sulfite were showed from n.d.to 16.4 ppm for 100 wooden chopstick samples. However, there were no samples detected OPP, BP, TBZ or IMZ in this study.

D-24

DETERMINATION OF THE SELECTED TRIAZINE HERBICIDE RESIDUES AND THEIR METABOLITES IN WATER USING SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY EQUIPPED WITH A TANDEM MASS SPECTROMETRIC DETECTION

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Modern chromatographic techniques allow determination of pesticide residues in samples of environment origin at very low level. One of them is ultra performance liquid chromatography with a tandem mass spectrometric detection (UPLC-MS/MS). The technique allows rapid analysis of residues of target compounds and simultaneous identification of them. In this paper, a simple, rapid, very sensitive, highly repeatable and inexpensive method for determination of triazine residues in water using RP-UPLC-MS/MS technique has been described. Therefore, we present analyses of seven basic compounds belonging to a very resistant in environment group of triazine herbicides (atrazine, cyanazine, propazine, simazine, terbuthylazine, prometryn, terbutryn) and their selected dealkylated metabolites (desisopropyl atrazine, deethyl atrazine, deethyldesisopropyl atrazine, deethyl terbutylazine) and hydroxyl derivatives (hydroxy atrazine, hydroxy desisopropyl atrazine, hydroxy deethyl atrazine, hydroxy propazine, hydroxy terbutylazine). The whole procedure consists of a solid phase extraction (SPE) of triazines from water samples using a carbon black column. A ultra performance liquid chromatograph ACQUITY UPLC system of Waters equipped with tandem quadrupole mass spectrometer Waters Quattro Premier XE, operated by a MassLynx software, Waters ACQUITY UPLC column BEH C_{18} were used for final LC analysis. Column was eluted with the mobile phase: water with 0.1% ammonium acetate (A) and methanol with 0.1% ammonium acetate (B) at the flow rate of 0.3 mL min^{-1} using gradient mode. Time of analysis was 6 min while recoveres of spiked samples averaged for level 0.01 ppm from 72 to 106% (excluding deethyl deisopropyl atrazine), and for level 0.1 ppm from 74 to 113%. Standard deviations (RSDs) were from 2.0 to 9,6% for the level 0.01 ppm and from 1.7 to 7,7% for the level 0.1 ppm. Limit of quantification (LOQ) for any compound analyzed was defined at the level of 0.01 $\upsilon g\ L^{-1}$ excluding deethyl deisopropyl atrazine for which LOQ was determined at the level of 0.05 $\upsilon g\ L^{-1}$.

D-25

MULTI RESIDUE ANALYSIS OF PESTICIDES IN BOVINE MILK BY SPE AND DSPE FOLLOWED BY LC-MS/MS

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A multi residue method based on Solid-Phase Extraction (SPE) and Dispersive Solid Phase Extraction (DSPE) techniques followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) was developed for the simultaneous determination of 50 pesticides in bovine milk. Since no CRM is available for those compounds in milk, analysis of fortified skimmed, half-skimmed and full fat milk samples were performed. Second order Doehlert designs were implemented to assess the influence of several factors on the SPE and to determine the optimal extraction conditions. The main factors and second-order interactions could be estimated with this design. On the basis of the literature and preliminary experiments, the following variables were selected to define the experimental field: Dilution factor of milk in organic solvent /water mixtures (A), relative organic solvent/water proportions (B), composition of the elution mixtures (containing methylene chloride, diethylether and methanol) (C).

As regards the DSPE protocols, Envi-Carb and C_{18} materials were tested both in combination with MgSulfate and Clean PSA.

The efficiency of the two extraction processes was studied at several concentration levels obtaining quantitative recoveries for all types of milk. The performance of the methods was also assessed and the limits of quantification (LOQs) often reached the ppb level, complying with the most recent MRLs.

Finally, the optimized extraction methods were applied to the analysis of raw milk samples collected in 23 farms of dairy cattle from NW Spain (Galicia).

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[1] S. L. C. Ferreira, W. N. L. Dos Santos, C. M. Quintella, B. B. Neto, J. M. Bosque-Sendra. *Talanta*, **63**, 2004, 1061-1067. "Doehlert Matrix: a chemometric tool for analytical chemistry- review".

[2] S. Bogialli, R. Curini, A. Di Corcia, A. Lagana, A. Stabile, E. Sturchio. *Journal of Chromatography A*. **1102**, 2006, 1-10. "Development of a multiresidue method for analyzing herbicide and fungicide residues in bovine milk based on solid-phase extraction and liquid chromatography–tandem mass spectrometry".

D-26

THE USE OF DISPERSIVE SPE HPLC MS/MS FOR THE ANALYSIS OF ORGANOPHOSPHATE PESTICIDES AND ACIDIC HERBICIDES IN FRUIT AND VEGETABLES BY LC/MS/MS SYSTEM

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Organophosphate insecticides and acidic herbicides are pesticides used to maximise the yield and enhance the quality of fruit and vegetables such as tomatoes, grapes, strawberries, apples etc. To ensure they are applied using good agricultural practice and that any residues are within relevant legal limits, food products are routinely screened using targeted methods.

Dispersive SPE is a rapid analytical technique for the extraction of pesticides from fruit and vegetables which contain high levels of water and low sulphur content. It simply involves emulsifying the sample and shaking it with an equivalent volume of acetonitrile. The acetonitrile is phase separated by the addition of magnesium sulphate and salt. The resulting acetonitrile layer is cleaned up further by shaking with a SPE sorbent and more magnesium sulphate. The final extract is diluted 1 to 5 into water and analysed by LCMS.

The poster investigates the use of this crude workup on the analysis of these two groups of pesticides in fruit and vegetables down to and below the EU limit of 10 ppb in food. Acid herbicides and organophosphate pesticides were chosen as they ionise with different polarities.

Organophosphates are known to be more difficult to ionise in LCMS when compared to other pesticide classes e.g. phenylurea pesticides.

The data presented discusses robustness using CV data from replicate extraction of spiked foods such as strawberries and tomatoes. The effect of the food matrix and the sensitivity achieved for a series of contaminants is discussed.

D-27
ESTIMATION OF THE DIETARY EXPOSURE TO PESTICIDE RESIDUES IN POLISH CROPS IN 2006

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One of the most important factors influencing the human development and health condition are nutrition habits. The quality of food including first of all health safety as well as usable attributes of products often decides about the consumer choice. Safe food should be characterized by both, adequate nutritive value and the tolerably low content of substances which presence could be a risk and threat for health, e.g. pesticide residues.

The aim of the study was to estimate long-term and short-term intake of pesticide residues in Polish fruit and vegetables in 2006. The estimation of dietary exposure was based on pesticide residue data from official control of domestic crops carried out by the Institute of Plant Protection and on British food consumption data.

The estimated dietary intake has shown that the chronic dietary exposure of consumers to the pesticide residues in 2006 in Polish crops was relatively low.

For example:

- in apples where pesticide residues of fourteen compounds were found the long-term intake did not exceed for adult 1.4% and for toddlers 7.6% of the ADI calculated for each compound and respectively 5.1% and 28.3% of the ADI for the sum of all compounds,
- in black currants where pesticide residues of eleven compounds were found the long- term intake did not exceed for adult 1.1% and for toddlers 3.3% of the ADI calculated for each compound and respectively 3.3% and 10.3% of the ADI for the sum of all compounds.

An acute dietary exposure was estimated for residues of endosulfan in black currants, fenhexamid in raspberries and captan in sour cherries. An acute dietary exposure did not exceed 18.9% for adults and for toddlers 43.2% of the ARfD.

The results show that Polish fruit and vegetables are safe in long- as well as in short-term nutrition.

D-28
PESTICIDE RESIDUES IN POLISH CROPS OF DIFFERENT PRODUCTION SYSTEMS ORIGIN IN 2006

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One of the most important factors influencing the human development and health condition are nutrition habits. The quality of food including first of all health safety as well as usable attributes of products often decides about the consumer choice. Safe food should be characterized by both, adequate nutritive value and the tolerably low content of substances which presence could be a risk and threat for health, e.g. pesticide residues.

Official control was carried out in 2006 on the order of Plant Health and Seed Inspection Service, financed through grants of the Ministry of Agriculture and Rural Development and included the determination of 104 compounds in 954, 344 and 89 samples from conventional, integrated and organic production sites, respectively. Laboratories of the Institute of Plant Protection analysed 32, 20 and 34 commodities produced under conventional, integrated and organic conditions, respectively.

Pesticide residues were found in 21% and 48% of samples from conventional and integrated production. Violations of MRLs were detected in 2.7% and 2.6% of samples, respectively. Residues of plant protection products not registered for use were found in 0.8% and 0.9% of samples from conventional and integrated farming.

Pesticide residues were also found in 4.4% of samples from organic farming.

Comparison of the samples from different production sites showed that residues in the organic samples were significantly lower than in two other categories of samples. An integrated production in Poland is now more intensively protected than the conventional one.

D-29
DYNAMIC CHANGES OF PESTICIDE RESIDUES IN APPLES

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Pesticides are wide group of organic contaminants representing different physico-chemical properties widely used for treatment of crop. Even if applied in accordance with Good Agriculture Practise (GAP), under certain circumstances they still might leave detectable residues in commoditieseven if contents of pesticide residues applied for treatment of crops is in respect to Maximal Residual Limits (MRLs). Acute and/or chronic exposure cause potential risk to consumer inclunding humans.

In this study two experiments were carried out. The aim of the first experiment was to realize monitoring distribution of selected pesticide residues (fungicides and insectisides) which were applicated on the four different apple varieties (Gloster, Idared, Melrose and Golden delicious), differing in amount of upper layer waxes in peels. The aim of the second experiment was to cooking experiments illustrating the fate of selected pesticide residues (fungicides captan, tetraconazole and trifloxystrobine and insectiside chlorpyrifos-methyl) during apples processing with the intention to calculate the values of the processing factors. Due to a large variety of registered pesticides possessing different physico-chemical properties, application of multiresidue methods (MRM) is unavoidable solution for convenient and flexible monitoring of their residues.

Multiresidue GC-MS method for determination of 103 pesticides representing several chemical classes (e.g. organophosphorus, modern organochlorine, pyrethroids, dikarboximids and carbamates) employed within this study consists of following steps: (i) extraction of homogenized sample aliquot by ethyl acetate, (ii) purification of crude extract by high performance gel permeation chromatography (HPGPC), (iii) identification/quantitation of analytes by gas chromatography coupled to mass spectrometry (GC-MS).

This study was carried out within the project NAZV 1G46073.

D-30
FAST SCREENING OF MORE THAN 150 PESTICIDE RESIDUES IN FOOD BY UPLC TOF MS

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In recent last years, liquid chromatography–tandem mass spectrometry (LC-MS/MS) operated in multiple-reaction-monitoring mode, has become the main tool for pesticide residues analysis both in food and environmental area. LC-MS/MS allows fast, selective and robust determination of a wide range of pesticides at low levels. However, this technique is usable neither for non-target screening nor for analysis of pesticide metabolites, because of unavailability standards for most of them. LC systems hyphenated with time-of-flight mass analyzers are much more suitable for this purpose. Due to combination of high mass resolution (above 10 000 FWHM at *m/z* 199) and mass accuracy (below 3 ppm RMS accuracy) with full scan spectra acquisition in a wide range of *m/z* (from 50 to 1000 Da), selective detection of analytes (both for pesticides and their transformation products) is enabled. However, confirmation of positive findings might represents problem when LC-TOF-MS system is used. In accordance with Commission Directive 2002/657/EC it is necessary to acquire at least three identification points (IP). With TOF MS in-source fragmentation, although fragmented mass spectra are rather poor (as compared to gas chromatography-mass spectrometry with electron ionization), it is possible to get characteristic fragments of analytes, e.i. three IP. Moreover, in many pesticides molecules chlorine atom is present, providing characteristic isotope at *m/z* M+2.

Degradation products of pesticides can be used as markers of pesticide application (theirs use is banned in products of organic farming, hence control is needed. For some pesticides such imazalil, prohloraz [1], amitraz and malathion [2] indicator breakdown compounds were earlier reported in studies employing HPLC-TOF-MS.

In the current study, application of ultra–performance liquid chromatography (UPLC) coupled to TOF-MS for fast screening of pesticide residues in food is presented. During development of this method, separation and fragmentation of more than 150 compounds was optimized in order to achieve low detection limites for as many fragments as possible. The list of MS/MS transitions shown for more than 400 compounds in paper by L. Alder et al. was employed for search of *m/z* of characteristic fragments. It should be noted, that in-source fragmentation usually provided the same fragments as observed in tandem mass spectrometry systems.

This study was carried out with support from the Ministry of Education, Youth and Sports, Czech Republic - partly from the project MSM 6046137305 and partly from project FRVS G4 1839.

[1] E.M. Thurman, I. Ferrer, J.A. Zweigenbaum, J.F.García-Reyes, M. Woodman, A.R. Fernández-Alba, *J. Chromatogr. A* 1082 (2005) 71
[2] J.F. García-Reyes, A. Molina-Diáz, A.R. Fernández-Alba, *Anal. Chem.* 79 (2007) 307
[3] L. Alder, K. Greulich, G. Kempe, B. Vieth, *Mass Spectrom. Rev.* 25 (2006) 838

D-31

EVALUATION OF INSECTICIDE CONCENTRATIONS IN THE FOOD STORAGE/PROCESSING FACILITIES AFTER TREATMENT BY SOLID AEROSOL

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Treatment of food storage and processing facilities by insecticides is often considered as a cheap and efficient alternative of pest control to avoid the excessive damage of raw commodities and to comply with hygienic requirements. The control of stored-product arthropods is usually accomplished through the use of aerosol formulations of organophosphate and pyrethroid insecticides. The aim of the presented study was to assess the dynamics of insecticide residues applied in a sealed experimental room by an aerosol smoke generator. The used pesticide formulation (Ultimate Super SG) contained pirimiphos-methyl (71.3 g/kg) and cypermethrin (13.1 g/kg). Methods for monitoring of air concentration of these insecticides after their application are based on the sorption on polyurethane foam (PUF) placed in the air sampling pump (flow rate 2 l/min). Nevertheless, more than 90 % of airborne pesticides were found on membrane pre-filter as the result of sorption on solid aerosol and dust particles in the first hours after application. Samples of wheat and wheat flour placed in thin layer simultaneously in different positions from the smoke generator were also analyzed by GC/MS method as well as the surface layer of wooden board. All samples were extracted by hexane-diethylether (95:5, v/v) using SOXTEC™ (Foss, DK) automatic extraction unit. Sampling and analytical methods used for this model studies are discussed together with final contamination levels. This strategy will be used for optimization of application procedures and techniques to increase insecticidal efficiency and to decrease exposition of operators as well as stored food.

This study was supported by MSMT: project NPV2-CHEMKONTAM-2B06099

D-32

ALTERNATIVE ANALYTICAL TECHNIQUES FOR DETERMINATION OF STROBILURIN PESTICIDES IN COMPLEX FOOD MATRICES

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In this study, three different sample preparation techniques used as a part of multiresidue methods in analysis of pesticides in food matrices were evaluated. The tested methods were: (i) conventional ethyl acetate extraction, (ii) QuEChERs method and (iii) pressurized liquid extraction (PLE). Identification and quantitation of target analytes, modern pesticides, namely strobilurins in cereal matrices, was performed by orthogonal two-dimensional gas chromatography coupled with high-speed "time-of-flight" mass spectrometry (GC×GC/TOF-MS). An alternative analytical approach – LC-MS/MS – for determination of strobilurins in wheat grain was also tested. Comparison of the extraction efficiency of PLE with those of the other techniques is presented together with the assessment of generated data and validation parameters. Appraisal of mentioned isolation techniques with respect to the amount of co-extracts, cost, time and solvent volume demands was also done. The results, from measuring incurred residue samples, proven the substantial reduction of solvent and time consumption while using PLE over „classic" method without decrease of analyte recoveries and more automatization procedure over QuEChERs method.

This work was realized as a part of the European Commission-funded Integrated Project FOOD-CT-2004-06988 "BIOCOP (New Technologies to Screen Multiple Chemical Contaminants in Foods)" co-ordinated by Queen's University (Belfast, Ireland), and also within the scope of research projects MSM 6046137305 supported by the Ministry of Education, Youth and Sports of the Czech Republic.

RESIDUES – DRUGS et al.

(E1 – E25)

E-1

IMPROVING THE MS DETECTION OF MALACHITE GREEN IN FISH PRODUCTS USING AUTOMATED ON-LINE SPE COUPLED TO LC/ION TRAP MS

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Malachite green (MG) is a traditional agent used in aquaculture to treat and prevent fungal infections. MG, which is structurally related to other carcinogenic triphenymethane dyes, is reduced to leucomalachite green (LGM) and deposited in the fatty tissue of the fish. MG can cause significantly health risks for humans after consumption of contaminated fish. In 2003 the European Commision decided that the MRPL (minimum required performance limit) of MG and LMG is 2 µg/kg. The present work describes the configuration and the method set-up of a LC/Ion Trap system with fully automated online SPE in order to improve the detection limits for the two compounds,but also to increase the lab productivity.

After homogenization of fish fillets, the sample is extracted with ACN/H2O, centrifuged and the liquid phase is collected. The extraction process is repeated two times. Collected extracts are evaporated to dryness and resolved in methanol/water. The clean-up step was performed on-line using a Gerstel MPS-3 autosampler with its automated SPE options. The MPS-3 was integrated in an Agilent 1100 LC/MSD Ion Trap system, consisting of a binary pump, thermostatted column compartment, diode array detector and a XCT+Ion Trap MS. The LC/ITMS was used with electrospray ionization in positive ion mode. Complete system control (including the autosampler) and data evaluation were carried out using the Agilent ChemStation (Rev.A.10.03).

Malachite green as well as it´s main metabolite leuco malachite green are easily ionized using electrospray in positive mode. In contrast to malchite green itself the metabolite forms a doubly charged ion besides the molecular ion [M+H] = 331. This is due to the non-planar sterical structure of the central carbon in the leuco form in contrast to the planar orientation of the central carbon in malachite green. For sensitive detection of both compounds a LC/ITMS system has been used. For quantification the formation of fragment ion m/z = 313 from the precursor m/z 329 has been observed, while the formation of fragment ion m/z = 158.5 from the doubly charged precursor m/z = 166 has proved to be highly selective and can be used for extremely sensitive determination of leuco malachite green. Using these transitions it was possible to reach limits of determination of 0.5 µg/kg for malachite green and 0.05 µg/kg for leuco malachite green.Automated online Solid Phase Extraction allowed to combine a high recovery rate of more than 90 % with an excellent reproducibility of the analysis. Moreover the time consumption of the sample preparation has been reduced by approximately 50% compared to a manual preparation on SPE cartridges, which increases significantly the lab productivity.

E-2

FLUOROQUINOLONE RESIDUES IN FOOD PRODUCTS BY LC-MS/MS USING A UNIQUE FLUORINATED STATIONARY PHASE

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Fluoroquinolones are widely used broad-spectrum antibiotics. Because they have been found in food products such as honey, shellfish and beef liver, accurate screening methods are necessary to ensure the safety of our food supply. Fluoroquinolones are derived from the parent compound, nalidixic acid, which results in compounds that are amphoteric and polar in nature. Traditional methods for assaying fluoroquinolones use highly aqueous mobile phases and ion-pairing reagents with alkyl (e.g. C18) stationary phases. However, adequate resolution of the fluoroquinolones from the sample matrix is often difficult using this approach; additionally, non-volatile ion pair reagents are not compatible with MS detection.

In this presentation, we will discuss a simplified assay for fluoroquinolones in food products, using both MS/MS and fluorescence detection. By optimizing the stationary phase chemistry for these small, polar analytes, acceptable resolution can be achieved without the need for ion pair reagents. A unique fluorinated stationary phase, which uses a pentafluorophenyl propyl ligand, provided enhanced selectivity and retention when compared to traditional alkyl and cyano-based stationary phases. This allows the use of a simplified, two-component mobile phase, which is much more compatible with MS detection.

E-3
STABILITY STUDY OF LINCOMYCIN AND TYLOSIN IN HONEY

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American foulbrood disease (AFB) is a devastating disease of honey bees, *Apis mellifera*, which affects apiculture world-wide. Certain strains of the bacterial causative agent, *Paenibacillus larvae,* have developed a resistance to oxytetracycline, the only antibiotic registered in Canada for control of AFB in beekeeping operations. The use of alternative antibiotics such as tylosin and lincomycin was recently investigated, however the stability and persistence of potential residues of these antibiotics in honey has not been fully investigated. A complicating factor is that tylosin A, the major component of "tylosin," degrades to desmycosin (or tylosin B) which also has antimicrobial properties.

In recent work carried out in Canada, LC-MS/MS was used to determine the stability of lincomycin, tylosin A and desmycosin in honey samples over a period of one year. Three different temperatures were studied: average hive temperature (+34^{o}C), normal room temperature (+20^{o}C) and typical laboratory storage conditions (–18^{o}C).

Results indicate that both antibiotics were very stable in frozen honey, with no significant degradation after one year. At +34^{o}C and +20^{o}C, lincomycin degraded at a slower rate than tylosin A. Desmycosin was also found to be more stable in honey than tylosin A, and as such, desmycosin concentrations in honey may approach or even exceed tylosin A levels depending upon honey storage conditions and the time after antibiotic application. Protocols for monitoring tylosin in honey should consider concentrations of both the parent molecule and it primary breakdown product.

E-4

EXTRACTION OF BETA AGONISTS AND BETA BLOCKERS FROM FOOD AND OTHER COMPLEX MATRICES USING MOLECULARLY IMPRINTED POLYMERS

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In the analysis of trace residues in food and other complex matrices, sample preparation is often both elaborate and time-consuming. One rapidly developing technology that obviates the need for multiple clean-up and extraction steps, thereby simplifying the sample preparation procedure, is solid-phase extraction phases based on *molecularly imprinted polymers* (MIPs). The use of such phases allows a wide range of solvents and reduced need for additional sample pre-treatment steps. The total sample handling time is less and thereby the analysis time is reduced and the cost of sample preparation is significantly lower.

MIP sorbents are designed to contain artificial 'receptor sites' that are complementary both in shape and chemical properties to desired target analytes. By targeting discreet chemical differences between molecules through the use of 'smart' templates, MIPs can be used for selective extraction of either single molecular species, or 'classes' of molecules containing the same functional or chemical motif. Because MIP materials show high selectivity to the specified compounds, they enable development of fast and sensitive methods for trace compound determination in complex samples.

In this work, we present examples of the extraction and separation of trace compounds from complex matrices that will illustrate the growing use of MIPs in trace analysis.

In veterinary, food and environmental monitoring sensitive methods for determination of Beta-receptor agonists and antagonists are required. MIP materials both for class selective screening and for highly selective analysis of the Beta agonist, Clenbuterol, will be shown. Also a MIP material has been developed and simultaneous extraction of both the Beta-blockers and Beta agonists is achieved. Applications in urine, muscle tissue and waste water are available. The methods give clean extracts with good recoveries and low ion suppression, facilitating low detection limits.

MIP based SPE materials have been shown as a very valuable technique in clinical, veterinary and environmental monitoring. MIPs are also known to simplify the extraction, quantitation and analysis of pharmacologically active compounds, carcinogenic compounds as well as banned compounds in foodstuffs and in the environment.

E-5
A NOVEL TIME-OF-FLIGHT MASS SPECTROMETRY APPROACH FOR THE SCREENING OF QUINOLONES AND FLUOROQUINOLONES IN MEAT TISSUE

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Time-of-flight mass spectrometry (TOF-MS) screening is described in conjunction with a new software tool (TargetAnalysis). This system enables screening for unlimited numbers of compounds based on their molecular formula, using highly stable accurate mass measurements and analysis of mass spectrum isotope patterns. Results will be shown for screening and quantification of quinolone compounds in spiked matrix and in tissue samples, with TargetAnalysis applied to the processing and display of the data.

Classical liquid chromatography – tandem mass spectrometry (LC-MS/MS) analysis for pre-selected targets allows the screening of limited numbers of species simultaneously. All possible targets must be taken into account when setting up the multiple reaction monitoring (MRM) experiment. Screening for fresh targets requires repeat analysis of the samples, which is often not possible, due to limited sample quantity or storage constraints. Since the number of targets is continually increasing, alternative approaches for non-presumptive screening are desirable, without making compromises in sensitivity or selectivity.

Electrospray time-of-flight (ESI-TOF) and quadrupole time-of-flight (ESI-QTOF) mass spectrometers have been developed which fulfil all of these criteria[1,2]. This new generation of ESI-TOF instruments combine high sensitivity analysis, in the sub-ppb to low ppb range, with novel selectivity. High resolution (15,000) plus the ability to make accurate mass measurements with very high stability permits extremely discrete separation of target from matrix, using extracted ion chromatograms with a tolerance as exacting as 2 millidalton. As full scan spectra are acquired, the information for an unlimited number of targets is always available; information on unknown species is also obtained, hence data can be retrospectively interrogated for new components. In addition to the high mass accuracy, the use of the precise isotopic pattern (True Isotope Pattern; “TIP”), characteristic for every molecular formula, gives a high confidence in the identification of a compound, e.g. in the characterisation of new metabolites.

The quinolone and fluoroquinolone antibiotics are of concern as residues in meats, particularly poultry and seafood, due to the risk of bacterial resistance. The system described above has been applied to the detection of the compounds of interest at concentrations down to 0.25 x MRL. Data will be presented to demonstrate the selective detection of quinolone and fluoroquinolone species using the TargetAnalysis system. Quantitative data from accurate mass extracted ion chromatograms will be presented.

[1] Opjanperä, S., Pelander, A., Pelzing, M., Krebs, I., Vuori, E. and Opjanperä, I., Rapid Commun. Mass Spectrom., 20 (2006) 1361-1167
[2] Kolomonen, M., Leinonen, A., Pelander, A. and Opjanperä, I., Anal. Chim. Acta., 585 (2007) 94-102

E-6
ELISA FOR THE DETERMINATION OF SEMICARBAZIDE IN FOOD

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Tissue bound metabolite semicarbazide (SEM) is a residual marker for nitrofurazone, a drug banned for use in animal food production in the European Union. SEM released from tissue is used for 1) monitoring illegal use of nitrofurazone and 2) veterinary residue monitoring in samples of animal origin at concentrations below the EU MRPL of 1mg/Kg. Recent studies report SEM contamination in glass container baby foods and bread, both of which are not related to nitrofuran abuse. These new stipulations, linked with thermal decomposition of azocarbonamide in lid gasket production, indicate an obvious need for a rapid assay capable of SEM detection in matrices of various origins. The common method of SEM analysis involves acid hydrolysis for the release of tissue bound residues in a sample, followed by derivatisation with o-nitrobenzaldehyde (o-NBA) to increase molecular mass prior to detection. The formed structure is the derivatised analyte nitrophenyl semicarbazide (NPSEM). In this laboratory, five rabbits produced specific antibodies against NPSEM (in indirect and direct ELISA formats) exhibiting a 50% binding inhibition (IC_{50} value) of 0.06 - 4.30 µg/L in assay buffer. The antibody MVK39 exhibited superior sensitivity in indirect ELISA format having a high dynamic range and providing linear readout in the calibration range of 0.01 to 0.2 µg/L. The sensitivity of the antibody MVK31 for SEM in direct ELISA was a somewhat lower (IC_{50}=0.14 µg/L), however, this format was used for evaluation and validation of this immunoassay method using both fortified and incurred samples. The recovery of added SEM to porcine muscle samples at concentrations of 0.3, 0.8 and 1.0 varied around 100%. Additionally, incurred whole egg samples were analysed using ELISA method within validation study. An excellent correlation between ELISA and LC/MS-MS results was found for incurred egg samples ($y = - 0.055 + 1.038x$, $r=0.992$, $n=14$). Thus, the assay is considered a quantitative analysis tool for the measurement of SEM in eggs. Preliminary results obtained in this laboratory indicate that the ELISA is well suited for determination of SEM in baby food matrices such as fruit puree and vegetable or meat samples.

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E-7

VALIDATION OF AN ANALYTICAL METHOD TO DETERMINE FOUR TETRACYCLINES IN MEAT BY PRESSURIED LIQUID EXTRACTION AND LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Tetracyclines (TCs) are given to animals destined for human consumption not only to prevent and treat certain diseases but also to fraudulently promote growth. However, the abundant and, in some cases, improper use of TCs may result in the presence of residues in edible animal tissues, which can be toxic and dangerous for humans. In order to protect consumers' health, the European Union has enacted permitted limits (PL) or maximum residue levels (MRLs) for the presence of TCs in animal products. The use of veterinary drugs in the EU is regulated through Council Directive 96/23/EC.

The present study is aimed at developing and validating an analytical method to determine tetracycline, oxytetracycline, chlortetracycline and doxycycline, which are 4 members of the TC antibiotics group commonly used in food-producing animals. TCs were extracted by pressurized liquid extraction (PLE) using water as extractant, followed by an Oasis HLB cleanup and liquid chromatography tandem mass spectrometry (LC-MS/MS) determination. Two MS systems were compared: a triple quadrupole (QqQ) Quattro LC mass spectrometer (Water, Milford, MA, USA) and a ion trap (IT) Esquire3000 LC/MS(n) (Bruker Daltonik GmbH, Germany).

The performance was evaluated according to the 2002/657/EC Commission Decision. Potential of each technique for verifying the identity of residues detected in meat samples is discussed using the concept of identification points (IPs). Mass spectra obtained in the two systems are very similar showing the same fragmentation pattern.

The decision limits (CC_α) and the detection capabilities (CC_β) were determined by the calibration curve procedure. The percentage of recovery, the repeatability (within-day precision) and the reproducibility (day-to-day precision) were determined at three spiked levels (MRLs/2, MRLs and 2MRLs). Recoveries are 70-94%, with reproducibility ranges from 8 to 19% by LC-QqQ/MS, and 72-92% with reproducibility ranges from 12 to 19% by LC-QIT/MS. Although recovery is similar by both mass analyzers and precision iss within the range of the EU guidelines, it should be noted the best precision and sensitivity obtained using QqQ.

The proposed method provides simultaneous quantification and confirmation. The extraction by PLE with water combined with SPE to isolate and enrich the analyte is a valuable alternative to long and tedious liquid-liquid extraction. Any of the mass spectrometry techniques achieves a proper identification and quantification. Robutness and feasibility of the method allows its successful application as routine procedure to identify and quantify TCs in laboratories of food quality and safety control.

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E-8

INVESTIGATION OF MATRIX INTERFERENCES FROM HONEYS IN THE BIOSENSOR ANALYSIS OF STREPTOMYCIN

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The antibiotic substance streptomycin is not approved for use in apiculture within the European Union. Hence, its absence in honey is legally required and has to be monitored analytically. Immunochemical screening methods play a major role in this field. In the analysis of honey for streptomycin by an immunochemical biosensor method a matrix influence was observed which disturbs the results. The influence correlated with the coulour of the honeys, an effect which is also known from other immunochemical methods, e.g. enzyme immunoassays (EIA). Different hypothesis on the nature of the interfering factor were tested aiming at the elucidation of the interfering compound. Subsequently the elimation of the interference from the samples was targeted in order to lower the detection limit of the method. Most efficient reduction of matrix effects was achieved by reliable pH-control and ultrafiltration of the dissolved honey samples. The resulting analytical method is rapid and simple and yielded a detection limit of 10 µg/kg.

E-9

NORFLOXACIN IGG RAISED IN RABBITS USING MULTICONJUGATES ANTIGEN AND THEIR APPLICATION FOR ELISA ASSAY

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Fluoroquinolone antibiotics, such as norfloxacin, are considered very effective antibacterial agent specially against Gram-negative bacteria and some Gram-positive bacteria. They have been introduced in Europe and the United States for human uses and were approved for livestock treatment in 1990`s and nowadays are considered the most commonly prescribed antibiotics. The extensive use of these drugs in the animal industry makes necessary the control of their residues mainly for health purposes. A rapid screening and monitoring of these compounds can be achieved by using immuno-based assays. Direct competitive enzyme-linked immunosorbent assays (ELISA) have been developed for the detection of a broad range of (fluoro)quinolones present in different food samples, enabling the detection of major fluoroquinolones under he regulatory levels and therefore ensuring food safety.

This work present a novel approach for norfloxacin conjugation. With this aim, immunization steps were optimized. Several protein carriers were used for bioconjugation linked with the same hapten using active ester method. Rabbits were alternatively immunised with these conjugates at different times for increasing the specificity of the polyclonal antibodies developed. The IgG fraction from crude serum was purified by affinity chromatography and used for the immunoassay development. Several parameters such as, reagents composition, antibody and antigen concentration and incubations times were optimized to improve the performance of the immunoassay making it suitable for an efficient control of norfloxacin in food samples.

E-10

QUANTITATIVE HIGH-THROUGHPUT ANALYSIS OF 16 (FLUORO)QUINOLONES IN HONEY USING AUTOMATED EXTRACTION BY TURBULENT FLOW CHROMATOGRAPHY COUPLED TO LC- MS/MS

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(Fluoro)quinolones belong to the current arsenal of antibiotics developed to treat various infections in animal therapy. In the EU, several of these drugs have been regulated for different food matrices of animal origin, but not for honey. In the last five years, considerable problems related to the finding of veterinary drug residues in this commodity have arisen and such issues have had a serious impact on both raw material suppliers and food manufacturers, resulting in rejection and potentially destruction of honey batches, but has also endangered the image of bee products as natural and clean.

Current analytical methods devoted to the analysis of (fluoro)quinolones encompass a traditional scheme, i.e., liquid-liquid extraction (LLE) followed by an clean-up/enrichment step by solid phase extraction (SPE) before final quantitation either by ultraviolet, fluorescence or MS detection. Though sensitive, these procedures are still time-consuming and are not fitted for high throughput analysis.

A new multiresidue method, making use of turbulent flow chromatography (TFC), was developed for the analysis of 16 (fluoro)quinolones in honey. The sample preparation was limited to a simple solubilisation of the honey test-portion in water (in a 1:1 ratio) followed by a filtration before subsequent analysis by high throughput liquid chromatography tandem mass spectrometry (HTLC-MS/MS). The extract was on-line purified on a large particle size extraction column where the sample matrix was washed away while the analytes were retained. Subsequently, the analytes were eluted from the extraction column onto an analytical column by means of an organic solvent prior to chromatographic separation and MS detection. Validation was performed at three fortification levels (i.e., 5, 20 and 50 µg/kg) in three different honeys (acacia, multiflower and forest) using the single-point calibration procedure. Satisfactory recoveries and within- and between-day precisions were obtained whatever the level of spike. The limit of quantification (LOQ) for all compounds was arbitrarily set at the lowest fortification level considered during the validation, i.e., 5 µg/kg. This rapid method has been successfully applied in a mini-survey of honeys of Chinese origin, showing that ciprofloxaxin and norfloxacin are the main (fluoro)quinolone antibiotics administered to treat bacterial diseases of bees.

Turbulent flow chromatography coupled to high sensitive MS/MS detectors was shown to be a good alternative to traditional analytical procedures to deal with the growing numbers of samples to be analyzed for drug residues in a continuously expanding global trading of food.

E-11

AMPROLIUM DETECTION IN COMPOUND FEEDINGSTUFF FOR POULTRY

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INTRODUCTION

The presence of residue of veterinary drugs in food has received much attention in recent years because of growing concern for safety by consumers. This prompted the European Commission to ban a variety of compounds, including coccidiostats and histomonostats. Amprolium is a thiamine analog used as coccidiostats and histomonostats in poultry, this drug is no longer authorized as an additive for feedingstuff since 2005 (Racc 2005/925/EC).

Since an official method for the detection of Amprolium in feedingstuff is lacking, we developed a rapid and effective chromathografic (LC-MS) method for the determination of this drug in coumpound feedingstuffs for poultry, with a quantification range from 0.8 µg/g to 25 µg/g.

AIM

Aim of this work is to report the results of the validation of a LC-MS method for the detection of Amprolium in compound feedingstuffs for poultry.

MATERIAL AND METHODS

20 samples of compound feedingstuff for poultry were extracted with methanol and water 4+1 (v+v) and filtred with inorganic membrane filter (0.2 µm, 10 mm). The anaysis was then carried out by HPLC using a mass spectrometer as detector in ESI mode, molecular ion 243, fragment ions 150; 94. Separation was carried out on PURSUIT XRs C_{18} (150x2mm, id 3µm) column in gradient mode with epta-fluorobutirric acid –methanol solution. Validation was carried out according to the Reg 2004/882/CE.

RESULTS

Mean recovery was 96.8% with RDS of 0.127%, LOD was estimated at 0,061 µg/g and LOQ was evaluated at 0.2 µg/g.

DISCUSSION

The LC-MS method above described was characterized according to the Reg 2004/882/CE showing good performances. Therefore it is going to be employed in the detection of Amprolium in compound feedingstuffs for poultry as official test in Italy.

E-12

MULTI-DETECTION AND QUANTIFICATION OF BANNED STEROIDS IN FOOD BY A LIQUID CHROMATOGRAPHY QUADRUPOLE-TIME-OF-FLIGHT MASS SPECTROMETRY METHOD

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The European Union has banned the use of steroids in food producing animals since 1998(96/22/EC). For banned compounds there is zero tolerance and testing must meet the minimum required performance level (MRPL- for hormones at 1-2 µg/kg). For the detection of 11 banned anabolic steroid residues in food matrix, such as milk and honey, a highly sensitive liquid chromatographic/quadrupole-time-of-flight mass spectrometry (LC/QTOF MS) method was developed following a simple clean-up step. The procedure included enzymolysis, ultrasonic homogenization, and then extraction with tert-butyl methyl ether. The extract was then cleaned-up with C18 solid-phase extraction (SPE). Analyses were performed on an LC/QTOF MS equipped with electrospray ionisation with an analytical run time of 15 min using targeted MS/MS for quantitation. The use of a 4 Da bandbass with the quadrupole mass analyzer for the selection of precursor ions allowed full spectra with at least A+3 isotopes to be collected. The high resolution and accurate mass capabilites of the QTOF allowed the generation of reconstructed ion chromatograms having a narrow acurate mass window (20 ppm), thus providing very high selectivity in complex sample matrices. The mass accuracy of the MS/MS quantitation ion was better than 5 ppm for all the analytes tested. The limits of detection (LOD) for the LC/ QTOF MS method used for testing 1-dehydrocortisol, betamethasone, dexamethasone, trenbolone, cortisol acetate, cortisone acetate, prednisone acetate, methyltestosterone, beclomethasone, 17β-hydroxy-4-androsten-3-one 17-propionate, and nanarolonepheylpropionate varied between 0.1 and 0.5 ng/mL and the limits of quantification (LOQ) were from 0.22 to 0.8 µg/kg. Experiments using spiked samples at 1.0 µg/kg in milk and honey showed that the average recovery of the steroids ranged from 51.5% to 72%. This work demonstrates that LC/QTOF MS can provide quantitative results with the specificity of full-spectra accurate-mass MS/MS to meet EU requirements.

E-13

IDENTIFICATION AND QUANTITATION PFOS AND PFOA IN MILK AND HONEY USING LIQUID CHROMATOGRAPHY QUADRUPOLE-TIME-OF-FLIGHT MASS SPECTROMETRY

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Fluorinated monomers are used in the manufacture of fluoropolymers that have found broad use in many products such as paints, polishes, packaging materials, lubricants, fire-fighting foams, cookware, and stain repellents. These compounds are potentially toxic to the environment and humans. PFOS (perfluorooctane sulfonate) and PFOA (perfluorooctanoic acid), have been detected in human blood worldwide. Paul Lam of the City University of Hong Kong and colleagues reports detecting PFOS, PFOA, and several other PFCs in the breast milk of 19 new mothers in the rural seaside town of Zhoushan, China. For these nonvolatile or polar fluorinated coumpounds, LC with ESI MS in negtive ion mode is now the most widely accepted method for analysis. In this study, we discuss the result obtained using an LC/MS Q-TOF to detect and quantify these compounds in milk and honey samples. The linearity for these compounds with the QTOF was R^2 > 0.99 in the real food samples. Excellent mass accuracy and peak area reproducibility were obtained in milk and honey also discussed. In addition, an injection programme that avoided ”carry-over”will be described.

E-14

LC-MS INTERCOMPARISON ON NITROIMIDAZOLES IN MEAT: A CRUCIAL FEASIBILITY STUDY TOWARDS A CERTIFIED REFERENCE MATERIAL

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Certified Reference Materials (CRMs) are important tools for method validation (e.g. trueness determination) and method performance verification. Apart from other necessary data which are needed to establish a suitable certified reference material (e.g. various commutability studies investigating animal species, matrix, and matrix type), validated analytical methods of high performance are required for accurate determination of the property value(s) in the material.

In a recent cooperation between the BVL in Berlin (responsible CRL for these compounds, organizer of preliminary stability and commutability studies) and the IRMM in Geel (responsible for the entire process of reference material certification) it was decided to launch a laboratory inter-comparison to evaluate the performances of currently used LC-MS methods in the concentration range of 0.5 – 3 µg/kg (foreseen concentration of analytes in the CRM, values close to proposed recommended concentrations).

Small batches of lyophilised turkey meat materials were produced at BVL, checked for sufficient homogeneity and stability during dispatch, and delivered to the participants. Each lab received 4 lyophilised meat samples (3 incurred materials, 1 blank) which had to be analysed in duplicate. Laboratories used their in-house protocols (different samples preparation procedures, LC-MS methods). No false positive results were obtained. The three incurred samples containing three different nitroimidazoles/hydroxymetabolites in the range of 0.5 to 3 µg/kg were detected by all laboratories (according to their stated method performance parameters). Partly very low reproducibility standard deviations (significantly below 20%) could be observed.

In conclusion, a sufficiently large number of laboratories - independent of the different methods applied – are capable of performing high-quality measurements required for establishing a certified reference material.

E-15

DEVELOPMENT OF A RAPID AND SENSITIVE LC/MS/MS ROUTINE METHOD FOR DETERMINATION OF CHLORAMPHENICOL IN HONEY

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Chloramphenicol (CAP) is a broad spectrum antibiotic applied in the treatment of veterinarian and human diseases. However, CAP exhibits some toxic effects in certain susceptible individuals such as those with bone marrow depression including fatal aplastic anemia. As this condition is dose independent, CAP has been banned for use in livestock in which honeybees are included. In this study, a quick and simple low cost routine method of analysis was developed for determination and confirmation of chloramphenicol in honey by LC/MS/MS. The sample clean-up takes only two steps without SPE procedure with recoveries above 97%.

The samples were selected from several producers in Brazil and diluted in a small amount of water. After addition of d5-chloramphenicol as internal standard, the samples were extracted with ethyl acetate. The complete validation of the method was performed on the basis of EU-decision 2002/657. The within-laboratory CV reproducibility at lowest concentration was below 10%. An evaluation of two different methods to calculate Ccalfa and Ccbeta was carried out obtaining 0,08 µg/Kg for Ccalfa and 0,12 µg/Kg for Ccbeta.

E-16
DETERMINATION OF ZERANOL AND METABOLITES IN URINE OF SLAUGHTERED ANIMALS BY GC-MS

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Zeranol (α-zearalanol-ZER) is a non-steroidal synthetic oestrogenic growth promoter related to the oestrogen mycotoxin zearalenone (ZON). It belongs to the group of substances known as beta resorcyclic acid lactones. It increases liveweight gain in food producing animals following implantation. The use of zeranol for growth promotion was banned in the European Union (EU) in 1985. All Member States have been required, since 1986, to establish National Surveillance Schemes to monitor animals for possible abuse of banned substances including zeranol. The New Zealand study suggested that naturally occurring *Fusarium spp*. toxins could be metabolised to zeranol.

The GC-MS method was presented for determination of zeranol, its major metabolite zearalanone (ZAN), its minor metabolite taleranol (β-zearalanol-TAL) and metabolites of zearalenone (α-zearalenol/α-ZOL and β-zearalenol/β-ZOL) in urine of animal origin and used as a confirmatory. Because the method was quantitative the internal deuterated standards: zeranol-d_4/taleranol-d_4, α-zearalenol-d_4 and β-zearalenol-d_4 were added to the samples before extraction procedure. After this process double diethyl ether liquid-liquid extraction and clean-up of octadecyl (C_{18}- 100 mg, 1 ml) and amino (NH_2- 500 mg, 3 ml) solid phase extraction cartridges were performed. The residues were derivatised with 50 µl of a mixture containing MSTFA/NH4I/Dithiotreitol (1000:2:5, v/w/w) for 20 min at 60°C (± 2°C). After derivatisation samples were not evaporated, collected in autosampler vials and applied onto GC-MS instrument.

The GC-MS analysis was carried out on a 6890N gas chromatograph directly coupled to a MSD 5973 (Agilent). The GC capillary column was HP-5 ms with a length of 30 m, an internal diameter of 0,25 mm and a film thickness of 0.25 µm. The carrier gas was helium at 0.6 ml/min flow. The oven temperature program was as follows: 120°C (2 min) - 20°C/min - 300°C (6 min). Injection (2 µl) was pulsed splitless. The injector temperature was 260°C. The mass spectrometer was operated in electron impact (EI), selected ion monitoring mode.

The described method was validated according to the Decision Commission 2002/657/EC. Satisfactory chromatography separation was obtained. For analytes the diagnostic ions were performed; ZER and epimer form TAL: 538-523-433-379-335-307; α-ZOL and β-ZOL: 536-446-431-333-305; ZAN: 534-519-464-449-335-307; ZER-d4 and TAL-d4: 542-523-437-379-335-307; α-ZOL-d4 and β-ZOL-d4: 540-450-431-333-305.

The concentration of analytes in samples was counted using calibration curves. Average recoveries of analytes at MRPL level were between 78.8 and 104.0% with the relative standard deviation < 15%. The limits of detection ranged from 0.12 to 0.24 µg/l.

E-17
THE ANALYSIS OF TRENBOLONE IN MUSCLE TISSUE WITH GC-MS

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Trenbolone acetate (TBA), 17β-hydroxyestra-4,9,11-triene-3-one, is a synthetic steroid with anabolic properties. It is 8 to 10 times as potent as testosterone. In animals, TBA, alone or in combination with 17β-estradiol, is used to improve weight gain and feed efficiency. The most popular implants containing TBA are Torelor, Revalor, inaplix. TBA upon entering the circulatory system is rapidly metabolised to its free active form, alfa and beta trenbolone (TBOH). In the bovine species, the 17α-epimer is the major metabolite occurring in the excreta, bile and liver; the 17 β-epimer is the major metabolite occurring in muscles.

A sensitive and selective method was developed for the confirmatory analysis of 17β-trenbolone in muscle tissue. To a minced muscle tissue sample (10g), 10 ng internal standard β-TBOH-d_3 is added (corresponding to 1 µg/kg). Tissue is homogenized in sodium acetate buffer (pH 5.2), digested with glucuronidase AS-HP, mixed with methanol, heated at 90°C and then centrifuged. The methanol layer is deffated with n-hexane and diluted with water. Then liquid-liquid extraction with diethyl ether is performed, and ether phase after washing with sodium carbonate buffer and water respectively is evaporated to dryness under a stream of nitrogen. The residues is then recovered in acetate buffer (pH 4.8), and this extract is further purified using both C_{18} and NH_2 SPE columns. The purified, evaporated extract is derivatized. For trenbolonetwo derivatization steps were used: first with MSTFA/I_2 (1000 µl: 10 mg) solution and second with MSTFA. The gas chromatographic-mass spectrometric analysis were performed using an Agilent MSD 5973 detector, interfaced to an GC 6890 N gas chromatograph equipped with an Agilent 7683 autosampler and HP-5 capillary column (30m, 0.25 mm i.d., 0.25 µm film thickness). The method has been validated according to Commission Decision 2002/657/EC. The CCα and CCβ values are based on the detection of the most abundant ion and the values: 0.18 µg/kg and 0.28 µg/kg were obtained respectively. The repeatability for the validation level 1 µg/kg was 0.08 µg/kg (CV 7.7%) and within laboratory reproducibility – 0.17 µg/kg (CV 15.8%). The accuracy 106.1% was obtained and uncertainty of measurement – 0.11 µg/kg for this validation level. For identification and confirmatory purpose following diagnostic ions for β-TBOH were obtained: m/z 323, 380, 442, 449, 524.

E-19

OPTIMIZATION OF SPE CLEAN UP AND VALIDATION OF QUANTITATIVE DETERMINATION OF CORTICOSTEROIDS IN URINE BY LC-MS/MS

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A solid phase extraction (SPE) method for extraction and clean up of 9 synthetic corticosteroids was optimized for quantification by reversed-phase high-performance liquid chromatography/ negative electrospray ionisation mass spectrometry (LC-MS/MS).

The final method was validated for bovine urine according to EU regulations for determination of residues of veterinarian drugs in products of animal origin.

Initially, acceptable recoveries with negligible matrix interference for five synthetic corticosteroids (triamcinolone acetonid, flumethason, dexamethason, betamethason and prednisolon) were produced from porcine urine (absolute recoveries: 74-125%) with a simple clean up procedure based on application of urine on a mixed mode polymeric strong anion exchange SPE column (Oasis MAX), washing with water and elution with methanol. From bovine urine, however, recoveries were low (10-27%).

Dilution experiments showed that the low recovery from bovine urine was caused by suppression of the signal during the measuring process and not due to losses during clean up.

Washing with acid or alkaline solutions in combination with elution with neutral methanol shoved little effect towards reducing the suppression. By combining an alkaline washing procedure (0.1N ammonia in water) with elution with alkaline methanol (0.1N ammonia in methanol), suppression was effectively eliminated for all analytes except prednisolon.

By reducing the amount of cleaned up sample injected to the chromatographic system from the equivalent of 70 µl urine to approximately 20 µl, suppression of prednisolon was minimized (absolute recoveries from porcine urine: 92-115%, from bovine urine: 99-111%).

To include conjugated corticosteroids in the analysis, the sample was hydrolysed with Helix Pomatia β-glucuronidase/aryl sulfatase. This process showed some effects on the recoveries, so for the final method, which also included fluocinolon acetonid, 6α-methylprednisolon, beclomethason and prednison, a quantification based on spiked samples put through the entire analytical procedure was used. For quantification of triamcinolone acetonid an internal standard (triamcinolon acetonid-D6) was used.

During validation, the method showed relative average recoveries from 96 to 103% with the exception of beclomethason (113%). Absolute average recoveries were 81-99%. Quantification limits (decision limits, CCa) were demonstrated to be not higher than 1 µg/l (3 µg/l for prednison and prednisolon). The internal reproducibility, determined by triplicates from spiking at three different levels in six analytical series was 7-19% (at 2-4 µg/l) except for prednison and prednisolon (26-27% at 3-6 µg/l).

E-20

DEVELOPMENT OF A LC-MS/MS CONFIRMATORY ASSAY FOR THE SIMULTANEOUS DETERMINATION OF SEVERAL TETRACYCLINES IN MILK TAKING INTO ACCOUNT KETO-ENOL TAUTOMERISM AND EPIMERIZATION FENOMENS

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The failure to follow good veterinary practices can lead to unsafe residue levels of antibiotics in food of animal origin, with potential adverse effects on human health. In Brazil, none of the tetracyclines (TCs) are allowed as feed additive for growth promoting purposes, but oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC) are currently approved for the treatment of mastitis and other infections in dairy cattle, while doxycycline (DC) can be administrated just for poultry, swine and calves. Residues of demeclocycline (DMC) and metacycline (MTC) should not be detected either in milk or edible tissues, as they are licensed for use in human medicine only. Although, there have been concerns over the use of unauthorized drugs in lactating species.

The MRLs harmonized between Brazilian's Ministry of Health and Ministry of Agriculture are those recommended by *Codex Alimentarius*, 100μg kg^{-1} in milk, either for the sum of OTC, TC and CTC or each drug individually. In European Union the same value has been established considering the marker residues as the sum of the parent drug compounds and its 4-epimers.

A liquid chromatography-positive electrospray ionization tandem mass spectrometric (LC-ESI-MS/MS) method for the analysis of several tetracyclines residues in bovine milk was developed. Milk deproteinization/extraction of samples was performed with acidified acetonitrile. After diluting and purification by solid-phase extraction (SPE), the extracts were injected into the instrument operated in Selected Reaction Monitoring (SRM) acquisition mode. The reversible epimerization at C-4 of oxytetracycline, tetracycline and chlortetracycline and the keto-enol tautomerism of chlortetracycline between C-11a and C-12 were considered for reliable quantification. Degradation was also taken in account and minimized for the same purpose. By using DMC as internal standard recoveries in excess of 81% were achieved in spiked samples at 100ng mL^{-1}, with RSD lower than 14%. The method is still being validated (according to criteria established by Commission Decision 2002/657/EC) and was tested in naturally contaminated samples with TC. Besides the parent drug, 4-epitetracycline (4-ETC) could be detected. Since it was verified on method development that the 4-epimers are not formed during sample preparation, these data indicate that 4-ETC was probably formed *in vivo* after TC treatment of lactating cow.

E-21

VALIDATION OF A ROBUST LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MSMS) CONFIRMATORY METHOD FOR 13 SULPHONAMIDES

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Codex Alimentarius and other national and regional bodies have set maximum residue limits (MRL) for sulphonamides in various animal tissues. To monitor compliance with these MRLs, highly specific and selective methods must be available to confirm the presence of any sulphonamide(s) at a level greater than the MRL in a sample. A fast, easy, and robust confirmatory method was developed for 13 sulphonamides in porcine kidney and chicken muscle tissues. The method employs a simple extraction protocol using an inexpensive extraction solvent (ethyl acetate), concentration by evaporation, a hexane wash and reconstitution in LC mobile phase. All compounds eluted from a reversed-phase column within 8 minutes. Identification and quantitation was achieved using a triple-quadrupole mass analyzer with positive mode electrospray ionization. Quantitation was based on matrix-matched calibration and the inclusion of a stable isotope internal standard (d4-sulphadimidine) to resolve matrix ion-suppression effects and run to run variation in instrument response and improves precision. The method was fully validated for 13 sulphonamides in porcine kidney, with a single day validation in chicken muscle. The method meets the identification criteria specified by the European Union in Commission Decision 2002/657/EC, and included in the current draft revision of the Codex guidelines, for confirmatory methods for compounds licensed for use in food-producing animals. Recoveries, calculated for all compounds using the d4-sulphamethazine internal standard, ranged between 69% and 108.9% for 11 of the analytes, with lower values for sulphaguanidine (23% in chicken muscle and 36% in pig kidney) and sulphaquinoxaline (59% in chicken muscle and 60% in pig kidney). Method performance would be improved for these latter analytes by the inclusion of stable isotope labeled internal standards for each compound. Precision was acceptable for all analytes, with reproducibility ranging between 1.5 and 8.9% in chicken muscle and 2.3-7.9% in pig kidney, and decision limits (CCα) between 103 and 113 ng/g (MRL=100 ng/g). The robustness of the method was demonstrated by applying the Youden approach with variations in seven method parameters (e.g. extraction pH, evaporation temperature, hexane wash volume).

The method presented is rapid and relatively inexpensive in comparison to many other published methods for confirmation of sulphonamides and is suitable for use in both developed and developing country regulatory laboratories that are equipped with LC-MS/MS.

E-22
HUMAN EXPOSURE TO ANDROGENIC/ANTI-ANDROGENIC COMPOUNDS (AACS) IN EUROPE

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The food market is a growing economic sector very profitable as well as the application of pesticides, which is an unavoidable necessity in order to improve the agricultural production. On the other hand, consumers are becoming more concerned about food contaminants; and pesticide residues feature high in their list of worries. For these reasons food monitoring and human exposure data should be used to illustrate what regulators are doing to ensure that the food we eat is safe. Within the COMPRENDO project an attempt to identify the potential threat of AACs exposure in human populations originating from the food is carried out.

About 390 samples were analysed for the presence of organochlorine pesticides (pp'DDT, pp'DDE), organotins (MBT, DBT, TBT, MPT, DPT, TPT) as well as pesticides (vinclozolin, fenarimol, linuron, diuron and their metabolites-DCA, DCPU, DCPMU), total, 15 different chemical substances.

63.2% of the samples analyzed, contained no detectable AACs residues. Detectable residues at or below the MRL were found in 35.6% of the samples. The data show that there were exceedances of MRLs in approximately 1.1% of all samples, for all chemicals analyzed.

The percentage of samples with pesticide residues (linuron, diuron and their metabolites, as well as vinclozolin and fenarimol), was considerably higher in fruits and vegetables (69.2 %), compared to other commodities.

On the other hand, meat (100%), eggs (97%), fish (97%), cereals (90%), shellfish (87%), fat/oil (83%), and dairy products (64%) were contaminated with organochlorine pesticide residues (pp'DDE).

Results on the analysis of organotin compounds have shown the presence of TBT in fish (23.3%) and shellfish (46.7%). Wine samples (16.7%) were found to be contaminated at low concentration levels by MBT and DBT, respectively.

Consequently the number of samples with pesticide residues exceeding the MRL was higher in fruits and vegetables (7 samples, 1.8%), while for fatty commodities, three samples (0.8%) were contaminated with organochlorines residues above the MRL, respectively.

In general a significant proportion of the samples analysed contain AACs residues but the levels present are considered generally low.

E-23

MULTI-RESIDUE METHOD FOR THE SIMULTANEOUS DETERMINATION OF BENZIMIDAZOLES IN BOVINE MILK BY ONLINE SPE-LC-MS/MS

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Benzimidazoles are veterinary drugs widely used for prevention and treatment of parasitic infections in agriculture and aquaculture. Residues of benzimidazoles are most likely to be found in milk for which the withdrawal periods have not been strictly observed. Maximum residue limits (MRLs) in milk range from 10 µg/l for oxfendazole to 100 µg/l for albendazole. For the determination of residues of benzimidazoles in biological samples very frequently a solid-phase-extraction (SPE) technique is combined with a liquid-chromatographic separation and mas-spectrometric detection using triplequadrupole MS (LC–QqQ MS) [1].

Online SPE–LC–QqQ MS is becoming more and more of interest for pharmaceutical, environmental and clinical applications. The online technique offers many advances over offline techniques *viz.* method development and sample analysis can take place overnight which saves time and the repeatability of the final method will increase due to reduction of the manual sample-handling-steps. As far as we know there are no analytical methods published, recently, using online SPE–LC–QqQ MS for residue analysis of veterinary drugs in biological samples. In this study an analytical method was developed and validated for the determination of 17 benzimidazoles (including metabolites) in milk by using online SPE– LC–QqQ MS.

For the analytical method developed, sample pre-treatment and extraction were very simple. After a defatting step the analytes were online extracted, separated and detected. SPE-Oasis™ MAX cartridges from Waters were used for the extraction. The chromatographic separation was achieved using a Waters Xbridge™ C_{18} column with an ammonia formate buffer (pH 3.5)/methanol gradient. The analytes were detected in multiple reaction monitoring mode by positive electrospray ionisation tandem mass spectrometry (MRM, (ESI(+)QqQ mode).

The method was validated according to the revised European Union requirements (2002/657/EC) and all relevant parameters were found in conformity with the criteria. The method characteristics and validation results will be presented and discussed.

[1] M. Danaher, H. De Ruyck, S.R.H. Crooks, G. Dowling, M. O'Keeffe, J. Chromatogr. B 845 (2007) 1.

E-24

SIMULTANEOUS LC-MS/MS DETERMINATION OF SILDENAFIL AND RELATED ANALOGUES ADDED ILLEGALLY TO HERBAL PRODUCTS INTENDED FOR THE TREATMENT OF ERECTILE DYSFUNCTION

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Introduction

Sildenafil citrate (Viagra), vardenafil hydrochloride (Levitra) and tadalafil (Cialis) are phosphodiesterase-5 (PDE-5) inhibitors approved for the treatment of erectile dysfunction (ED). Despite the efficacy of PDE-5 inhibitors as a treatment of ED, their drawbacks are notable as well. Adverse effects such as headache, visual disturbances, dyspepsia and muscle aches have been reported. Therewithal, patients with hypertension, hyperlipidemia and ischemic heart diseases, which use nitrate medication, should not take synthetic PDE-5 inhibitors. These patients may resort to herbal remedies and dietary supplements for treatment of ED. However, it was discovered that some herbal products had contained illegally added synthetic PDE-5 inhibitors and their analogues (viagra-like drugs), without any declaration on labeling.

Methods

A liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS/MS) method was developed for the simultaneous analysis of six synthetic PDE-5 inhibitors, namely sildenafil, tadalafil, hydroxyhomosildenafil, acetildenafil, hydroxyacetildenafil and ethyltadalafil, i.e. compounds with chemical structures similar to the sildenafil. These compounds have been extracted from herbal products by acetonitrile, centrifuged (3500 rpm) and diluted prior analysis. Identification and quantification of PDE-5 inhibitors and their analogues was elucidated using liquid chromatography/mass spectrometry (LC-ESI-MS/MS) in multiple reaction monitoring (MRM) scan mode. The method has been validated for accuracy, precision, linearity and sensitivity. The prospective presence of PDE-5 inhibitors was subsequently confirmed by exact mass measurement of precursors and their fragments using liquid chromatography coupled with electrospray/time-of-flight mass spectrometry (LC-TOF MS).

Preliminary results

The contribution presents chromatographic method for separation of group of six synthetic PDE-5 inhibitors, including hydroxyacetildenafil and ethyltadalafil whose separation and detection conditions have not been published yet. In the study LC-TOF MS and LC-ESI-MS/MS techniques have been applied for fast and effective analysis of six PDE-5 inhibitors in different samples of herbal products taken by CAFIA for testing. Six samples of herbal products were tested during the last year. Five of them were proved to contain acetildenafil although the presence of PDE-5 inhibitor was not declared. The concentration levels of acetildenafil in these samples varied from 9.1% to 28.7% per capsule. Furthermore, the data of routine analysis of these compounds collected during the year 2007 will be presented as well.

E-25

CONFIRMATORY ANALYSIS OF PHENYLBUTAZONE IN BOVINE PLASMA USING GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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A new method for the determination of phenylbutazone in bovine plasma has been developed. Plasma samples are mixed with phosphoric acid and are cleaned up on a Oasis HLB solid phase extraction cartridge. Extracts are analysed by gas chromatography/tandem mass spectrometry (GC-MS/MS). The method was validated according to criteria defined in Commission Decision 2002/657/EC. The method allows for quantitation in the range of 1-10 ppb. The Decision Limit, CCα is 0.2 ppb and the Detection Capability, CCβ is 0.34 ppb.

NATURAL TOXINS, MYCOTOXINS

(F1 – F49)

F-1

COACERVATIVE EXTRACTION OF OCHRATOXINE A IN WINES PRIOR TO LIQUID CHROMATOGRAPHY/FLUORESCENCE DETERMINATION

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Ochratoxin A (OTA) is a mycotoxin produced by several species of fungi, including Aspergillus and Penicillium. Depending on both environmental and manufacture conditions, OTA occurs at different concentration levels in various foodstuffs and beverages (e.g. cereals, coffee, wine, cocoa, spices, etc). According to a 2002 report on the assessment of dietary intake of OTA by European people, wine resulted in one of the main dietary source (10-20%). Because of the long half-life of OTA in the organism, its non-reversible disturbances in kidneys, its possible carcinogenic effect and its immunosuppressive and neurotoxic properties, there is a growing need to monitor OTA in food samples, including wine, for which the EU has set a maximum level at 2,0 µg/l.

Analytical methods currently available for OTA determination in wines consist of a concentration/clean-up step followed by reverse-phase LC separation and fluorescence detection or in some cases mass spectrometry with electrospray ionization. The most frequently used concentration/clean-up techniques are solid-phase extraction (SPE) and liquid-liquid extraction (LLE). For LLE techniques, the most important disadvantages are the high organic solvent consumption and the poor recoveries obtained. With regard to SPE, inmunosorbents or inmunoaffinity columns (IAC), which offers high selectivity, are the sorbents more extensively used. In fact, IAC is recommended by the Office International de la Vigne et du Vin (OIV) and the Association of Official Analytical Chemists (AOAC). However inmunosorbents are not recyclable, have a limited storage time (commonly 12 months) and, in some cases, show cross-reactivity with Ochratoxin C.

This research deals with the use of coacervates, colloid-rich liquids, for the extraction/concentration of OTA from white, rosé and red wines, prior to LC/fluorescence quantitation. Water-induced coacervates made up of reverse micelles of decanoic acid in tetrahydrofuran (THF) were used for this application. Extraction of OTA was carried out at pH 2 and it was based on both hydrophobic and hydrogen bonds analyte:extractant interactions. Parametres affecting extaction efficiency and concentration factors were studied. Concentration of decanoic acid and THF were the most influential parametres, being 0,5% of acid and 5% of THF the selected concentrations. The procedure was very robust, so that the extractions were not significantly influenced by the pH and the nature or concentration of the matrix components. Recoveries of the target compounds ranged between 80% and 94% and the concentration factors varied from 128 to 150 for sample volumes of 19 mL. The precision of the method expressed as relative standard deviation was about 5% and the detection limit was 4,5 ngL^{-1} in white and rosé wines and 15 ngL^{-1} in red wines, values which are far below the maximun level established for OTA by EU directives (Commission Regulation (EC) no. 123/2005, Official Journal of the European Union L25 (2005) 3). This method offers a simple, cheap and rapid alternative for the usually tedious and time-consuming wine pre-treatment. The approach developed was applied to the determination of OTA in different wine samples from south of Spain.

F-2

APPLICATION OF AN LC-MS/MS BASED MULTIANALYTE METHOD FOR SCREENING MYCOTOXINS IN SPOILED FOOD

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Although 300-400 fungal metabolites are currently recognized as mycotoxins, the large majority of the related quantitative analytical methods (including most of the recently developed multi-mycotoxin approaches) investigate only those toxins for which regulations already exist and some of their derivatives, i.e. deoxynivalenol and other B-trichothecenes, HT-2 and T-2 toxin and other A-trichothecenes, zearalenone and its derivatives, fumonisins, aflatoxins, ochratoxin A and patulin. This adds up to a comparatively small fraction comprising approximately 20-25 compounds.

Most of the remaining mycotoxins have not been addressed sufficiently by quantitative analysis so far. This stands clearly in contrast to the health hazards posed by these substances, particularly as they are produced by molds that are known to be involved in spoilage of food, e.g. *Penicillium*, *Aspergillus*, *Alternaria* species. As a result, they are of no lesser relevance for a typical consumer than the abovementioned “classical” mycotoxins.

In our group, an HPLC-MS/MS based multi-analyte method has been developed that currently comprises 107 fungal metabolites and has recently been validated for 87 analytes. This presentation focuses on the application of the method to (moldy) food samples and will show that the simultaneous determination of such a wide range of mycotoxins is of great value for acquiring comprehensive data on their occurrence in the food and feed chain. This will facilitate a sound assessment of the hazards that these fungal metabolites pose to the human and animal health.

F-3

LC-MS PROFILING OF LIPID SOLUBLE BIOTOXINS IN SCOTTISH SHELLFISH DURING SUMMER 2006

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Bivalve shellfish can provide a nutritional and appetising food. However, the safety of the human consumer may be compromised by the consumption of shellfish contaminated with marine phycotoxins. To ensure consumer protection and to reduce the economic impacts to the shellfish industry, toxin monitoring of shellfish from classified production areas is a requirement. The approved method for detecting lipophilic toxins is a bioassay and this constitutes the European Union's 'reference method'. Under this regulation, a number of analytical and *in vitro* methods may be used as alternatives or supplementary to the assay provided they give an equivalent level of public health protection, they are not less effective than the assay and can, either alone or combined, detect a prescribed series of toxin analogues. Liquid chromatography with mass spectrometric (LC-MS) comprises one of the common, alternative techniques and has the advantage of sensitivity, precision, confirmation and quantitation of toxin identity. The combination of LC interfaced with electrospray ionisation (ESI) and tandem quadrupole MS is an invaluable alternative for determining the presence of these harmful compounds in seafood products.

Over the past five years and alongside the approved method for monitoring marine lipophilic toxins, LC-MS and MS/MS has been applied to detect and quantify a range of these toxins in shellfish harvested from coastal waters of England, Wales and Scotland. Presentation of recent method performance information will be given for the identification, confirmation and quantitation of a selection of lipophilic toxins. In view of regulatory limits, validation data will be described for parameters such as accuracy, limits of detection and quantitation, linearity, precision, recovery and selectivity. A deion of observed geographical distributions of shellfish contaminated by these toxins will also be presented together with an overview of toxin profiles determined in shellfish during several toxic episodes.

F-4

DEVELOPMENT OF AN ENZYME-LINKED IMMUNO-FLOW ASSAY (ELIFA) FOR ANALYSIS OF DOMOIC ACID IN SHELLFISH SAMPLES

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Domoic acid (DA) is a neurotoxic amino acid that has been identified in marine samples. For human consumption, a maximum content of 20 mg/kg of DA was established in the commission decision 2002/226/EC (OJ L 75, 16.3.2002, p. 65.). The techniques most extensively used are based on liquid chromatography; however, in recent years rapid detection methods are gaining popularity and importance in the field of food control because they are fast, cost-effective and time-saving compared to conventional methods. Nowadays they are widely used as screening tools as excellent complement to confirmatory methods.

In this study an Enzyme-linked immuno-flow assay (ELIFA), based on a competitive format, for the detection of DA in shellfish is presented. The immunoreagents used were a conjugated, consisting of a carrier protein linked to DA through a spacer arm, and a monoclonal specific antibody conjugated to horseradish peroxidase (Mab-HRP). The assay procedure was as follows: the conjugated was immobilized on Immobilon P membrane, the remaining binding sites were blocked with BSA 1% (w/v), subsequently Mab-HRP was spread and after a washing step the enzyme-substrate (TMB) was added producing a blue colour. To optimize parameters such as immunoreagent concentrations and assay time a checkerboard titration was conducted to visualize the best signal-to-noise ratio. The reaction between DA and Mab-HRP was established in 5 min in the dark. Once the working conditions were fixed competitive assays were performed over the 0-5000 ng/mL DA concentration range. A gradual variation in the blue colour intensity in the concentration range tested was appreciated. The visual detection limit, calculated as IC90, was 25 ppb. Possible matrix effects in the assay colour development were evaluated by performing competitive assays with mussel extract spiked with DA in the 0-500 ng/ml range. Colour developed was visually different accordingly to the spike concentration level.

The proposed flow immunoassay being simple, fast and capable to measure DA at regulatory levels could be used as screening tool for food control purposes.

F-5
A MULTI-ENZYME BIOSENSOR FOR DETECTION OF OKADAIC ACID

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Okadaic acid (OA) belongs to a group of liposoluble toxins produced by dinoflagellates belonging to the genera Dynophysis and Prorocentrum. Shellfish accumulating these toxic algae can cause diarrhoeic shellfish poisoning (DSP) in humans. In Italy Yasumoto's mouse bioassay is the current official method for DSP toxins in molluscs. Its major problems are variability and lack of specificity; for instance, it does not differentiate the various DSP toxins (okadaic acid and its derivative DTXs, pectenotoxins, yessotoxins).

We propose an electrochemical biosensor system for the detection of okadaic acid. It employs multiple enzymes, including PP2A, a serine/threonine protein phosphatase which dephosphorylates the α-subunit of phosphorylase kinase and also has a significant activity towards glycogen phosphorylase a (PHOS a). Importantly, PP2A is also inhibited by okadaic acid. Due to this combination of properties, PP2A was employed to develop an assay system involving a preliminary regime of enzymatic incubations followed by electrochemical measurement using a by-enzyme-biosensor for H_2O_2 inserted into a FIA system.

The enzymatic reactions involved are the following:

1. glycogen (n glucose units) + Pi --- PHOS *a* -- > glucose 1-P + glycogen (n-1 glucose units)

2. glucose 1-P ---AP --> glucose + Pi

3. glucose + O_2 ----GOD--- > gluconic acid + H_2O_2

Alkaline phosphatase (AP) and glucose oxidase (GOD) were immobilised onto the surface of this H_2O_2 probe, while incubations were performed in a micro-tube. After interaction between combinations of enzymes and substrates, a few µL were withdraw, mixed with carrier buffer to quench, and this mixture was then injected into the FIA system. In a control incubation, the glucose 1-P produced by reaction 1 above leads to an increased current signal from the probe. With the addition of an optimised concentration of PP2A to the micro-tube incubation scheme, glycogen phosphorylase a was converted into inactive glycogen phosphorylase b, thus strongly reducing the current signal. However, in the presence of the target toxin (OA), the PP2A was inhibited (to different degrees depending on toxin concentration) and higher residual levels of glycogen phosphorylase a activity led to a restoration of the current signal, proportional to the toxin concentration.

Preliminarily, all analytical parameters, such as concentration and pH of the buffer, enzymes immobilisation procedure, glycogen and phosphate concentrations, amount of glycogen phosphorylase a and PP2A, incubation times, were optimised. In a subsequent phase, different concentrations of OA were included in the incubation involving PP2A, prior to quenching and injection. The calibration of the system showed a working range for detection of the toxin between 30 and 500 ppt.

F-6
FAST AND RELIABLE PATULIN QUANTIFICATION BY LC-UV-MS/MS

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The mycotoxin patulin (4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one) is produced by fungi belonging to several genera, including *Penicillium*, *Aspergillus*, and *Byssochylamys* species. It can occur in many mouldy fruit, vegetables, grains and other foods but the major sources of contamination are apples and apple products. Owing to its toxicity and concerns for human health, authorities in many countries regulate patulin in food at levels ranging between 10 and 50 µg/Kg.

The contamination of fruits with patulin has not lonely caused health hazards but also resulted in economic losses. Due to this, there is increasing interest in developing methods for its elimination in foodstuffs. After the application of any decontamination process, a quantitative method is necessary to test its efficacy. In this work, a fast and reliable method by means of LC-UV-MS/MS (APCI in positive mode) method has been developed and validated.

Described method involves an Atlantis C18 analytical column (150mm × 2.1 mm, 5µ ID) operated at a flow rate of 0.4 mL/min at 30ºC. To avoid degradation products induced by UV light gradient method was run in 13 min using as mobile phase at the start 95% solvent A (water 0.1% formic acid) and 5% solvent B (95% methanol-0.1% formic acid). The final determination of patulin was performed by UV detection at 275 nm and multiple reaction monitoring of the product ions (m/z 137 and 127) resulting from collision induced dissociation of the protonated molecular ion (m/z 155) of patulin.

The method is sensitive with a limit of detection below 2 µg/L for patulin and limit of quantisation of 6 µg/L and 3 µg/L for UV and MSMS detection, respectively. Good linearity (r^2: 0.999) was observed for both kinds of detection over a linear range including the maximum admissible values in foodstuffs. All the validation data, such as accuracy, precision, and interday repeatability, were within the required limits. No interferences from the patulin degradation products were observed for both kinds of detections.

This quantitative method has been used in this work to test the efficacy of a new technology involving UV light in the degradation of patulin in aqueous solution. No interferences from patulin degradation products were observed in UV or MSMS quantitation by using the gradient method proposed here.

F-7
NOVEL LC METHOD FOR THE SEPARATION OF MARINE LIPOPHILIC TOXINS

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Several phytoplankton species can produce marine biotoxins which can accumulate in filter feeding bivalves such as mussels, scallops and oysters. When consuming the contaminated shellfish, depending on the type of marine biotoxin and the concentration, several severe intoxications can occur. Generally, the marine biotoxins are classified by the adverse effects they are causing. One of the intoxication syndromes is caused by the Diarrhetic Shellfish Poisoning (DSP) toxins, which causes gastrointestinal disorders. The most prominent members of the group of DSP toxins are okadaic acid (OA) and derivatives of this toxin; dinophysistoxin-1 (DTX1), DTX2 and their ester derivatives (DTX3). OA and derivatives are often coexisting with pectenotoxins (PTXs), azaspiracids (AZAs) and yessotoxins (YTXs). The DSP and coexisting toxins are so called marine lipophilic toxins. PTXs have shown to be hepatotoxic and mildly diarrhetic, while YTXs have an adverse effect on the cardiac muscle cells and on defattening of liver cells. The European Union has established legislation (2002/225/EG) on 12 lipophilic marine toxins. Some other marine lipophilic toxins, such as spirolides (SPXs) and gymnodimine (GYM), are members of the spiroimine group which are classified as “fast-acting toxins” they produce neurotoxic symptoms when administrated orally or injected intraperitoneally in mice.

In recent years much effort has been put in the development of LC-MS/MS methods that are capable of the detection of the class of DSP toxins. Most of these methods use a gradient of water/acetonitrile containing ammonium formate at acidic pH. With this mobile phase system the chromatographic separation of some of the 12 marine lipophilic toxins under legislation is problematic. YTX and its derivatives may elute as very broad peaks with a full-width-half-maximum of up to several minutes. Furthermore, DTX2 and PTX2, which are analyzed in negative and positive ionization mode, respectively, are co-eluting. When the mass spectrometer is not capable of fast ionization mode switching during analysis, the samples need to be analyzed in two separate runs.

In the newly developed LC method the DSP toxins are separated with an acetonitrile/water gradient containing 0.05 v/v% ammonia at pH 11. A Waters X-Bridge® C_{18} LC column is used as this column is very stable at high pH. Special attention was paid to the stability of the marine lipophilic toxins under these conditions. No degradation or loss of analytes was observed. By using the X-bridge column in combination with a basic water/acetonitrile gradient we were able to overcome the problems of poor peak shape and the necessity of fast ionization mode switching. The YTXs elute with greatly improved peak shapes and limit-of-detections (LODs). The toxins elute in such order that no overlap between positive and negative ESI occurs. With this new LC method 16 marine lipophilic toxins, including all 12 toxins stated in the EU legislation can be analyzed in a single analysis and with LODs that are sufficient for routine monitoring purposes.

F-8

USE OF A SURFACE PLASMON RESONANCE-BASED BIOSENSOR TO DETECT AND QUANTIFY YESSOTOXIN

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Yessotoxin (YTX) is a lipophilic polyether toxin originally isolated from the japanese scallop Patinopecten yessoensis. It was initially included in the diarrhetic shellfish poisoning (DSP) group although subsequently it has been reported that its chemistry and toxicology differ distinctly from the DSP family. In fact there is no clear evidence that YTX is toxic to humans, however it is a major cause of false positives in DSP toxin detection by mouse bioassay. We developed a new detection and quantification method for yessotoxin using a BiaCORE X Surface plasmon resonance (SPR)-based biosensor. The assay is based in the interaction of YTX with phosphodiesterase enzymes (PDE), one of its cellular targets. Phosphodiesterases from two different sources, PDE I from Crotalus atrox and PDE from bovine brain, were immobilized on a CM5 chip using EDC/NHS chemistry and compared for YTX binding properties. PDE I chip was more sensitive at low concentrations of YTX. The injection of several YTX concentrations showed a dose dependent binding signal, which Kobs (observed rate constant) allowed us to obtain a calibration curve with a linear fit, y = 0.00546x - 0.01248, R = 0.9669, for n = 4 experiments. The detection of yessotoxin with an SPR-based biosensor allows the quantification of the toxin with an automated and repetitive method at concentrations in the range of the 1 mg/kg European regulatory limit.

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KEY WORDS: SPR, surface plasmon resonance, phycotoxin, YTX, yessotoxin, phosphodiesterase, biosensor, diarrhetic shellfish poisoning, DSP.

F-9

DETECTION OF G-ACTIN BINDING MOLECULES USING A SURFACE PLASMON RESONANCE-BASED BIOSENSOR

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Actin, one of the most abundant and conserved structural proteins of eukaryotic cells, is a major determinant of cell shape, division, motility and adhesion. Actin can be found in two forms, G-actin, the globular form, and F-actin, the microfilament of G-actin monomers connected together. A large number of molecules are known to interact with G-actin and/or F-actin. G-actin binding molecules vary from mammalian proteins and peptides with a physiological role, such as DNAse I and β-thymosin, to natural toxins such as cytochalasin D, Latrunculin A, Misakinolide A and pectenotoxins, some of them being found as contaminants in food or water. A Surface Plasmon Resonance (SPR) based-Biosensor (BiaCore X), that allows the recording of direct interactions between two molecules in real time, was used to develop a method for the detection of G-actin binding compounds. G-actin covalently linked to biotin was bound to a CM5 chip surface previously activated with streptavidin. This surface showed binding activity to latrunculin A and DNAse I in several buffers, with enough sensitivity for detection of latrunculin A at concentrations ranging from 10 to 50 μM. Since latrunculin A is a small molecule and SPR-biosensors are sensitive to changes in mass concentration at the biospecific interface, these results suggest that the biotin-actin surface would be useful for detection and quantification of G-actin binding molecules with a higher molecular weight than 400 daltons, among them pectenotoxins, marine toxins originally associated with Diarrhetic Shellfish Poisoning (DSP).

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Key words: SPR, surface plasmon resonance, phycotoxin, pectenotoxin, latrunculin A, actin, actin binding molecules, biosensor, diarrhetic shellfish poisoning, DSP.

F-10

EFFICIENCY OF CHOSEN METHODS FOR WAVELENGTH SELECTION IN IMPROVEMENT OF MULTIVARIATE MODELS FOR SEVERITY OF FUSARIOSIS IN GROUND WHEAT BASED ON DIFFUSE REFLECTANCE SPECTRA

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Fusariosis is a grain disease caused by fungi *Fusarium* spp. invading crops during their growth in the field. Diseased grain has adversely changed compositional and technological properties that result in deterioration of quality both grain, grain based products and livestock feed. Moreover, developing fungi often cause contamination of grain with substances harmful for human and animal health known as mycotoxins. For these reasons considerable efforts have been made with the aim of development of the methods that could be useful in detection of fusariosis in cereal grain. Some of the methods are based on optical measurements followed by development of relevant calibration models. Errors of calibration models are a key question in such approaches, as their magnitude decides on potential usability of the approach. Our previous papers showed that most promising PLS1 models for the fine fraction of ground winter wheat (particle size less than 0.180 mm) were the models based on spectra recorded within the ranges that included the ultraviolet region. The present contribution aims to evaluate how much different methods developed for wavelength selection in PLS modelling can improve models based on diffuse reflectance optical measurements made within the UV and VIS spectral ranges.

Two categories of the material were used in measurements, i.e. control and damaged grain obtained from crop growing in natural conditions or from crop inoculated with *Fusarium culmorum* conidia during flowering stage, respectively. After harvesting, grain from both categories was dried in air, then ground and fractioned. Fractions of control and damaged material of particle size less than 0.18mm were blended in different proportions ranging from 0 percent (pure control) to 100 percent (completely damaged). This procedure was repeated for grain of five winter wheat cultivars. As a result, five series of blended samples, with number of samples within series ranging from 18 to 32, were at hand and optical measurements performed. Diffuse reflectance spectra in log(1/R) mode were recorded with Cary 5000 (Varian) spectrophotometer within spectral range from 200 to 800 nm, every 2 nm.

Sets of spectra were used for development PLS1 calibration models for particular wheat cultivars and obtained results were a reference values for the comparison of the results obtained when chosen approaches for wavelength selection were involved into modelling. From the variety of approaches for wavelength selection developed for use in PLS the most popular and most easy for implementation seem to be the Interval Partial Least Squares (IPLS) and Uninformative Variables Selection (UVE-PLS). Moreover, Genetic Algorithm (GA) was also applied to select the most informative variables.

The obtained results showed no considerable differences in efficiency of particular methods for wavelength selection. Typically, they resulted in lowering of RMSECV (root mean square error of cross-validation) of PLS1 models by ca. 10 percent of the value that could be obtained by application of ordinary PLS1 modelling. In some cases, however, lowering of the errors by even 40 percent was possible.

F-11

EFFECT OF PARTICLE SIZE AND SPECTRAL RANGE ON PERFORMANCE OF MULTIVARIATE CALIBRATION MODELS FOR SEVERITY OF FUSARIOSIS IN GROUND WHEAT BASED ON DIFFUSE REFLECTANCE SPECTRA

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Fusariosis is a grain disease caused by fungi *Fusarium* spp. invading crops during their growth in the field. Diseased grain has adversely changed compositional and technological properties that result in deterioration of quality both grain, grain based products and livestock feed. Moreover, secondary metabolites produced by developing fungi often result in contamination of grain with substances harmful for human and animal health known as mycotoxins. For these reasons considerable efforts have been made with the aim of development of the methods that could be useful in detection of fusariosis in cereal grain. Some of the methods are based on optical measurements followed by development of relevant calibration models. Errors of calibration models are a key question in such approaches as they determine ability of the method to detect fusariosis. Our previous papers showed that most promising PLS1 models for the fine fraction (particle size less than 0.180 mm) of ground winter wheat were the models based on spectra recorded within the UV range, or when the measurement covered additionally the VIS range. The present contribution aims to check whether that finding holds their validity when other fractions of ground wheat are used for diffuse reflectance measurements.

Two categories of the material were used in measurements, i.e. control and damaged grain obtained from crop growing in natural conditions or from crop inoculated with *Fusarium culmorum* conidia during flowering stage, respectively. After harvesting grain from both categories was dried in air, then ground and fractioned. Three fractions of particle size were sieved: fine (<0.180mm), medium (>0.180 and <0.355mm) and coarse (>0.355 and <0.710mm). Within the fractions control and damaged material was blended in different proportions ranging from 0 weight percent (pure control) to 100 percent (completely damaged). As a result three series of blended samples of different fractions were obtained. This procedure was repeated for grain of five winter wheat cultivars. Diffuse reflectance spectra in log (1/R) mode were recorded with Cary 5000 (Varian) spectrophotometer within spectral range from 200 to 2500 nm, every 2nm. PLS1 models were built on differentiated and centred spectra within the UV, VIS and NIR spectral ranges, and within all possible combinations of these ranges. For all wheat cultivars best models within particular cultivars were the models obtained for fine fraction and spectral range UV-VIS. Models for medium fraction were usually of better performance than for coarse, however much worse than for fine.

In conclusion, the most useful approach for detecting fusariosis in wheat grain with diffuse reflectance spectroscopy seems be to use fine fraction of ground material and to perform measurements in the UV-VIS spectral range.

F-12

ASSESSMENT OF APPLICABILITY OF THE MEASUREMENTS OF SINGLE KERNEL PHYSICAL FEATURES TO DISCRIMINATION AMONG HEALTHY AND DAMAGED WITH FUSARIOSIS GRAIN OF WINTER WHEAT

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Fusariosis is a grain disease caused by fungi *Fusarium* spp. invading crops during their growth in the field. Diseased grain has adversely changed compositional and technological properties that result in deterioration of quality both grain, grain products and livestock feed. Moreover, developing fungi often cause contamination of grain with substances harmful for human and animal health known as mycotoxins. For these reasons considerable efforts have been made with the aim of development of the methods that could be useful in detection of fusariosis in cereal grain. Most popular seem to be methods fully, or in part, based on optical measurements. However papers reporting efforts on applications of measurements of non-optical properties of healthy and damaged wheat grain were published as well. The present contribution reports results of the measurements of basic physical features (single kernel mass, volume, density, moisture, diameter and hardness) made on control and damaged batches of grain of five winter wheat cultivars, as well as the results of the analysis aimed to search for a set of features most useful in distinguishing between healthy (control) and damaged grain. Random samples of ca. 300 single kernels were taken from each batch of grain and the measurements of the mentioned above features performed with SKCS 4100 (Perten Instruments) single kernel characterization system and with a home made air micropycnometer. Mean values and standard deviations were calculated for each feature and sample, thus giving a set of original variables characterizing both five wheat cultivars and categories within each cultivar (control versus damaged) of grain. This set was subject to further analysis using Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). Values of discriminant function were used for evaluation of the ability of LDA models to discriminate between categories of grain. The measure of the ability was defined as the ratio of the distance between categories to standard deviation within categories (both calculated in values of discriminant function). In order to assess stability of the model based on given set of variables, all combinations of four from five cultivars were taken to develop model and the left out sample was used to test the model. Resulted changes in model parameters allowed to calculate mean ability for discrimination and its standard deviation, the latter being a measure of stability. This procedure was repeated for different subsets taken from the full set of original (measured) variables. In addition, scaled dimensionless variables based on original ones were also introduced and analysed with the same methods.

All obtained results showed: (i) possibility to discriminate among two categories of grain, (ii) there were several different sets of features leading to models of similar ability to discrimination, (iii) several single features (e.g. moisture) had special influence on LDA results.

F-13

OCHRATOXIN A IN ROASTED COFFEE PURCHASED IN FRENCH SUPERMARKET- TRANSFER IN COFFEE BEVERAGE: COMPARISON OF DIFFERENT METHODS OF ANALYSIS

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Ochratoxin A (OTA) produced by several fungi, notably *Aspergillus ochraceus*, could be found in grain of coffee. This mycotoxin is very stable and is not destroyed by roasting. The aim of this study was to evaluate the presence of OTA in roasted coffee and the transfer of OTA in the beverage. Thirty roasted coffee were purchased in French supermarket as would be done by a consumer. OTA content was evaluated by three different methods: two using IAC clean-up and alkalinisation [CEN 14132; Pittet et al, 1996] and the later using toluene extraction in acidic conditions [CEN 15141]. The major difference between the two IAC methods was the amount of Phosphate buffer saline (PBS) medium added to the extract before IAC. OTA recoveries ranged from 16% to 49% with 'CEN 14132' method (alkaline condition) depending on the concentration of OTA (0.5 to 5 µg/kg). The recovery with the 'CEN 15141' method (acidic condition) was of 55-60% whatever the concentration. The recoveries were better with Pittet method (75%) when the concentration was over 1 µg/kg. Recoveries of OTA from beverage were similar with all methods (75-80%).

All samples contain OTA ranging from trace (< LOQ, 5 samples) to 11.9 µg/kg. Nine samples were over 1.5 µg/kg. Some samples contain also OTB (dechlorinated OTA). The amount of OTA passing in the beverage ranged between 20- 140%. Higher recovery of OTA in beverage as expected is due to two types of interference (i) presence of OTB which cross-reacts with OTA-antibodies (ii) alkalinisation of OTA converted its in open ring OTA (OP-OA) which is no more recognized by antibodies. We observed also that more the coffee was roasted, more isomerization of OTA occurred. This isomer again is not recognised by antibodies. These analytical problems will have serious impact on the level of mycotoxin detected, especially at the levels close to those from the EU legislation. Underestimation could be highly dangerous for health

Consumption of 300 ml of the beverage made with the coffee containing the highest amount of OTA (11.9 µg/kg) correspond to an intake of 250 ng (83% of TDI for an individual weighing 60 kg). This indicates that OTA intake via coffee for a high consumer could be very important.

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F-14

OCCURRENCE OF FUMONISINS, ZEARALENONE, OCHRATOXIN AND CITRININ IN BREAKFAST CEREAL

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Crops may be simultaneously contaminated by several mycotoxins which can persist in the final products. Some species of mould genera (i.e. *Fusarium)* invade crops in the field; other (i.e. *Aspergillus, Penicillium)* may grow on food during storage under favourable conditions of temperature and humidity. These fungi may produce several mycotoxins such ochratoxin A (OTA), citrinin (CIT), Aflatoxin (AF), fumonisin (FB), zearalenone (ZEA), which are stable during the food processing. The aim of this study was to evaluate the simultaneous presence of OTA, CIT, AFB, FB, ZEA in breakfast purchased in French supermarket in 2007. OTA, CIT, AFB, ZEA were extracted under acidic conditions by acetonitril/chlorform, as described previously by our laboratory. FB was purified by immunoaffinity column (IAC). Two methods of extraction of ZEA have been compared (extraction under acidic conditions used for OTA, CIT, AF *versus* IAC method). Presence of mycotoxins has been confirmed by LC/ms/ms.

Thirty samples were purchased in French retails. All samples contain at least one mycotoxin. None contain AF. Two third contained OTA (ranging from 0.03 – 0.175 µg/kg) or FB1 (ranging from 29 – 160 µg/kg); one third contained CIT (0.08 – 1.8 µg/kg) or ZEA (3-22 µg/kg). Samples containing OTA were also contaminated either by CIT and/or FB. Samples containing ZEA were also contaminated by FB. Interestingly, FB and ZEA could be found in samples containing only rice product, confirming previous data indicating that maize in not the sole substrate for these toxins. The amounts of OTA and CIT were considerably lower than that found four years ago, far below the EU legislation. In contrast, the amount of FB was in the same range, even slightly higher. Trichothecenes were found only as trace (< 10 µg/kg), indicating that they are probably destroyed during food processing.

The simultaneous presence of these toxins is particularly important in regard to possible synergism and additive effects of these mycotoxins. Thus this may increase health problem. This should be taken into consideration as claimed by the European community (Scientific committee on Food opinion on Ochratoxin A. CS/CNTM/MTC/14 Final annex II to document XXI/2210/98, Brussels: CEC, 1998).

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F-15

HPLC DETERMINATION OF OCHRATOXIN A IN WINE AND EVALUATION OF INTAKE BY ITALIAN POPULATION

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A total of 1166 wine samples (290 white, 28 dessert, 75 rosé, and 773 red wines), mostly produced in 1988-2004, collected in 19 different Italian regions, was analyzed for ochratoxin A (OTA) content by HPLC-FL and immunoaffinity cleanup. The aim of this study was also to monitor the status of contamination of Italian wines and to evaluate the exposure of the Italian population to OTA. The obtained results are reported by year of harvest, geographical area of production, and type of wine. Red wine showed the highest levels of contamination, even if the dessert wines were characterized by an higher mean value. A gradual increasing concentration from north to south Italy was also observed. Exposure calculations performed using two different consumption databases, showed a daily intake for total population and consumers only of 0.40±0.69 and 0.72±0.79 ng/kg b.w. respectively. The obtained exposure values indicate that wine contribution does not represent a risk for the Italian population, especially taken into consideration the overall OTA dietary exposure.

F-16

IMMUNOAFFINITY COLUMN CLEANUP WITH LIQUID CHROMATOGRAPHY FOR DETERMINATION OF AFLATOXIN B1 IN CORN SAMPLES: INTERLABORATORY STUDY

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An interlaboratory study was conducted to evaluate the effectiveness of an immunoaffinity column cleanup liquid chromatography (LC) method for the determination of aflatoxin B_1 levels in corn samples, enforced by European Union legislation. A test portion was extracted with methanol-water (80 + 20); the extract was filtered, diluted with phosphate-buffered saline solution, filtered on a microfiber glass filter, and applied to an immunoaffinity column. The column was washed with deionized water to remove interfering compounds, and the purified aflatoxin B_1 was eluted with methanol. Aflatoxin B_1 was separated and determined by reversed-phase LC with fluorescence detection after either pre- or postcolumn derivatization. Precolumn derivatization was achieved by generating the trifluoroacetic acid derivative, used by 8 laboratories. The postcolumn derivatization was achieved either with pyridinium hydrobromide perbromide, used by 16 laboratories, or with an electrochemical cell by the addition of bromide to the mobile phase, used by 5 laboratories. The derivatization techniques used were not significantly different when compared by the Student's t-test; the method was statistically evaluated for all the laboratories. Five corn sample materials, both spiked and naturally contaminated, were sent to 29 laboratories (22 Italian and 7 European). Test portions were spiked with aflatoxin B_1 at levels of 2.00 and 5.00 ng/g. The mean values for recovery were 82% for the low level and 84% for the high contamination level. Based on results for spiked samples (blind pairs at 2 levels) as well as naturally contaminated samples (blind pairs at 3 levels), the values for relative standard deviation for repeatability (RSDr) ranged from 9.9 to 28.7%. The values for relative standard deviation for reproducibility (RSDR) ranged from 18.6 to 36.8%. The method demonstrated acceptable within- and between-laboratory precision for this matrix, as evidenced by the HORRAT values.

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F-17

OPTIMIZATION OF ELISA METHOD FOR DETERMINATION OF BARLEY PROLAMINS

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The prolamin protein fraction from wheat, barley and rye can cause some toxic effects when ingested by people with coeliac disease. The only treatment of coeliac sprue is still the adherence of strict diet free of toxic cereal proteins even in trace amounts. It is one of the most frequent food allergies occurring presumably in about one in 300 people in Europe. There is still a need for group of analytical methods with acceptable reliability and specifity in food control and food analysis of gluten i.e. prolamins.

Electrophoretic and chromatographic analytical methods are widely used for fractionation of the complex mixture of hordeins. For very sensitive and specific determination of hordein and hordein derivates immunochemical techniques are being involved. Recently a numerous of ELISA systems have been produced, mainly for the purpose of wheat gliadin detection. These systems are usually based on monoclonal antibodies.

Monoclonal antibodies (MAb) against hordein were prepared by immunisation of mice with the ethanol extract of barley proteins. The interactions between MAb and prolamins of different cereals (wheat, barley, rye, corn, rice, buckwheat) and ethanol soluble proteins from beer and malts were studied. Because of strong heterogeneity of homologous and heterologous antigen there is no suitable quantification of particular cross reactions. The cross reactivity within the group of gliadin, hordein and secalin is mainly equal due to high level of structural similarity.

An indirect competitive ELISA method for detection of barley prolamins was developed and working concentrations of immunoreagents were optimized. Detection limit of this method was assessed to 2 ppm of hordein, while linear range of concentrations for calibration curve was 1-100 ppm. Considering the choice of format for immunoanalytical method, competitive scheme seems to be more appropriate for analysis of sample with higher content of hydrolyzed or degraded proteins and peptides.

F-18

STRUCTURE-ACTIVITY RELATIONSHIPS FOR THE FLUORESCENCE OF OCHRATOXIN A: INSIGHT FOR DETECTION OF OCHRATOXIN A METABOLITES

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Ochratoxin A (OTA) is a *para*-chlorophenolic mycotoxin produced by strains of *Aspergillus* and *Penicillium* that is widely found as a contaminant of improperly stored food products. The toxin is a potent renal carcinogen in rats, especially male, and has an implicated role in the etiology of Balkan endemic nephropathy (BEN) and its associated urinary tract tumours. Although the mechanism of OTA-mediated tumour formation is not fully understood, and represents a hotly debated topic, OTA metabolism may play a critical role in OTA-mediated carcinogenesis. For OTA detection, liquid chromatography (LC) coupled with fluorescence detection (FD) is the most widely used analytical method because it is more sensitive than mass spectrometry (MS), easier to operate and lower in cost. The fluorescence spectrum of OTA peaks at 460-480 nm and exhibits no overlap with the absorption spectrum at 332 nm. The energy difference between the absorption and emission maxima (Stokes shift) of OTA is ~ 9000-10000 cm^{-1} and suggests that OTA undergoes an excited-state intramolecular proton transfer (ESIPT) process to generate the red shifted emission spectrum. The ESIPT process generates a tautomer of OTA that is highly emissive and fluorescence quantum yields are ~ 0.4. In this presentation the nature of OTA fluorescence and impact of structural modification on fluorescence maxima and intensity is addressed. Our findings show that replacement of the C5-chlorine atom of OTA with alternative substituents can cause significant variation in emission maxima, while groups that inhibit ESIPT also cause a marked decrease in fluorescence intensity. This new insight permitted the use of LC/FD for the detection of the hydroquinone metabolite (OTHQ) of OTA in the urine of rat fed OTA. The OTHQ metabolite contains an electron-donating OH group in place of the C5-chlorine atom of OTA and gives emission maxima at 510 nm; a red shift of ~ 40 nm compared to OTA. Our results highlight the advantages and limitations of LC/FD for the detection of OTA metabolites in biological fluids.

F-19

PRODUCTION AND EVALUATION OF BINDERS TO LIPOPHILIC PHYCOTOXINS

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Marine biotoxin contamination is the main factor which determines the safety of shellfish. Lipophilic phycotoxins, including the diarrheic shellfish toxins (DSP) okadaic acid and dinophysistoxin, yessotoxins (YTX), pectenotoxins (PTX), azaspiracids (AZA) and gymnodimine (GYM), are often found in high levels in contaminated seafood and may be a significant threat to human health if consumed. It is therefore necessary to monitor levels of these toxins in seafood. In Europe, the broad spectrum mouse or rat bioassay is the only official and the most widely used methodology for DSP detection but issues regarding lack of sensitivity and specificity, inability to distinguish between different toxin groups and animal welfare concerns have dictated that alternative methods of toxin detection are investigated.

The primary aim of this study is to develop a multichannel, high-throughput surface plasmon resonance based biosensor for the detection of lipophilic phycotoxins in shellfish residues. The biosensor exploits the phenomenon of surface plasmon resonance (SPR) to detect and measure the toxins. This would have the potential not only to eliminate the need for the ethically concerning bioassays currently used, but also to provide a simple, cheap and improved method for the detection of DSP reducing the damaging impact of phycotoxins to human health and the associated economic consequences to the shellfish industry.

Measuring the concentration of each toxin relies on a specific interaction between the toxin and a defined detecting molecule such as an antibody. The production of antibodies to each of the toxins is therefore a priority so they can be used as binding molecules in an assay. The DSP toxin molecules are small and difficult to measure directly with high precision at low concentrations by SPR so the assays must be developed as inhibition assays with the toxin immobilised onto the surface of a sensor chip. The antibody in excess concentration binds to the toxin on the chip surface and a standard curve is prepared from solutions of known toxin concentration. The concentration of toxin in an unknown sample is then derived from this standard curve. The production and evaluation of binders to lipophilic phycotoxins and subsequent assay development are described.

Keywords: Biosensors; Diarrhetic Shellfish Toxins; Azaspiracid; Gymnodimine; Yessotoxin; Pectenotoxin; Monoclonal and Polyclonal antibody production; Assay development

F-20

ADVANCES IN UTILISATION OF OPTICAL BIOSENSOR TECHNOLOGY TO DETECT A BROAD RANGE OF PARALYTIC SHELLFISH POISONS IN SHELLFISH EXTRACTS

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As part of the European Union Sixth work Programme, a major project, BIOCOP (www.Biocop.org) commenced in 2005. An important work package within this project is dedicated to the development of a biosensor based screening assay for the detection of paralytic shellfish poisons (PSPs) in shellfish. Saxitoxin is the parent PSP toxin however there are at least 21 structurally distinct analogues all with different toxic potencies. PSP monitoring in shellfish is currently performed using a mouse bioassay. This method is utilised in food safety laboratories throughout the world as these toxins can cause serious illness or even death if consumed but it can suffer from poor precision, lack sensitivity and there is a growing global consensus that this method is ethically unacceptable.

A surface plasmon resonance (SPR) optical biosensor, (Biacore Q®) was used as the detection technology in the present study. A feasibility study to modify the binding activity of these proteins in relation of PSP toxins was undertaken. In addition the effects on alterations to sample preparation techniques and assay conditions were explored to determine if improvements in the generic nature of the procedure could be found.

Data will be presented to demonstrate that a biosensor assay has been developed which has the capability of detecting a very broad spectrum of PSP toxins.

F-21

STUDIES OF FUNGAL METABOLITE PRODUCTION ON INOCULATED FIGS USING LC/TOF-MS

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Two toxigenic fungi, *A.flavus* (200-138) and *A.parasiticus* (2999-465) from reference collections were grown on Gamma radiation sterilised figs, for 7 – 20 days. Using a previously validated method involving direct extraction without clean-up and subsequent analysis by LC/TOF-MS the production of secondary metabolites were studied. On a daily basis levels of aflatoxin B_1, kojic acid and sterigmatocystins were monitored over 20 days. Levels of aflatoxin B_1, aflatoxin B_2 and kojic acid maximised at 5-6 days whilst levels of 5-methoxysterigmatocystin remained relatively constant. The pH values of the incubated figs were also monitored over the period of fungal growth. .In addition to the known metabolites expected to be produced by these fungi, aspergillic acid, aflatoxin G_1, aflatoxin G_2, gallic acid and tryptophan were also identified. The use of LC/TOF-MS has been demonstrated to be a rapid, and simple technique which has enabled time related measurements to be made which are not readily achievable by conventional analysis. This study indicates the potential of LC/TOF-MS for routine rapid screening for mycotoxins and other metabolites in dried fig samples which clearly can be extended for use as a tool for mycotoxin surveillance.

F-22

USE OF FULLY STABLE ISOTOPE LABELED MYCOTOXINS AS INTERNAL STANDARDS FOR MYCOTOXIN ANALYSIS WITH LC-MS

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The mass spectrometric detection of mycotoxins in analytical chromatography is a very powerful tool. Nowadays so called multitoxin analysis allows to determine more than 100 toxins in one run. Nevertheless interferences from matrix components lead to a different ionization of the analyte in a sample in comparison of pure standard calibrants and hence results in different signal intensity. This matrix effect limits the methods using a mass spectrometer as detector for liquid chromatography. Such a problem can be overcome by adding an internal standard (IS) to the sample, which behaves similar to the analyte and therefore can both correct recovery losses during the sample preparation process and ion suppression effects in the MS source. Stable isotope-labeled analogs of natural T-2 toxin provide the best IS for T-2 toxin when accurately quantifying it using MS based methods. However, it should be noted that deuterated compounds still run the risk of H/D exchange in protic solvents and retention time shifts relative to the natural toxin. Moreover, partially labeled toxins frequently contain considerable amounts of “lighter” isomers, leading to mass peaks that interfere with natural toxin isotopes. Therefore, fully ^{13}C-substituted compounds can be regarded as the best standard for quantification by LC–MS- and GC–MS-based methods.

Thus we focus our work on this topic and prepared fully isotope labeled analogues of the most important mycotoxins which contaminate cereals: $^{13}C_{15}$-deoxynivalenol, $^{13}C_{17}$-acetyldeoxynivalenol, $^{13}C_{24}$-T-2 toxin, $^{13}C_{22}$-HT-2 toxin, $^{13}C_{34}$-fumonisin B1, B2 and B3, $^{13}C_{18}$-zearalenone and $^{13}C_{20}$-ochratoxin A for the use as internal standards. The produced labeled mycotoxins were fully characterized and the usability was verified by developing stable isotope dilution LC-MS methods for a quantification of these toxins in cereals. Results showed that an isotope labeled IS can successfully correct fluctuations that may occur during extraction, clean-up, separation and ionization of the sample. It could be demonstrated that by applying an isotope labeled IS extensive clean-up steps are not necessary for obtaining accurate results by LC-MS.

F-23

FUSARIUM TOXINS AND CONJUGATED DEOXYNIVALENOL IN "HEALTH" WHOLEMEAL FLOUR AND BREAD

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Wholemeal bread is a staple food in many cultures worldwide. This report is the first that shows the occurrence of not only "free" but also "masked" *Fusarium* mycotoxins, mainly deoxynivalenol (DON) and its conjugate, deoxynivalenol-3-glucoside (DON-3-Glc), in fermented bread prepared from wholemeal flour, water, salt, yeasts and sucrose. High performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was validated for determination of these toxic secondary fungal metabolites in the raw materials (both naturally as well as artificially *Fusarium* infected wheat grains), all intermediates obtained during milling process of these wheat samples, wholemeal flours and final bakery products (wholemeal breads).

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F-24

ISOLATION OF SINGLE-CHAIN FV ANTIBODY FRAGMENTS (SCFVS) AGAINST AFLATOXIN M1 (AFM1) FROM A SEMI SYNTHETIC PHAGE DISPLAY LIBRARY

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Aflatoxins are a group of mycotoxins mainly produced by ubiquitous fungi (Aspergillus flavus, A. parasiticus and A. nominus) that can occur in a wide range of agricultural commodities, such as cereals, nuts, spices, dried fruit and in feedstuffs. Aflatoxin M1 (AFM1) is the principal hydroxylated Aflatoxin B1 (AFB1) metabolite and is detected in milk of mammals, after consumption of feed contaminated with AFB1. It is heat stable and no reduction of toxin level has been observed after pasteurization process. AFM1 has even been found in UHT milk and in some milk derivatives (yogurt and cheese) with a 3–5 fold enrichment. As it is classified as probable human carcinogen (IARC, group 2B), to reduce AFM1 risk the EU has fixed 50 ng/kg as the maximum allowed level in milk. The isolation of synthetic antibody fragments (particularly in the single-chain Fv antibody format, scFv) from large phage-display libraries, not requiring the need to immunise animals or to use the hybridoma technology, is emerging as a powerful tool for food management. In this system, the coupling of the scFv on the surface of the phage with the gene encoding it inside the phage, allows a rapid selection of antibodies with desired binding properties (i.e. specificity, affinity) by a series of recursive cycles of phage binding, elution and amplification ('biopanning'). Soluble antibodies can be then easily derived at high yields. This system is particularly valuable when antibodies against small, non-immunogenic molecules (haptens) or highly toxic substances (like mycotoxins) have to be produced. In this work, to isolate scFv antibodies specific to AFM1, we used a phage display library previously built in our lab and proved to be composed by intrinsically stable scFvs. This repertoire contains approximately 5x 107 different clones ("F8 library"). After four rounds of 'biopanning' on the immobilised AFM1 (conjugated to bovine serum albumin), a panel of different phages clones were obtained from the library and individual clones were selected and tested for binding to the AFM1 by enzyme-linked immunosorbent assay (ELISA). Four soluble clones, showing the highest ELISA values, were chosen for sequencing and purified by immobilized metal affinity chromatography for further biochemical characterization. The antibodies obtained might potentially be used for developing a rapid and affordable immunoassay for detection of food contamination and can be applied in immunoaffinity chromatography, usually carried out prior to HPLC analysis of mycotoxin-contaminated food and feed.

F-25

DETERMINATION OF VICINE AND CONVICINE IN VICIA FABA MINOR BY NMR SPECTROSCOPY

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Vicia Faba minor is cultivated in the semiarid environment of Central and Southern Italy mainly for grain production which contains principally stark (40-45%) and proteins (25-30%). Proteins have good biological values so *Vicia Faba* minor grain represents a valid alternative to soy bean in the animal diet, but the presence of antinutritional factors like vicine and convicine restricts its utilization, mainly in monogastric species.

The objectives of the present work are: (i) to evaluate the effect of genotype on vicine and convicine content in 14 varieties of *Ficia Faba* minor; (ii) to apply Nuclear Magnetic Resonance (NMR) spectroscopy as a quick method to discriminate varieties with high content of vicina and convicina.

The quantification of glucosides vicine and convicine were conducted with HPLC following the Quemener method. ^{1}H-NMR spectra were performed, with a spectrometer NMR Bruker – 600 MHz Avance, on *Vicia Faba* minor flours extracts in deuterium oxide.

^{1}H-NMR spectra were elaborated with multivariate analysis. Analysis of variance was carried out on data from HPLC and NMR spectra. Scheffé's test was used as mean separation test for HPLC data and discriminant analysis was used to separate NMR groups.

Convicine content was low in all fourteen varieties and was statistically different only between Irena and Diva *vs*. Lady. While The vicine content was higher than 1% (on grain dry matter) in all varieties except for SR 1061, Vulcain and Lady.

The discriminant analysis was carried out on spectra variables with Fisher's F values higher than 4. In figure 1 is showed the result of discriminant analysis, we can observe that the varieties with low content of vicine (SR 1061, Vulcain and Lady) are grouped in the left side of the graph. While no clear separation occurs between varieties with content of vicine higher than 1%.

On the base of these results we can state that SR1061, Vulcain and Lady varieties have low content of vicine, while the convicine content is low in all varieties. ^{1}H-NMR technique seems to be a reliable method to discriminate varieties of *Vicia Faba* with low content of vicine from that of high content and vicine seems to be the most disciminant factor.

F-26

UPLC-TOF FOR FAST SCREENING AND QUANTITATION OF MICROCYSTINS IN WATER AND BLUE-GREEN ALGAE PRODUCTS

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Cyanobacteria, commonly called "blue-green algae", are unicellular organisms often growing in colonies or filaments. Cyanobacteria may accumulate in surface water supplies as "blooms" and may concentrate on the surface as blue-green "scums". Some species of cyanobacteria produce toxins, which are classified according to their mode of action into hepatotoxins, neurotoxins, skin irritants, and other toxins. One of the groups of toxins produced and released by cyanobacteria is called microcystin named from the species *Microcystis aeruginosa*. The microcystins are a group of cyclic heptapeptide hepatotoxins and at least 50 congeners have been identified. Microcystins are of relevance to water supplies and to blue-green algae products sold as food supplement often in tablets, capsules or powder form. The WHO has fixed a maximum residue limit of 1 μg/L for drinking water and the Swiss federal office of public health proposed for algae products a maximum daily intake of 2 μg of microcystins for adults. A common variety of blue-green algae, Spirulina, has not been found to contain toxins at harmful levels. However, other algae that are harvested to manufacture blue-green algae products may be contaminated with toxins.

A fast analysis method has been developed for the determination of microcystins LR, RR, YR, LA and Nodularine by UPLC-TOF. Based on exact mass detection of protonated ion, sodium adduct and also doubled charged ions, other microcystins (e.g. LF or LW) which are not commercially available could also be detected even if reference standard is missing. Water samples were analysed by direct injection and on-column preconcentration without any sample preparation step resulting in a total analysis time lower than 5 min with sub-ppb limits of detection. For blue-green algae products, samples were extracted with methanol/water and sonicated to break up cells and release the toxins in solvent. Extracts were centrifuged, diluted and filtered before injection on UPLC-TOF. Limits of detection below μg/g were achieved.

F-27

CO-EXTRACTION OF THE MYCOTOXINS DEOXYNIVALENOL AND ZEARALENONE FROM WHEAT BY SOL-GEL IMMUNOAFFINITY CHROMATOGRAPHY

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Mycotoxins are fungal secondary metabolites which can cause acute and chronic toxic effects in animals and humans. In Austria the most prevalent mycotoxins in grain are deoxynivalenol (DON) and zearalenone (ZON), both produced by *Fusarium graminearum* and *Fusarium culmorum*. DON is known to be harmful to both humans and animals, eliciting a wide range of symptoms of varying severity, mainly by inhibiting protein synthesis. In contrast, ZON is not very toxic but can cause estrogenic effects in humans and animals by binding to the natural estrogen receptor.

In order to reduce the risk of intoxication of humans and animals the European Commission established maximum levels for DON and ZON concentrations in food and feed. Highly selective and sensitive analytical methods have to be applied to ensure that DON and ZON concentrations comply with the legal regulations. However, traces of DON and ZON can only be determined in complex food and feed matrices after carrying out selective sample pre-treatment steps. Immunoaffinity chromatography is one of the most frequently applied clean-up procedures. Up to now, most immunoaffinity columns are prepared by covalently binding the antibodies to solid support materials. However, covalent binding is carried out under rather drastic conditions frequently affecting the affinity of the antibody for the antigen. In order to overcome this problem, our group has recently presented an alternative method to prepare DON specific immunoaffinity columns (1). In this recently published study anti-DON antibodies were physically entrapped in porous glass matrices prepared by the sol-gel technique under mild conditions.

In the present paper we report on the preparation of immunoaffinity columns by entrapping both anti-DON and anti-ZON antibodies in the pores of a sol-gel glass. In spite of the different affinity constants of the anti-DON and anti-ZON antibodies, operation conditions could be found which enabled co-extraction of DON and ZON from wheat. The columns proved to be very stable and could be repeatedly used for sample clean-up.

[1] Z. Brenn-Struckhofova, M. Cichna-Markl, C. Böhm, E. Razzazi-Fazeli. Selective sample cleanup by reusable sol-gel immunoaffinity columns for determination of deoxynivalenol in food and feed samples. Anal. Chem. 79 (2007) 710-717.

F-28

FUMONISINS IN MAIZE AND THEIR DETERMINATION BY THE ELISA AND HPLC METHODS

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The fumonisin contents in six identical corn hybrids from variety trials grown at three different localities of the Czech Republic (CR) were analysed. An immunochemical fumonisin test procedure, ELISA, with the limit of detection (LOD) for total fumonisins of 200 ppb was employed for analyses in comparison with the HPLC reference method. VERATOX (NEOGEN CORPORATION) kits were used and manufacturers guidelines were observed. By means of certified reference material, the recovery for the ELISA method used at a rate of 124 % was determined. Because the ELISA method does not discriminate between different types of fumonisins, the results were compared with the sum of HPLC estimated values for fumonisin B_1 and B_2. The correlation coefficient of 0.95 was obtained.

Wide differences between the three localities and also between individual hybrids in the harvest year concerned, 2006, were observed. The highest level of fumonisins content was found at the typical maize-producing area of the south of Moravia, for particular hybrids these values were between 1237 ppb and 21935 ppb. The same hybrids at the locality in the middle of Moravia reached only from under LOD to 2831 ppb.

The question of fusarium mycotoxins occurence in maize is even more complicated than that in wheat. Besides deoxynivalenol and zearalenone, toxic products of fusarium species such as *Fusarium graminearum* and *F. culmorum*, also fumonisins such as the metabolites of *F. moniliforme* must be taken into consideration. In addition to the factors identified in wheat (climate, previous crop, cultivation system, cultivar susceptibility), there are others - insect protection, problems of the weather at the time of harvesting or possible period between harvesting and drying.

Because of their negative health consequences, fumonisins belong among the mycotoxins involved in Commission Regulation (EC) No. 1881/2006, which sets maximum levels for certain contaminants in foodstuffs. Limits for maximum content of the sum of B_1 and B_2 in unprocessed maize to a maximum of 2000 ppb shall apply from 1 October 2007. In order to protect public health, fulfil the requirements of the legislation and the need of better knowledge of their causal factors and mutual interactions, it is necessary to have reliable, simple and, as far as possible, cheap analytical methods. The tested ELISA method proved to be suitable for such practical analyses under the proviso that care of measurement traceability by means of certified reference materials is taken, and that appropriate recovery value is determined and applied. The manufacturers leaflet recommends confirming positive results with an additional approved method prior to taking action.

F-29
OCCURENCE OF OCHRATOXIN A IN DRIED FIGS

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Ochratoxin A (OTA) is a fungal secondary metabolite produced by several species of *Aspergillus* and *Penicillium*, and can be frequently found in different foods and beverages such as cereals, coffee, dried vine fruits, cocoa, nuts, wine and etc. OTA exhibits nephrotoxic, immunosuppressive, teratogenic and carcinogenic properties and considered possible carcinogenic for human, group 2B by IARC(1993).

Dried figs are foodstuffs, which are great economical potential in internal and external markets. The temperature in Aegean Region during the ripening, harvesting and drying stage of figs are favourable for mold growth and mycotoxin formation. Dried figs contain high carbohydrate levels; this property is more favorable toxin formation than for mold growth.

In this study ochratoxin A contamination in dried figs have been investigated by HPLC with fluorescence detection. One hundred and fifteen samples were collected from fields during drying stage in Aegean Region in 2003 and 2004. Fifty five of 115 samples were found to contain ochratoxin A. Ochratoxin A contamination frequency in dried figs harvested in 2003 and 2004 are 47% and 50 %, respectively.

F-30

DETERMINATION OF A COUMARIN RESIDUES IN FOOD USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY – ELECTROSPRAY-TANDEM MASS SPECTROMETRY

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An method based on the use of ultra performance liquid chromatography – tandem mass spectrometry interfaced with electrospray (UPLC/MS/MS) was devised for the determination of a coumarin residues in food samples. Sample treatment includes accelerated solvent extraction (ASE) uses dichlormethane followed by a clean up on a gel permeation chromatography. Ultra performance liquid chromatography (UPLC) was performed on a Acquity UPLC BEH C_{18} (100 mm x 2.1 mm), the mobile phase being a water – acetonotrile isocratic (each isocratic component containing 0.1 % formic acid) at a flow rate of 0.3 ml min^{-1}. For unequivocal identification of analyte, two ions were detected and choosen for multiple reaction monitoring (MRM). Validation was carried out on spiked vanilla milk rice and licorice sweets.

F-31

METHOD FOR ANALYSIS DRIED VINE FRUITS CONTAMINATED WITH OCHRATOXIN A

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The EU maximum limit of 10 μg kg^{-1} for dried vine fruits has been in force since 2002 (European Commision, 2005); 7% of the samples of dried vine fruits in the SCOOP-2 report exceeded the EU maximum limit. Dried vine fruits were reported as products that are often eaten by children being them a risk group of contamination by OTA. The exisiting screenig method still expensive and they are difficult to implement in a large scale (Krska *et al*., 2005). Spectroscopic methodologies as near-infrared mir-infrared had appeared in the last years as a possible tools of screening mycotoxins in grains (Kos *et al*., 2003; Pettersson and Äberg, 2003).

The present work had the objective to develop a methodology for screening OTA in dried vine fruits using the Fourier infrared spectroscopy.

Keywords: FTIR-ATR, clean-up procedure, sample preparation, mycotoxin, OTA.

Materials and methods:

A stock OTA solution of 20 μg ml^{-1} was prepared according EN 14132:2003. A work solution at 1 μg ml^{-1} was prepared by diluting the stock standard solution (20 μg ml^{-1}) 100 times with tolueno/acetic acid (99:1).

Two approaches were taken: i) a calibration curve using a model OTA solutions in a range between 5–50 μg kg^{-1} using appropiated volumes of OTA work solution in the solvent system methanol-sodium bicarbonate 1% and ii) a calibration curve for OTA, using the same range of concentrations using dried vine fruits spiked with a OTA work solution (1 μg ml^{-1}). The sultana samples were homogenized and extracted with methanol-sodium bicarbonate 1% (Möller and Nyberg, 2003). 2 mL of the sultana's extracts were eluted through C18 cartridges, previously conditioned with 2 ml of acetonitrile and 2ml of water (Sáez *et al*., 2004). The cartridges were cleaned-up with 2 ml of water, and after air drying, elution of OTA was carried out with 2 ml of methanol-acetic acid (95.5:0.5, v/v). A 20 μl of OTA extract was put on the surface of an ATR Golden Gate crystal and all spectra were acquired continously and at a real time until all the solvent was evaporated, making 20 spectrum for each replicate. A total of five replicates for concentration were registered. Principal component analysis and PLS1 algorithm were used for spectrum analysis.

Results and discussion:

The capability of using Fourier infrared spectroscopy (FTIR) for the detection of ochratoxin A (OTA) was first evaluated by the adquisition of the spectrum of a model OTA solutions with concentrations in a range between 5–50 μg kg^{-1}. The results of this assay indicated that the experimental points of the initial spectrum fitted well to a straight line in the range between 5–50 μg kg^{-1} (r^2 = 0.9569). The PCA analysis of the initial spectrum showed that exist a separation according the OTA concentration.

The possitive results showed by the OTA model solutions lead us to proceed with the determination of OTA in dried vine fruits, in this case an extraction and clean-up steps were necessary before spectrum acquisition. The analysis of the initial spectrum of sultanas spiked with differente OTA concentrations (5–50 μg kg^{-1}), showed that the experimental points fit well to straight line (r^2 = 0.9547), in this case a second derivative was applied and the concentration of 5 μg kg^{-1} was removed before analysis.

Conclusions:

It can be concluded that the presented method is well suited for the screening of dried vine fruits giving rapid results and could be used as a semiquantitative analysis for OTA.

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[1] European Commision 2005. Commission regulation (EC) No 123/2005 of 26 of January 2005 amending regulation (EC) No 466/2001 as regards ochratoxin A. Official Journal of the European Union L25:3–5.
[2] Krska, R., Welzig, E., Berthiller, F., Molinelli, A. and Mizaikoff, B. 2005. Advances in the analysis of mycotoxins and its quality assurance. Food *Additives and Contaminants*. 22(4):345-353.
[3] Kos, G., Lohninger, H. and Krska, R. 2003. Development of a method for the determination of Fusarium fungi on corn using mid-infrared spectroscopy with attenuated total reflection and chemometrics. *Analytical Chemistry* 75:1211-1217.
[4] Pettersson, H. and Äberg, L. 2003. Near infrared spectroscopy for determination of mycotoxins in cereals. *Food Control*. 14:229-232.
[5] Sáez, J. M., Medina, A., Gimeno-Adelantado, J. V., Mateo, R. and Jiménez, M. 2004. Comparison of different sample treatments for the analysis of ochratoxin A in must, wine and beer by liquid chromatography. Journal of Chromatography A. 1029:125-133.
[6] Möller, T.E. and Nyberg, M. 2003. Ochratoxin A in raisins and currants: basic extraction procedure used in two small marketing surveys of the ocurrence and control of the heterogeneity of the toxins in samples. *Food Additives and Contaminants*. 20(11):1072-1076.

F-32

ANALYSIS OF CYTOTOXIC CONJUGATED-TRIENE FATTY ACIDS IN PLEUROCYBELLA PORRIGENS

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Pleurocybella porrigens mushrooms are one of common edible mushrooms in Japan. In 2004, acute encephalopathy occurred in patients with renal dysfunction after ingestion of the mushrooms,

In order to investigate compounds that cause such toxicity, we carried out a chemical analysis of constituents in the mushrooms with a biological assay using NGF-differentiated rat adrenal pheochromocytoma PC 12 cells.

Like other mushrooms, *P. porrigens* mushrooms contain a wide variety of fatty acid such as paltimic acid, linoleic acid, and oleic acid. In addition, we found that *P. porringens* contained specific fatty acids; ostopanic acid, three novel dienones, conjugated trienes. Among them, two conjugated trienes, α- and β-eleostearic acids (α- and β-ESA), which other mushrooms do not contain, exerted cytotoxicity.

The mushroom extracts were partitioned into four fractions by liquid-liquid partition. Lipophilic fractions were subjected to further biological assays (cytotoxicity tests) and active fractions were investigated. The biologically active fractions were analyzed and identified as α- and β-ESA (λmax = 262, 271, 283 nm in α-ESA, λmax = 260, 269, 281 nm in beta-ESA) using HPLC coupled with photodiode array detector (PDA). HPLC analysis was performed on C_{18} column (2.0 x 250 mm) and gradient mobile phase (solvent A:CH_3CN/THF = 55/30, solvent B:0.1% HCOOH).

P. porrigens mushrooms included the conjugated triens in seven samples from different areas in northern Japan, ranging from 7.8 to 172 μg/g dry in α-ESA.

α- and β-ESA inhibited neurite outgrowth and induced apoptosis-like cell death at a concentration of less than 1 μg/mL (3.6 μM) in NGF-mediated PC12 cells. In contrast, non-conjugated trienes (e.g. linolenic acid) and conjugated dienes (e.g. conjugated linoleic acid (CLA)) showed no cytotoxic activity.

Moreover, in order to investigate cell distribution of these triene fatty acids, α-ESA was added to NGF-differentiated PC12 cells and phospholipids were extracted at a intervals, and then five phospholipids (PC, PE, PS, PI, SM) were analyzed using neutral loss scan or precursor ion scan LC/MS (AppliedBiosystems API-3000) in positive mode. Separation of four phospholipids classes was carried out using silica-gel column (2.0 x 150 mm) with a gradient system of hexane-isopropanol-water containing 1 mM NH4OAc. The results show that alpha-ESA is not incorporated into five phospholipids.

*PC, phosphatidylcholine; PE, phosphatidyethanolamine; PS, phosphatidylserine; PI, phosphatidyinositol; SM, sphingomyelin

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FUMONISIN B1 CONTAMINATON IN DRIED FIGS

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Fumonisin B_1, mycotoxin produced by *Fusarium* sp. Fumonisins commonly contaminate frequently corn, other cereals and their products. Fumonisins have been related with leukoencephalomalacia in horses, pulmonary edema, cardiovascular diseases in pigs also they have nephrotoxic, hepatoxic and immunosuppressing effects in various animal species. Fumonisins are considered possible carcinogenic for human, group 2B by IARC (1993).

Dried fig, very nutritional and a healthy food, is one of the most widely produced fruits in the world. Occurence of aflatoxins and ochratoxin A in dried figs are previously determined in dried figs by various researchers. Because of high carbohydrate level and temperature in the growing regions and water activity in drying stage, dried figs are susceptible to mycotoxin contamination.

In this study fumonisin contamination in dried figs has been investigated using HPLC with fluorescence detection. One hundred and fifteen dried fig samples were collected from fields during drying stage from seven orchards in Aegean Region in 2003 and 2004. Seventy seven percent of the samples were contaminated with fumonisin B_1. Fumonisin B_1 contamination in dried figs harvested in 2003 and 2004 were determined fifty tree (75%) of 71 and thirty six (82%) of 44 samples, respectively.

F-34

A VALIDATED LC-MS/MS METHOD FOR THE DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN ANIMAL FEED

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Pyrrolizidine alkaloids (PAs) are secondary plant metabolites produced by a number of plants from the families Asteraceae, Boraginaceae and Fabaceae. Many of these alkaloids have been shown to be highly toxic, causing hepatic veno-occlusive disease, liver cirrhoris and ultimately death. Some PAs have also mutagenic and carcinogenic potential. Amongst livestock, cattle and especially horses are known to be susceptible to the toxic effects of the PAs. There is ample documentation of incidents where contamination of animal feed by PA-containing weeds has resulted in the death of animals. Humans may also be at risk by the consumption of honey originating from PA-containing plants, herbal teas and herbal medical products prepared from PA-plants and by the consumption of milk of cows or goats fed on PA-contaminated feeding stuffs. Tansy ragwort (*Senecio jacobaea*) and common ragwort (*S. vulgaris*) are common (bi)annual weeds occurring world-wide in pastures, (marginal) grasslands, along the borders of rivers, roads and highways. Ragworts are known to contain substantial concentrations of PAs.

EFSA has recently put out a scientific opinion on the presence of pyrrolizidine alkaloids as undesirable substance in animal feed [1]. One of the important conclusions of the EFSA report is that analytical methods for the detection and quantification of PAs in animal feed are largely lacking. Suitable methods should be developed in order to carry out surveys on the occurrence of PAs in animal feeding stuffs.

A validated LC-MS/MS method is presented for the determination of 14 PAs in dry and wet forage (grass, hay). The selected PAs include tertiary bases and N-oxides present in ragwort. Blank forage samples spiked at three levels (50, 250 and 1000 µg/kg) were extracted with an acidic aqueous solution. The extracts were purified and concentrated by solid phase extraction. The individual compounds were separated with an acetonitrile/water gradient with addition of a small amount of ammonia. Two precursor – product ion transitions were monitored with positive electrospray ionization. Recoveries for wet forage (n = 22 samples) at the 250 µg/kg level were quite good, averaging 98 ± 23% for the N-oxides and 90 ± 11% for the tertiary bases. For dry forage (n = 8 samples) slightly lower recoveries were obtained: 74 ± 5% for the N-oxides and 78 ± 5% for the tertiary bases. Detection limits typically were 5 to 10 µg/kg, while limits of quantification ranged from 5 to 45 µg/kg.

The application of the method to forage samples -intended to be used as animal feed- collected from different sites in The Netherlands will be discussed.

[1] Opinion of the scientific panel on contaminants in the food chain on a request from the European commission related to pyrrolizidine alkaloids as undesirable substances in animal feed, The EFSA Journal (2007) 447, 1-51.

F-35

THE USE OF SOLID PHASE EXTRACTION AS A CLEANUP STEP FOR THE LC-MS/MS ANALYSIS OF LIPOPHILIC MARINE TOXINS

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Marine Biotoxins are produced by several phytoplankton species and can accumulate in filter feeding bivalves such as mussels, oysters and clams. The consumption of these contaminated shellfish can lead to various syndromes with different levels of severity, depending on the type and concentration of marine biotoxins. Regulations have been put in place at the European levels to protect human consumers. The mouse bioassay is still the reference method for the analysis of the regulated lipophilic marine biotoxins (okadaic acid (OA) and dinophysistoxins (DTXs), azaspiracids (AZAs), pectenotoxins (PTXs), yessotoxins (YTXs)) but in the last decade several LC-MS/MS based methods have been developed for the detection of these toxins. Most of these LC-MS/MS methods analyse the crude shellfish extract with only minor cleanup such as filtration and when cleanup procedures are involved liquid-liquid extraction (LLE) is the most applied technique. The need for cleanup procedures is dictated by the fact that the lipophilic marine biotoxins are prone to matrix effects when analysed by LC-MS/MS, affecting the accuracy and precision of the measurement.

The work reported here investigated the use of solid phase extraction (SPE) as a cleanup step prior to the analysis of the lipophilic toxins. Of the different types of SPE sorbents that were tested the most promising were the polymeric sorbent-based cartridges. Further work was done in order to optimize the washing and elution procedures. The final SPE protocol includes a neutral wash and a basic elution step. With this method it is possible to retain the following groups of toxins on the cartridge; OA & DTXs, PTXs, AZAs and YTXs. For those toxins where standards were available recoveries and matrix effects were determined (n=5). This was done for three different matrices; mussel, scallop and oyster. The recovery of the toxins from the SPE was around 90% for all three matrices. In the crude extracts OA, AZA1 and YTX suffer from matrix effects (ion suppression or enhancement) varying between 0 and 50%. For PTX2 a strong ion enhancement effect was observed (up to 100%). For the first three toxins SPE cleanup resulted in a substantial reduction of those effects (to less than 20%), for PTX2 the cleanup effect was less pronounced.

It can be concluded that the developed SPE method has a good potential in reducing matrix effects associated with the analysis of some lipophilic marine toxins. However, as matrix effects are variable and dependant on a number of factors such as the LCMS conditions, the effectiveness of the cleanup is also subject to variability.

F-36

COMPARATIVE STUDY OF PATULIN DETERMINATION IN APPLE PRODUCTS USING HPLC/DAD AND UPLC/MS/MS

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The mycotoxin, patulin (4-hydroxy-4H-furo[3,2c] pyran-2[6H]-one), is produced by a number of fungi common to fruit- and vegetable-based products, most notably apples. A rapid, simple and economical method was described for determination patulin in apple products. The sample was extracted with ethyl acetate and the extract was then cleaned up by extraction with a sodium carbonate solution. Patulin was determined by reverse-phase liquid chromatography using a C_{18} column and a photodiode array detector and ultra performance liquid chromatography with electrospray-tandem mass spectrometry. Patulin was separated HPLC/ DAD method on 125x4 mm I.D Purospher STAR RP-18 endcapped column (Merck) with 5 µm C_{18} stacionary phase and it was detected at 275nm. The mobile phase was using acetonitrile - 1% acetonitrile in water and flow-rate 0,5ml/min. Ultra performance liquid chromatography (UPLC) was performed on a Acquity UPLC BEH C_{18} (100 mm x 2.1 mm). The mobile phase for UPLC/MS/MS analyse was using acetonitrile-0,01 M Amonium acetate and flow-rate 0.3ml/min. The limits of detection for patulin were found to be <0,001 mg/kg for HPLC/DAD method and <0.004 mg/kg for UPLC/MS/MS method.

F-37

AUTOMATED EXTRACTION OF MICROCYSTIN-LR RESIDUES FROM BLUE GREEN ALGAE HEALTH FOOD SAMPLES WITH DETECTION BY LC-MS/MS

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A method was developed for the determination of microcystin-LR residues in blue green algae (BGA) products (powder, capsule, tablets and liquids). Samples (1 g) were extracted with methanol/water (75/25, v/v, 10 ml) after homogenisation for 2.5 min using a FASTH 21^{TM} automated homogenisation unit. The system was capable of unattended homogenisation of 24 samples in a 15 min period. Sample extracts were centrifuged (3000 rpm) and filtered. An aliquot of the sample extract (0.5 ml) was diluted with water (2 ml) and applied to a preconditioned Oasis® HLB SPE cartridge (60 mg, 3 ml). The cartridge was washed by methanol/water (30/70, v/v, 5 ml) and eluted with methanol/water (80/20, v/v, 4 ml) for AFA clean-up. For *Spirulina* clean-up, the cartridge was washed with 3ml of water followed by methanol/water (30/70, v/v, 3 ml) and eluted with methanol/water (50/50, v/v, 6 ml). Sample extracts were evaporated to dryness at 50°C under nitrogen. Extracts were reconstituted by sequential addition of methanol (400 µl) and water (600 µl) and vortexing after each addition (2 min). Samples extracts were analysed by LC-MS/MS operating in positive electrospray mode using a gradient of 0.1% formic acid and acetonitrile. The suitability of the method was evaluated through application of samples naturally contaminated with microcystin-LR. The limit of quantitation of the method was 0.5 mg/kg.

F-38

SENSITIVE METHOD FOR THE DETERMINATION OF ARISTOLOCHIC ACIDS IN TEAS USING HPLC WITH FLUORESCENCE DETECTION

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Aristolochic acid (AA) is a known nephrotoxin and suspected carcinogen that may be found in a number of botanicals. A sensitive method was developed to determine AA I and II residues in herbal teas. AA I and II residues were extracted from ground tea products (1.0 g) in the presence of methanol (20 ml) by sonication (30 min) and shaking (15 min, 200 oscillations/min). The extracts were incubated at room temperature for approximately 1 h. An aliquot of the sample extract (4 ml) was diluted with water (9 ml) and applied to a preconditioned Strata-X C18 cartridge (60 mg, 3 ml). The cartridge was washed by methanol/water (30/70, v/v, 10 ml) and eluted with methanol (8 ml). Sample extracts were evaporated to dryness at 60°C under nitrogen and reconstituted in methanol (1 ml). Extracts were reduced by shaking samples in the presence of 0.01N HCl (9 ml) and iron powder (10 mg) on a shaker (200 oscillations/min, 40 min). Derivatised extracts were partitioned into dichloromethane (10 ml) and centrifuged (3000 rpm). An aliquot of the organic layer was removed (5 ml) and evaporated to dryness under nitrogen (35°C) prior to reconstitution in methanol (0.5 ml). Filtered extracts were analysed by HPLC with fluorescence detection (excitation 390 nm and emission 467 nm). Recovery was typically greater than 70%. The limit of quantitation of the method was 2 µg/kg for AAI and 4.55 µg/kg for AAII based on the lowest calibrant level.

F-39

DEVELOPMENT OF EARLY WARNING TOOLS FOR THE DETECTION OF TOXIC ALGAL BLOOMS PRIOR TO CONTAMINATION IN SHELLFISH BASED ON THE EXPRESSION OF GENES ASSOCIATED WITH TOXIN PRODUCTION

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Over the last 25 years the consumption and trade of shellfish has increased globally as well as in Europe. However, an increase has been observed in the frequency and diversity of toxic algal blooms. These algae are accumulated by most shellfish species and can cause serious health problems for the consumers. The lack of reliable analytical methods for the determination of toxins that cause DSP is one of the main problems for dealing with the potential problems associated with toxic shellfish. The BIOTOX project is a multi-disciplinary project in which the lack of analytical methods for the determination of marine lipophylic shellfish toxins is addressed.

Here, we will describe our results so far in developing biomarkers for the detection of toxic algal blooms, based on gene expression. cDNA microarray analysis of gene expression in a toxic and non-toxic strain of Protoceratium reticulatum will be presented.

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F-40

INVESTIGATION OF ORGANIC CONTAMINANTS AND NATURAL TOXINS IN FOODSTUFFS USING TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH TIME-OF-FLIGHT MASS SPECTROMETRIC DETECTION

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Comprehensive two-dimesional gas chromatography with time-of-flight mass spectrometric detection (GCxGC-TOF MS) have become popular in a scientific community during the last decade. This analytical technique has several unique features in comparison with a common one-dimensional GC-MS. Enhanced sensitivity and chromatographic resolution resulted from separation of a sample in two capillary columns with different polarities as well as unique identification of compounds due to the collection of full mass spectra are well known characteristics of this sophisticated technique.

Czech Agriculture and Food Inspection Authority (CAFIA) is responsible for checking the quality of food that is placed on a Czech and/or European (EU) market. Besides currently running monitoring programmes focused on pesticide residues, mycotoxins etc., non-target analysis of samples suspicious from contamination or presence of organic compounds and natural toxins is important part of CAFIA activity.

For non-target analysis of samples GCxGC-TOF MS is employed in our laboratory. Separation is performed using non-polar column (DB-5 MS, 30m x 0.25mm x 0.25um) in the 1st dimension and moderately polar column (BPX-50, 2m x 0.1mm x 0.1µm) in the 2nd dimension. The comprehensive sampling from the 1st column and introduction into the 2nd column is achieved with 2-stage thermal modulator (5 sec. modulation period). Acquisition rate of mass spectrometer is ranging from 50 to 100 spectra/sec. based on the kind of analysis.

Practical examples and results of non-target analysis of real samples demonstrating theoretical aspects and possibilities of GCxGC-TOF MS will be presented. Identification of unknown compounds (2-isopropylthioxanthone in a yoghurt, morphine and other alkaloids in poppy seeds, furanocoumarins in alcohol beverage containing herbal extract) by means of the comparison of deconvoluted spectra with library database will be shown.

F-41

AGRICULTURAL PRACTICES AFFECTING FUSARIUM TOXINS LEVELS IN BARLEY AND MALTED BARLEY - RAW MATERIAL FOR BEER PRODUCTION

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Barley being used in beer production is often infected by toxinogenic *Fusarium* species and thus contaminated by their secondary metabolites - mycotoxins. *Fusarium* toxins may pose to health risk and so maximum limits for major mycotoxins in various cereals have been established. Althought *Fusarium* toxins cannot be completely prevented by the way of cereals cultivation, adopting of the "Good Agricultural Practice" (GAP) can minimize their concentrations. The impact of agricultural practices: (i) usage of pesticides, (ii) the type of preceding crop in the rotation and (iii) resistance of barley cultivars on *Fusarium* mycotoxin levels in both naturally and artificially infected barley was investigated within 2 years study. Samples were analyzed using the method of high performance liquid chromatography coupled with tandem mass spectrometry. Results show that the weather conditions during growing season are the key factor of mycotoxins contamination.

This study was carried out within the project MSM 6046137305 granted by the Ministry of Education, Youth and Sports of the Czech Republic.

F-42
LC/MS-MS DETERMINATION OF CALYSTEGINES IN FOOD PLANTS

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Calystegines are water-soluble biologically active northropane alkaloids, their structure resembles atropine. Until now, 17 calystegines differing in number and position of hydroxyl groups in their molecule have been identified. They occur in plant families *Convulvoceae, Maraceacea* and *Solanacea,* especially in *Solanum melongena* (eggplant) and *Solanum tuberosum* (potato). Calystegines are selective glycosidase inhibitors and the levels in plants can reach up to 180 mg/kg.

For determination of calystegine levels in food crops HPLC method with MS-MS detection in APCI+ mode was developed and validated in our study. Since standards of calystegines are not commercially available, the mixture of calystegines (A_3, B_2, B_4) was isolated from potato sprouts, after LC separation their identity and purity was confirmed by NMR method. Limit of detection (LOD) for individual calystegines A_3, B_2 and B_4 in plant matrices was 0.4 mg, 0.25 mg/kg and 0.5 mg/kg respectively. Repeatability, expressed as relative standard deviation, was in the range 4-6%.

Interestingly, the levels of calystegines (sum of A3, B2 and B4) was higher than that of α-solanine and α-chaconine, well known potato toxic glycoalkaloides. Concentrations up to 140 mg/kg were found in variety Dita. Alike solanine, also calystegines are distributed in tubers unevenly, most of them (75-92%) is located in the peel.

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F-43

INFLUENCE OF CHEMICAL TREATMENT ON FUSARIUM TOXINS IN BARLEY HARVESTED 2005-2006

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Mycotoxins are toxic secondary metabolites produced by certain toxigenic microscopic fungi in various crops used as raw materials for the production of foodstuffs and fodder. *Fusarium* toxins have been shown to cause a variety of toxic effects (both acute and chronic) in humans and animals (vomiting, dermal irritation, haemorragic lesions, weight reduction, etc.).

The main objective of this study was to investigate the influence of key agricultural practices (resistance of barley cultivars, chemical treatment use, pre-crop and different growing locality) on *Fusarium* mycotoxins in barley. Field trials were realized in cooperation with the Agricultural Research Institute Kroměříž (harvest 2005 and 2006).

For the determination of the most important *Fusarium* mycotoxins (deoxynivalenol, HT-2 toxin, T-2 toxin, zearalenone, nivalenol, 15-acetyldeoxynivalenol, fusarenon-X and 3-acetyldeoxynivalenol), the high-performance liquid chromatography employed with tandem mass spectrometry (LC-MS/MS) was used. The analytical procedure includes following steps:

(i) Extraction with an acetonitrile:water mixture for one hour by a shaker

(ii) Clean-up by solid phase extraction empolying the MycoSep # 226® cartridges

(iii) Identification and quantification by LC-MS/MS

The trueness of the generated data was successfully demonstrated in proficiency test (Food Analysis Performance Assessment Scheme, FAPAS®) organized by Central Science Laboratory (CSL, York, UK).

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F-44

DETERMINATION OF DON AND ITS METABOLITES IN BEER AND MALT SAMPLES - COMPARISON OF HPLC-MS/MS AND ELISA

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Deoxynivalenol (DON) is mycotoxin produced mainly by *Fusarium graminearum* a *F. culmorum*. It belongs to the most well known mycotoxins due to its toxicity and especially due to its frequency of occurrence. Almost all species of common cereals are naturally affected by this mycotoxin and from the infected barley it can be easily transferred into malt and beer. Not only DON figure in malt and beer contamination, but also its metabolites, e.g. acetylated and glycosilated forms occur together with it. The maximal residual limits are determined for DON only, because the glycosilated DON metabolites were till lately not possible to identify by using of common analytical methods. However, due to the fact, that DON metabolites are largely biologically available, their identification is very important. The comparative study between DON determination using ELISA immunoassay and HPLC-MS/MS was performed.

At present, by the LC-MS/MS method is possible reliably quantify DON, deoxynivalenol-3-glucoside (DON-3-Glc) and sum of acetylated-DONs (3-ADON + 15-ADON). As regards ELISA, this is very easy and quick screening method, but its cross-reactivity with DON metabolites mentioned above can significantly overestimate the results.

10 malt and 25 beer samples were investigated by both methods. For ELISA, the performance characteristics like recovery and cross-reactivity in different matrices were determined. Concerning HPLC-MS/MS results, concentrations of DON, DON-3-Glc and acetylated-DONs were recalculated as a total DON and this result was compared with ELISA kits answer. It can be concluded, that in most cases the ELISA answer was higher than the total DON obtained by HPLC-MS/MS. Regarding beer samples, results of two tested kits were quite similar to each other and they were also in relatively good agreement with HPLC-MS/MS. Results of other two kits were relatively similar to each other, however with respect to HPLC-MS/MS, the ELISA answer was several times higher.

It was confirmed, that anti- DON antibodies do not react with DON only, and the ELISA kits differ each from other, first of all by their sensitivity to masked DON forms. The difference between ELISA results and total DON concentration obtained by HPLC-MS/MS could be explained by the presence of another hidden DON forms. This problematic will be further investigated in the near future.

F-45

NEW QUECHERS LC-MS/MS METHOD FOR FUMONISINS QUANTIFICATION; MONITORING OF CORN BASED FOODS AVAILABLE FROM THE CZECH MARKET

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Fumonisins are the group of mycotoxins, which are produced predominantly by microscopic filamentary fungi *Fusarium moniliformis* and *F. proliferatum*. They occur mainly in maize and maize products, but their incidence was proved in rice and millet, too. The International Agency for Research on Cancer evaluated them as potential human carcinogen (2B class). That is why the development of better qualitative and quantitative methods for the fumonisins analysis is necessary. In this context, liquid chromatography coupled with tandem mass spectrometry means a significant progress in the mycotoxins analysis area.

The QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, Safe) is well described for pesticide analysis, however it starts to be employed also in other branches including mycotoxins determination. In QuEChERS method for fumonisins B1 and B2 determination acetonitrile and water were used as the extraction solvents. After extraction, NaCl and $MgSO_4$ was added and shaked in hand. After centrifugation, two phases (water and acetonitrile) were separated and an aliquot from the acetonitrile layer was taken and analysed.

The separation of analytes was realized on the reverse phase with polar endcaping. As a mobile phase gradient of methanol and water acidified by formic acid due to the better ionisation and also obtaining of better peak shapes. Tandem mass spectrometry was performed by Quattro Premiere XE with positive electrospray ionisation in MRM mode.

Regarding the Czech market monitoring, several groups of foods like maize flour, corn flakes, extruded products, nachos, baby food, sterilized corn and feeds were investigated. As was found, the highest level of contamination had the nachos - 4 from 4 investigated samples were contaminated on relatively high levels (on average 691 µg/kg of FB1 + FB2), 2 samples exceeded the maximal residual limit (400 µg/kg), one of them even more than 4 times! As far as corn flour and polentas, 7 from 10 samples were contaminated and two of them exceeded the maximal residual limit. As regards extruded and puffed products, cornflakes, baby foods, sterilized corn and some foods intended for special diet, they mostly showed no contamination. Concerning animal feed, 5 samples were tested and only 1 showed rather high value (548 µg/kg of FB1 + FB2).

F-46
MYCOTOXIN DON AND ITS MASKED FORMS IN BEER

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Trichothecene mycotoxin deoxynivalenol (DON), produced predominantly by *Fusarium graminearum*, is known worldwide for its frequent contamination of cereals and its toxic effects on humans and animals health. Mycotoxin DON can survive the brewing technological parameters and is transmitted from infected barley and other adjucts into the beer. As a "masked DON" is called conjugates of DON to more polar substances (carbohydrates, aminoacids). Up to now the occurrence of masked mycotoxin DON have been studied entirely in unprocessed grains. Our research was focused on determination of DON, deoxynivalenol-3-ß-D-glucopyranoside (D3G) and 3-acetylDON and 15-acetylDON (ADONs) in malt and beer.

A total number of 176 beer samples of various types from 15 European and North America countries have been collected from retail markets in 2007. New method was developed and validated for DON, D3G and ADONs determination in beer. The extent of beer contamination was investigated by means of high performance liquid chromatography coupled with a tandem mass spectrometry (APCI, ion trap analyzer). The limit of detection was 1 µg/l for given analytes, the recovery rates were in range of 75 - 93 %, depending on the types of beers.

The presence of fusariotoxin DON and its masked forms have been proved in examined beers. Generally, 86% of beers contained at least one of the investigated analytes. DON, D3G and ADONs were found in 63; 74 and 49% of samples, respectively. When results are expressed as a contents of DON in all toxins forms, the overall levels of contamination ranged from 0.9 – 85.9 µg/l, with the mean value 15.3 µg/l. The contribution of D3G to the whole extent of contamination was 34%. The levels of masked D3G were comparable with levels of DON, in majority of measured samples.

F-47
ALKALOIDS AND MYCOTOXINS IN LUPINS

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Lupins and isolates have gained growing interest in recent years. Flour and isolates are applied among other things to replace potentially genetically modified soya, roasted beans are used as coffee surrogates free of coffeine and some lupin containing foods claim their health benefit. On the other hand, the allergenic potential of lupins seems to be of major concern since the European Commission has recently decided to include lupins in the list of food allergens, whose declaration on food labels is mandatory [1]. Additionally, naturally occurring alkaloids of the quinolizidine, piperidine and indole group and mycotoxins, known as phomopsins, represent antinutritive compounds in lupins, which deserve supervision. The Australian New Zealand Food Authority (ANZFA) assessed the risk of lupin alkaloids for humans and derived a provisional tolerable daily intake (PTDI) of 0.035 mg/kg bw/day [2]. The ANZFA has set a maximum allowed limit of 200 mg/kg for alkaloids in lupin flours and foods [2, 3] and of 0.005 mg/kg for phomopsins in any food [3].

In this context, lupin seeds, flours and lupin containing food have been analyzed in order to assess the relevance of a potential health hazard given by mycotoxins and/or naturally occurring alkaloids.

The alkaloids were analyzed by GC-MS/GC-FID in parallel. In some cases, matrix effects (flours of *L.angustifolius*, roasted beans) and/or specific alkaloids (angustifoline, 13α-hydroxylupanine, lupinine) required derivatization of the analytes prior to quantification. Trimethylsilylation of alkaloids was not adequate, since partial CO_2-incorporations occurred as artifact. In foods where lupin was only a minor constituent, the alkaloid content was of no concern. However, roasted lupin beans intended as coffee surrogate had alkaloid contents close to the intervention limit of 200 mg/kg. Switzerland considers to regulate this issue. From the alkaloids found in the Swiss market products it can be concluded that mainly *L.albus*, rarely *L.angustifolius* and never *L.luteus* had been used for production.

The phomopsin mycotoxins were analyzed by ELISA [4], since chromatographic methods applied (LC-DAD-ECD, LC-MS/MS) were not sensitive enough and required time-consuming sample cleanup. The analyzed lupin containing foods were free of phomopsins. However, additional efforts should be taken to develop a chromatographic confirmation method for phomopsins in the low ng/g range, especially to identify which one of the five phomopsins is present since the differences in their toxicity is of relevance [5].

[1] http://eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_368/l_36820061223en01100111.pdf
[2] http://www.foodstandards.gov.au/_srcfiles/TR3.pdf
[3] http://www.foodstandards.gov.au/_srcfiles/P158_FAR.pdf
[4] K.A. Than, A.L. Payne, J.A. Edgar, in: L.F. James, E.M. Bailey, P.R. Cheeke, M.P. Hegarty (Eds.), Poisonous Plants, Iowa State University Press, Iowa, US, 1992, pp 259-263.
[5] J.G. Allen, G.R. Hancock, J. Appl. Toxicol. 1989, 9, 83.

F-48

EARLY DETECTION OF MICROBIAL CONTAMINATION IN PROCESSED TOMATOES AND IN FRUIT JUICES BY ELECTRONIC NOSE

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Fruit and vegetable products can be easily spoiled by microbial contaminants, as bacteria and fungi, which not only alter flavour and taste, but could also be dangerous for human health. A fast and reliable detection of such contamination is a relevant goal both for customer health protection and for improving producer's business.

The pattern of volatile compounds produced by the secondary metabolism of the microorganisms can be exploited as a marker of microbial spoilage. In this work we performed a systematic study to prove the ability of an Electronic Nose (EN) to detect the contamination by different microorganisms in peeled tomatoes and fruit juices. The aim is to reveal contamination at early stages before the microorganisms start to produce gases (e.g. CO_2) or visible moulds on the product which are the usual indicators of contamination.

The headspace of pure and artificially contaminated products was analysed by means of the EOS835-EN based on an array of semiconducting metal oxide sensors. Contaminated samples were incubated at 37°C for a minimum of 48 h and a maximum of 2 weeks, according to the standard protocol for quality control used by the producer company.

We investigated contamination by different organisms, two bacteria and three fungi, which have different growth rates. In this preliminary study we did not quantify the number of inoculated cells.

The most relevant result is that the EN was able to distinguish between pure and contaminated products disregarding the type of microbial contaminant provided that we wait until the number of colonies is above the EN detection threshold. The intrinsic growth rate of microorganisms plays a crucial role in their detection. Yeast Saccharomyces, producing of huge amount of volatiles during its growth, and bacteria E. Coli, which doubles its colonies number in one hour, can be detected and identified just after 48h from inoculation. Instead, we need a longer growth time (at least 7 days) to detect contamination by Aspergillus, Penicillium, and Lactobacillus, which have a slower life cycle. These results were consistent for both tomatoes and fruit juices suggesting that it can be possible to training the EN for early diagnose microbial presence on the food.

F-49

CO-EXTRACTION OF THE MYCOTOXINS DEOXYNIVALENOL AND ZEARALENONE FROM WHEAT BY SOL-GEL IMMUNOAFFINITY CHROMATOGRAPHY

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Mycotoxins are fungal secondary metabolites which can cause acute and chronic toxic effects in animals and humans. In Austria the most prevalent mycotoxins in grain are deoxynivalenol (DON) and zearalenone (ZON), both produced by *Fusarium graminearum* and *Fusarium culmorum*. DON is known to be harmful to both humans and animals, eliciting a wide range of symptoms of varying severity, mainly by inhibiting protein synthesis. In contrast, ZON is not very toxic but can cause estrogenic effects in humans and animals by binding to the natural estrogen receptor.

In order to reduce the risk of intoxication of humans and animals the European Commission established maximum levels for DON and ZON concentrations in food and feed. Highly selective and sensitive analytical methods have to be applied to ensure that DON and ZON concentrations comply with the legal regulations. However, traces of DON and ZON can only be determined in complex food and feed matrices after carrying out selective sample pre-treatment steps. Immunoaffinity chromatography is one of the most frequently applied clean-up procedures. Up to now, most immunoaffinity columns are prepared by covalently binding the antibodies to solid support materials. However, covalent binding is carried out under rather drastic conditions frequently affecting the affinity of the antibody for the antigen. In order to overcome this problem, our group has recently presented an alternative method to prepare DON specific immunoaffinity columns (1). In this recently published study anti-DON antibodies were physically entrapped in porous glass matrices prepared by the sol-gel technique under mild conditions.

In the present paper we report on the preparation of immunoaffinity columns by entrapping both anti-DON and anti-ZON antibodies in the pores of a sol-gel glass. In spite of the different affinity constants of the anti-DON and anti-ZON antibodies, operation conditions could be found which enabled co-extraction of DON and ZON from wheat. The columns proved to be very stable and could be repeatedly used for sample clean-up.

[1] Z. Brenn-Struckhofova, M. Cichna-Markl, C. Böhm, E. Razzazi-Fazeli. Selective sample cleanup by reusable sol-gel immunoaffinity columns for determination of deoxynivalenol in food and feed samples. Anal. Chem. 79 (2007) 710-717.

PROCESSING AND PACKAGING CONTAMINANTS

(G1 – G24)

G-1

DETERMINATION OF BISPHENOL A IN CANNED VEGETABLE AND FRUIT SAMPLES BY COACERVATIVE EXTRACTION, LIQUID CHROMATOGRAPHY AND FLUORESCENCE DETECTION

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Bisphenol A (BPA) is used in the resins that coat the inside of some food cans. Human exposure to BPA occurs from the migration of this contaminant into foods. The tolerable daily intake (TDI) level established by the U.S. Environmental Protection Agency as well as that recently recommended by the European Food Safety Authority (EFSA) is 0.05 milligrams per kilogram of bodyweight. The amount of BPA legally permitted to migrate from packaging into food, known as the specific migration limit (SML), is based on the TDI and it was set at 600 ng/g by the EU Commission in 2004.

Up to now, liquid-liquid extraction and solid-phase extraction (SPE), followed by purification of extracts, evaporation with nitrogen and so on have been the most common sample preparation procedures applied to the determination of BPA in foodstuffs. The quantification is usually carried out by liquid chromatography in combination with either UV or fluorescence detection, or by gas chromatography coupled to mass spectrometry (GC-MS). The amount of sample handled usually ranges between 1 and 10 g and typical detection limits obtained vary from about 1to 20 ng BPA/g foodstuff.

The present research was intended to simplify the sample treatment required for the determination of BPA in canned food samples. For this purpose, colloid-rich liquids, named coacervates, were assessed for the first time as extractants of contaminants from solid foods. Coacervates made up of decanoic acid reverse micelles in tetrahydrofuran (THF) were proven to efficiently extract BPA on the basis of the hydrophobic and hydrogen bond interactions that were established between the analyte and the extractant. Factors affecting the extraction efficiency were optimised and the most influential ones were the decanoic acid and tetrahydrofuran concentration and the stirring time. The coacervates were formed by using 0.2 g decanoic acid and 2 mL of tetrahydrofuran. The whole procedure was developed at room temperature and the extraction time was 15 minutes. Using minute quantities of sample food (around 300 mg), the detection limit for BPA in canned vegetables and fruits was about 6 ng/g. No matrix effects were observed in any of the sample analyzed, which permitted the use of external calibration. No clean-up steps were necessary to achieve selectivity. The amount of BPA found in the solid portion of a variety of canned food samples, namely fruit salad, peach in syrup, mango slice, red pepper, sweetcorn, green bean and pea cans, ranged between 10 and 103 ng BPA/g canned food. These amounts were far below to the SML established by the EU Commission. Main assets of the use of coacervates for this application were simplicity, rapidity and low cost.

G-2
MODELLING THE FORMATION OF SOME POLYCYCLIC AROMATIC HYDROCARBONS DURING ROASTING OF ARABICA COFFEE

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Roasting is a crucial step for the production of coffee, as it enables the development of colour, aroma and flavour which are essential for the characterization of the coffee quality. Both the temperature and time conditions of the roasting step need to be optimised and controlled to achieve maximum aroma and flavour development. In practice, the roasting conditions differ depending on the coffee quality expected and the type of roaster used.

Chemical reactions are known to be responsible for the development of aroma and flavour during the roasting step, such as the Maillard reaction. Consequently, the formation of toxic compounds may not be excluded, and very recently acrylamide (a Maillard product) has been reported in coffee samples. The possible formation of polycyclic aromatic hydrocarbons (PAHs) is also of great concern due to their suspected carcinogenic and mutagenic properties. Therefore, the present work was undertaken to study the influence of roasting conditions on the PAH content of coffee samples. To avoid variations in PAH concentrations related to the coffee origin, only *Arabica* samples from Cuba were considered in this study.

Roasting of the green coffee beans was performed in a pilot spouted bed roaster. The roasting temperature was varied (from 180 to 260°C for the inlet air temperature) as well as the roasting time (5-20 min range). PAHs were determined in both roasted and green coffee samples, to take into account possible presence of PAHs in the green coffee due to atmospheric deposition during the drying step of the beans. Samples were extracted using an accelerated solvent extraction system, and the obtained extracts were saponified, concentrated and cleaned-up on silica solid-phase extraction cartridges. Final analysis of the extracts was performed using HPLC-fluorescence detection, and identification of PAHs was confirmed by HPLC-UV/DAD as well as GC-MS/MS analysis.

Formation of phenanthrene, anthracene and benzo[*a*]anthracene in coffee beans was observed at temperatures above 220°C, while formation of pyrene and chrysene required 260°C. Low levels of benzo[*g,h,i*]perylene were also noted for dark roasting under 260°C, with simultaneous partial degradation of 3-cycle PAHs, suggesting that transformation of low molecular PAHs to high molecular PAHs occurred as the roasting degree was increased. Modelling was investigated based on either a kinetic model, or an artificial neural network. Both models showed good correlation between experimental and predicted values as presented for benzo[*a*]anthracene and chrysene.

Keywords : Arabica ; coffee ; fluorescence detection ; liquid chromatography ; PAHs ; polycyclic aromatic hydrocarbons ; roasting.

G-3

OCCURRENCE OF MUTAGENIC/CARCINOGENIC HETEROCYCLIC AMINES IN OFFAL PRODUCTS (ANIMAL VISCERAL) PROCESSED AFTER TRADITIONAL RECEPIES FROM SPAIN

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Heterocyclic amines (HAs) are high mutagenic and carcinogenic microcontaminants [1] that are formed at ppb levels from creatine, amino acids and reducing sugars via Maillard reaction during heat-processing of proteinaceous food such as meat and fish. The International Agency on Cancer Research has classified several HAs as possible and probable human carcinogens [2]. Therefore, in order to estimate the intakes and risks to human health is important to quantify HAs in different meat products prepared in different ways, since it was proved that their formation varies with cooking conditions, amount of different precursors present in meat, and also with the presence of compounds with enhancing or inhibiting effect used during cooking process.

There are no previous reports on HAs analysed in cooked offal. Therefore, in order to assess the HAs formation in these foodstuffs several offal products such as beef tongue, beef liver and lamb kidney were prepared according to traditional recepies from Spain. The studied HAs were DMIP, IQ, MeIQ, MeIQx, 4,8-DiMeIQx, Harman, Norharman, PhIP, Trp-P-1, Trp-P-2, AαC and MeAαC and the sample analysis was performed using solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques [3]. Harman and Norharman were detected in all the samples in levels ranging from 0.28 to 8.87 ng g^{-1}. PhIP was found in low amounts in tongue, liver and kidney (0.001–0.12 ng g^{-1}), whereas DMIP, IQ, MeIQ, MeIQx, 4,8-DiMeIQx, Trp-P-1, Trp-P-2, AαC and MeAαC were found not detected to below limit of quantification. The low levels of HAs might be explained by the use of different ingredients that may contain components which inhibit the HAs formation.

[1] T. Sugimura, Environmental Health Perspectives, 67 (1986) 5-10.
[2] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 56 (1993).
[3] E. Barceló-Barrachina, E. Moyano, L. Puignou, M.T. Galceran, Journal of Chromatography A, 1023 (2004) 67-78.

G-4

RESEARCH CONCERNING FLUCTUATIONS OF CONTAMINATING MICROORGANISM POPULATIONS IN TRADITIONAL ROMANIAN CHEESES DURING AGEING

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In order to evaluate the influence of cheese ageing process over the initial microorganism population, two samples of traditional, raw milk Romanian cheeses were investigated: a brined Feta-like cheese, called “telemea” and a kneaded cheese called “burduf”. Over a three months ageing period, three sets of determinations were performed, concerning the number of *E. coli,* coliforms, and *Staphylococcus aureus,* both in the outer layer and inside the cheese samples*.* During the ageing period, the two cheese assortments suffered significant decrease of initial microorganism populations. For “telemea” cheese, outer layer *E.coli* dropped from 1.3 log CFU/g, to 0 after the first month of ageing. For this cheese sample, no other types of contamination were identified inside or in the outer layer. For “burduf” cheese, *E.coli* and coliforms populations in the core of the product dropped from 4.07 log CFU/g and 4.34 log CFU/g respectively, to 0 at the end of the investigation period. No *S.aureus* was isolated from “burduf” cheese core. The number of microorganisms in the outer layer of “burduf” cheese dropped with 1.97 log CFU/g for *E.coli,* 2.29 log CFU/g for coliforms and 3.14 log CFU/g for *S.aureus*, the latter not being identified after three months of ageing.

Key words: traditional cheeses, raw milk, ageing, *E.coli,* coliforms, *S.aureus*.

G-5
CONTRIBUTION TO THE CHROMATOGRAPHYC ANALYSIS (GC-MS) OF CHLOROPROPANOLS IN FOOD SAMPLES

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In the last years, it has increased of significant way the product consumption like the soybean sauce and food elaborated from soybean, due to the properties that these present, they are rich in proteins, nutritious and relatively cheap [1].

During the acid hydrolysis process that is carried out in the industrial elaboration of the soybean sauce, its produced chloropropanols, 1,3-dichloropropan-2-ol (1,3-DCP)and 3-monoclhoropropane-1,2-diol (3-MCPD) are the most abundant [2]. Due to the toxic properties of chloropropanols, it has been necessary to establish maximum levels of consumption, according to its, the Food and Drug Administration of United States specifications of 1mg/kg 3-MCPD and 0.05 mg/kg 1,3-DCP. The Europe Community specifications are 0.02 mg/kg 3-MCPD [3].

In this study, two methods using GC-MS and derivatization with N-methyl-N-trimethylsilyl)trifluoroacetamide (MSTFA) and bis(trimethylsilyl)trifluoreacetamide (BSTFA) + trimethylchlorosilane (TMCS) were optimized. The factors: temperature, derivatization time and volume of agent derivatizant were optimized by means of an experimental design of spherical domain with α=1.682. Using desirability functions, the optimal conditions of derivatization reactions were 50 minutes, 25ºC and 50 µL of MSTFA with MSTFA as derivatization agent, and 70 minutes, 60ºC and 55 µL of derivatization agent BSTFA.

The product of derivatization reaction (1µL) was injected in the GC-MS. The characteristic ions used for the cuantification were m/z 151 and 185 for the 1,3-DCP-TMS and m/z 219 and 239 for the 3-MCPD-dTMS. Also PCB-30 and d5-MCPD have been used like internal standards using for the quantification m/z 186, 221, 256 and 258 and m/z 244, respectively.

A good linearity in the concentration range from 8.0 to 190 ng/mL 1,3-DCP and 3-MCPD with coefficient $R^2 > 0.995$ was achieved. Detection and quantification limits were obtained at the ng/mL, below the legislation limits.

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[1] Angelika M. Tritscher,"Human healt risk assessment of processing-related compounds in food", Toxicology Letters 149 (2004) 177-186
[2] Colin G. Hamlet, Peter A. Sadd and David A. Gray. "Generation of monochloropropanols (MCPDs) in model dough systems. Leavened doughs". Journal of Agricultural and Food Chemistry, (2004), 52, 2059-2066
[3] Comisión del Codex Alimentarius. Documento de debate sobre cloropropanoles obtenidos por fabricación de proteínas vegetales hidrolizadas (PVH) mediante ácido y procesado térmico de los alimentos. Febrero 2007.

G-6
PACKAGING CONTAMINANTS: ISOPROPYLTIONXANTONE (ITX) IN DAIRY PRODUCTS

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In September 2005 the Italian competent authorities alerted the European Commission about the detection of isopropylthioxantone (ITX) in some baby milk and other dairy products at concentration levels in the range of about 100 – 400 ng/ml. This compound is known to be used as photoinitiator in inks applied to food packaging material.

As this substance is not regulated by specific EU legislation, EFSA was inquired for a toxicological evaluation, but due to a lack of adequate information, a definitive position in terms of maximum residual level could not be assessed, until now.

Therefore in Italy a campaign of controls for monitoring purposes was proposed in 2005 – 2006.

Under this perspective a method for the determination of ITX in dairy products was developed and validated and its characteristics are presented in this work.

Sample preparation consists in a simple extraction with acetonitrile.

Chromatographic separation was performed on a Gemini C_{18} column (100 x 2.0 mm, I.D. 5 µm) using a gradient of aqueous ammonium formate 20 mM pH 4.5 and acetonitrile as mobile phase at a flow rate of 0.25 ml min^{-1}.

The method was validated according to the guidelines laid down by Commission Decision 2002/657/EC using an in-house validation.

Validation parameters such as $Cc\alpha$, $Cc\beta$, repeatability, within-laboratory reproducibility and recovery were studied spiking three different series of 18 yogurts at 5, 10, 15 µg kg^{-1} (six replicates for each concentration level).

The selectivity/specificity was tested by analysing 20 representative blank samples of different types of yogurts (whole milk, low fat, non-fat, blended) and 10 representative blank samples of different types of milk and pudding.

The ruggedness of the method was tested both for minor changes (minor reasonable method variation) and for major changes (different matrices like milk, pudding, yogurt at different fat concentrations).

A total of 50 samples were analysed according to the proposed method.

G-7

LOSS OF NATURAL CONTAMINATING LISTERIA MONOCYTOGENES IN ROMANIAN TRADITIONAL CHEESE BURDUF IN CONNECTION WITH CHANGES IN LACTOBACILLUS SPP. POPULATION DURING AGEING

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Listeria monocytogenes was identified in traditional Romanian, raw milk cheese named *burduf*. The cheese was subjected to traditional ageing for three months. After each month, samples of the outer layer and the core of the cheese were tested for *L. monocytogenes* and were also used for *Lactobacillus spp.* counting. The first two sets of investigations revealed the presence of *L. monocytogenes,* while *Lactobacillus spp.* counting showed an increase of 0.731 log CFU/g in the outer layer and 0.98 log CFU/g in the core of the cheese sample. The third set of analysis revealed the absence of *L. monocytogenes* both in the cheese core and the rind. Also, a very slight decrease in *Lactobacillus spp.* number was shown (0.1 log CFU/g in the outer layer and 0.07 log CFU/g in the core), that may be associated to a constant level maintenance between the last two sets of determinations. The present study supports the theory of traditional raw milk cheese sterilization through ageing on one hand and highlights a positive correlation between lactic acid bacteria activity and pathogen inhibition in dairy products. Thirdly, this study shows that an approximately 90 days ageing period is necessary for *Listeria monocytogenes* elimination of raw milk cheese. Further studies are necessary in order to evaluate the behavior of *L.monocytogenes* in other traditional cheese types during ageing and also to statistically support the 90 days elimination period required for this pathogen.

Key words: traditional cheese, raw milk, *Listeria monocytogenes, Lactobacillus spp.,* cheese ageing.

G-8

FURAN EVALUATION IN BABY FOOD BY HS-GC-MS METHOD – IN-HOUSE VALIDATION STUDY

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The discovery of furan in food caused public concern, since this substance was classified by the International Agency for Research on Cancer (IARC) as possible carcinogenic to humans [1]. The European Food Safety Authority (EFSA) published an opinion on provisional findings on furan in food in 2005 [2] and is currently collecting data on furan levels in food. Analytical methods for the determination of furan in food are most frequently based on headspace gas chromatography mass spectrometry (HS-GC/MS) [3]. The paper will present results of an in-house validation study of a HS-GC/MS method for the determination of furan in selected types of baby food. The method is based on isotope dilution with [$^2H_4$]-furan. The method performance was evaluated for the concentration range of 5 µg/kg to 100 µg/kg. Precision of the analysis was high over the whole concentration range with relative standard deviations below 5 %. Recovery of furan was evaluated from spiking experiments at 3 concentration levels covering the whole working range. The overall recovery of 6 replicate samples at each concentration level was 100 % ±4 %. Besides presenting details on method performance, the paper will also pay special regard to problems related with sample handling.

[1] International Agency for Research on Cancer IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 63 (1995), Lyon, France
[2] Report of the CONTAM Panel on provisional findings on furan in food. (2005) http://www.efsa.europa.eu/en/science/contam/contam_documents/760.html
[3] Determination of furan in foods. (2006) http://www.cfsan.fda.gov/~dms/furan.html

G-9
DETERMINATION OF FURAN IN AUSTRIAN FOOD SAMPLES BY HEADSPACE GC/MS: FIRST RESULTS

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Furan is a volatile compound of weak aromatic character and of major toxicological relevance since it is classified as possibly carcinogenic to humans (IARC, 1995). Its formation in food samples usually occurs during thermal processing.

In May 2007 the ministry of health started a monitoring program to survey Austrian food samples concerning their furan contents. Samples were provided by local food control authorities.

A headspace gas chromatography/mass spectrometry (HS/GC/MS) based method published by the U.S. Food and Drug Administration (FDA) in 2004 was applied by us for furan identification and quantitation. Additionally a re-validation was made.

This methodology was used for analyses of 22 Austrian food samples including different kinds of baby food (of vegetable or of animal origin), bakery products, ground coffee and canned food. The samples were analyzed either as purchased in local supermarkets or as commonly consumed after further preparation (e.g. toasting).

The results of the monitoring program indicate furan contents up to 2200 µg/kg determined in coffee beans. In baby food contents are about 30 µg/kg, but further preparation steps seem to diminish the furan content.

These findings are comparable to furan contents reported in literature. The results of the Austrian monitoring program are a first overview and are no reflection of the actual distribution of furan in food. They should not be interpreted as suggesting possible risks associated with eating certain foodstuffs. The number of samples and the variety of food products examined in this study are far too limited to draw any conclusion with respect to human health risks. At the moment there is no justification to recommend any changes in dietary habits.

In its report (2004), EFSA concluded that a reliable risk assessment would need further data on toxicity and exposure. It is necessary to generate more data across the European Community on levels of furan in heat treated foodstuffs. Further studies and further data collection are therefore planned in Austria to get more background information of furan levels in Austrian foodstuffs.

G-10
DETERMINATION OF 1,3-DICHLOROPROPAN-2-OL AND 3-CHLOROPROPANE-1,2-DIOL FATTY ACID ESTERS

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3-Monochloropropane-1,2-diol (3-MCPD) and 1,3-dichloropropan-2-ol (1,3-DCP) are members of group of chemicals known as chloropropanols. 3-MCPD and 1,3-DCP are well-known contaminants of acid hydrolysed vegetable protein (acid-HVP), a frequently used savoury food ingredient is produced by treating protein-rich materials with concentrated hydrochloric acid at high temperatures. Monochloropropanediols and their esters have been shown to be precursors to dichloropropanol formation. This phenomenon has led industry and regulatory experts to predict that manufacturing changes to reduce or eliminate 3-MCPD also could control 1,3-DCP in acid-HVP.

3-MCPD occurs either as a free compound or bound in fatty acid esters. Fatty acid esters of 3-MCPD are stable intermediates or by-products of the pathway leading to 3-MCPD. They were found in raw acid-HVP and recent findings indicate that the formation of 3-MCPD esters is characteristic of a variety of processed and unprocessed foods and various food ingredients. These compounds represent a new class of food contaminants, the bound form of 3-MCPD, which could be released by a lipase-catalyzed hydrolysis reaction. Generally, the amount of 3-bound MCPD in any food or food ingredient largely exceeds that of free 3-MCPD.

The aim of this work was to develop the method for determination of total amount of 3-MCPD and 1,3-DCP and of their fatty acid esters in different foods. This method is based on separation of the fat isolated from food to the individual fractions using chromatography on a silica gel column. These fractions are concentrated and analysed by GC/MS using deuterated internal standards. Total amount of 3-MCPD mono- and diesters and 1,3-DCP esters is determined after acid-catalysed methanolysis of individual fractions to release 3-MCPD and 1,3-DCP. Validation of the method was carried out by analysing palm oil spiked with 3-MCPD monoesters and diesters and 1,3-DCP esters. This method has been applied for the monitoring mono- and diesters of 3-MCPD and esters of 1,3-DCP in a range of different foods and for the determination of the total amount of 3-MCPD and 1,3-DCP bound in esters with higher fatty acids.

G-11

STRUCTURAL CHARACTERIZATION OF BOVINE BETA-LACTOGLOBULIN-GALACTOSE/TAGATOSE MAILLARD COMPLEXES BY SPECTROSCOPIC METHODS

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Although beta-lactoglobulin (β-lg) has diverse applications in the food industry, considerable efforts are being made to improve its functional properties and, hence, to increase its degree of applicability. Glycation by Maillard reaction can be an efficient method to generate new modified proteins with great industrial and technological interest, as it avoids the use of chemical compounds. Since modifications of the native structure of proteins induced by glycation can influence their functional properties, structural studies on glycated proteins can give useful information about their structure-function relationship. Among the established methods for the structural characterization of glycated proteins, the spectroscopic techniques have demonstrated to be very suitable due to their easy handling, short time of analysis and relatively easy interpretation of the results.

The aim of this work was to investigate by spectrophotometric and spectrofluorometric methods the structural changes of β-lg following glycation with galactose and tagatose, and to evaluate the influence of the sugar carbonyl group on the extent of glycation. To promote the formation of covalent complexes, the reaction was performed in solid state (aw 0.44) and pH 7.0 at 40 and 50ºC. Assessment of protein glycation extent was performed by free amino groups determination (TNBS method). The structural changes were analysed through determination of the surface hydrophobicity (using ANS and retinol as fluorescent ligands) and the intrinsic (tryptophan) fluorescence, as well as by studying the Maillard reaction in its advanced state with respect to colour formation and accumulation of characteristic fluorescence of the so-called advanced glycation end products (AGEs). In the initial stages, a faster loss of free amino groups, a less surface hydrophobicity and a greater shift of the emission maximum of tryptophan towards longer wavelengths with galactose (aldose) than with tagatose (ketose) were observed. These results indicated a higher reactivity of galactose, probably due to that aldehyde carbonyl groups are relatively more electrophilic than ketose ones. However, in the advanced stages of the reaction, no differences between aldose and ketose were found regarding the loss of free amino groups, AGEs fluorescence, and colour formation, which could be explained by the greater reactivity of the Heyns compound formed from tagatose. The methods used in this study allowed the evaluation of the structural changes occurred during β-lg glycation, which could have a significant impact on the improvement of β-lg functionality.

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G-12

DEVELOPMENT OF HEAD SPACE SAMPLING/GAS CHROMATOGRAPHY-MASS SPECTROMETRY (HSS/GC/MS) FOR RESIDUAL SOLVENTS IN HEALTH FUNCTIONAL FOODS

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The analytical method to screen for the remaining solvents in health functional foods was developed using a Head Space Sampling/Gas Chromatography-Mass Spectrometry (HSS/GC/MS). Eleven volatile organic solvents including acetone, hexane, trichloroethane, heptane used to extract the functional compounds were aimed. Gas Chromatograms for eleven compounds were acquired and specified. The head space sampling method developed was compared with liquid-liquid extraction (LLE) and solid phase micro extraction (SPME) by carrying out recovery and reproducibility. The validation of the developed HSS/GC/MS method was carried out for precision, accuracy, recovery, linearity and adequate sensitivity. Therefore, the above method could be applied for the monitoring for the remaining solvents of the health functional foods in current market

G-13

DEVELOPMENT AND OPTIMISATION OF A SUB ROOM TEMPERATURE SPME-GC-MS METHOD FOR THE ANALYSIS OF FURAN IN FOOD

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Furan is classified as "*Possibly carcinogenic to humans*" (Group 2B) by the International Agency for Research on Cancer (IARC) since 1995. In 2004 the United States Food and Drug Administration (US FDA) find this undesirable compound in a variety of canned and jarred food. Recently, European authorities as well as member states need information about the contamination levels of the food chain.

First studies show that some matrices like baby food and instant coffee are contaminated from sub-ppb to hundreds ppb's levels. Other like bread and milk have levels below the current detection limits.

According to the physicochemical properties of furan the fit for purpose technique is a gas phase extraction coupled to gas chromatography – mass spectrometry (GC-MS); the isotopic dilution technique (ID), with a D_4-furan, is useful for the quantitative approach. The most promising extraction technique reported in the literature is the headspace Solid Phase Micro Extraction (SPME) because it usually provides better detection limits than static headspace.

Time and temperature are generally the most influencing parameters on SPME extraction efficiency. Stirring and salt addition might also have some influence. However, a Plackett-Burman experimental design reveals a slight influence of stirring and salt addition while it shows a sound interaction between extraction time and temperature. A Central Composite Design (CCD) experiment plan is further used to optimise them together. First results show that the temperature elevation has a negative effect on the extraction efficiency. Further experiments demonstrate that the optimum conditions are at sub room temperature (r.t.). Tests carried out under different matrices show that the optimum conditions are matrix dependant. For example, the optimum conditions for carrot baby food are an extraction of 26 minutes at 4°C and for water are 15 minutes at 12°C. These settings allow to detect quantities until sub-nanograms in the first case to 10 picograms in the second.

G-14

ON-LINE STACKING TECHNIQUES FOR THE NONAQUEOUS CAPILLARY ELECTROPHORETIC DETERMINATION OF ACRYLAMIDE IN PROCESSED FOOD

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The discovery of acrylamide formation in cooked food is a recent development. Consequently intensive activity began examining the many different types of food and thousands of analyses have been undertaken worldwide. Thus, efforts should also be focused on the development of inexpensive, convenient, and rapid screening methods that would be easily used by many laboratories. Capillary zone electrophoresis (CZE) is a separation method known for easy method development, low sample consumption, fast analysis times, and simple experimental apparatus. Since CZE separation is based on the differentiated migration in an electric field of analytes, based on charge/size ratios, and since polar but uncharged acrylamide cannot achieve electrophoretic mobility in an aqueous background electrolyte, the CZE method cannot be used for acrylamide. However, in the present study, a nonaqueous capillary electrophoresis method (NACE) was introduced for the quantitative determination of acrylamide in processed food. The method is premised on the modification of the aqueous acid-base character of acrylamide in an organic solvent. Acrylamide, which is a polar molecule in aqueous solution, in a low pH environment in acetonitrile acquires a proton and thereby migrates under its own electrophoretic mobility in capillary electrophoresis. Accordingly, nonaqueous separation of acrylamide was achieved employing 30 mmol/l $HClO_4$ in acetonitrile as the running electrolyte A field-amplified sample stacking method was developed for the on-line concentration of the acrylamide to improve acrylamide detection at 200 nm by diode-array detection. The conditions of stacking were systematically optimized and applied to the determination of acrylamide in several foodstuffs.

G-15

GAS CHROMATOGRAPH-MASS SPECTROMETRIC ANALYSIS OF RESIDUAL SOLVENTS IN HEALTH FUNCTIONAL FOODS WITH HEAD SPACE SAMPLING

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The analytical method to screen for the remaining solvents in health functional foods was developed using a Head Space Sampling/Gas Chromatograph-Mass Spectrometer (HSS/GC/MS) system. Eleven volatile organic solvents including acetone, hexane, trichloroethane, heptane used to extract the functional compounds were aimed. After homogenizing and weighing of health functional food, the fraction of analyte was pour into the vial in HSS. The gas Chromatograms for eleven compounds were acquired and specified. The head space sampling method developed was compared with liquid-liquid extraction (LLE) and solid phase micro extraction (SPME) by carrying out recovery and reproducibility. The validation of the developed HSS/GC/MS method was carried out for precision, accuracy, recovery, linearity and adequate sensitivity. Therefore, the above method could be applied for the monitoring for the remaining solvents of the health functional foods in current market.

G-16

APPLICATION OF RT-PCR AND GC-MS METHODS FOR DETECTION OF WINE SPOILAGE BY BRETTANOMYCES

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Brettanomyces is a non-spore forming genus of yeast in the family *Saccharomycetaceae*, and is often referred to as "Brett". *Brettanomyces* is important to both the brewing and wine industries due to the sensory compounds it produces. When *Brettanomyces* grows in wine it produces several compounds that can alter the bouquet. There seems to be a world-wide tendency that more and more winemakers accept the presence of these compounds at low levels as they have a positive effect on wine, contributing to complexity, and giving an aged character to some young red wines. The sensory threshold may differ between individuals, and so some find the compounds more unattractive than others. However, when the levels of the sensory compounds exceed the sensory threshold, their perception is most often negative. Wines that have been contaminated with *Brettanomyces* taints are often referred to as "Bretty", "mousy", or as having "Brett character"

From a winemaking point of view there is no guarantee that high levels will not be produced – compared to an initially tolerable level. Unfortunately a single analysis cannot tell whether the spoilage has just started or already finished, that is why it is advised to monitor the *Brettanomyces* population and/or its metabolic products over time. This requirement makes the speed and robustness of the applied methods a key issue.

In our studies the same set of wine samples have been analyzed by three different approaches: real-time polymerase chain reaction (RT-PCR), gas chromatography – mass spectrometry (GC-MS) and sensory evaluation. The used simple RT-PCR technique provides a present/absent result, with a sensitivity of 1 cell/ml sample[*]. The concentration of the two key metabolites (4-ethylphenol and 4-ethylguaiacol) was determined by GC-MS, and the sensory characteristics were also described. The comparison of the results obtained shows that the RT-PCR technique is more reliable (sensitive) when the infection needs to be detected in its early stage. However, all the three methods (including sensory analysis) have their own role in the detection of *Brettanomyces* infection and in the quality control of wines.

* Real-time PCR assay for detection and enumeration of Dekkera bruxellensis in wine, Applied and Environmental Microbiology, 69 (2003) 7430?7434.

G-17
MIGRATION OF NONYLFENOLS FROM POLYMER PACKAGING MATERIALS INTO FOOD SIMULANTS

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p-Nonylphenol (NP) is widely used in many industrial applications (detergents, latex paints, pesticides and plastics), but its presence in the environment has become of increasing concern since it has been shown to be an estrogenic compound besides its persistence and toxicity. Four samples of stretch PVC films for food packaging obtained from the food producers were analyzed for the presence of NP, two of the PVC films contained NP at concentrations 1.28 and 1.72 mg/g respectively, in remaining films NP was not detected (detection level being 5 μg/g). The NP positive films were used for the migration studies into the food simulants. The amount of NP migrating into the used food simulants: distilled water, 3% acetic acid solution and 95% ethanol were 0.041 - 0.091 (3.2 – 5.3%), 0.040 – 0.079, (3.2% - 4.6%) and 0.371 - 0.449 mg/g (21.5 - 35%). The potential safety risks arising from the obtained results as well as the possible sources of the NP contamination in the analyzed stretch PVC food films are discussed.

G-18
DEODORISATION OF TRIPE BY PRESSURISED COOKING

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Tripe is a type of edible offal made from the stomach of various domestic animals. Beef tripe is typically made from the first two of cattle's four stomachs, the rumen and the reticulum. Indole and skatole (3-methylindole), tryptophane metabolites, are responsible for the typical unpleasant fecal smell of tripe. These substances are formed in rumen by microbial degradation of dietary protein and cause the off – flavor in food products. The formation and/or retention of tryptophane metabolites poses a serious problem in the processing of meat and offal of ruminants and pigs.

The aim of the presented work was to evaluate the effect of pressurised cooking of tripe on the retention of indole and skatole. The pressurised cooking can be an alternative to the conventional boiling of tripe, which is time, energy and water consuming. The efficiency of treatment in tripe deodorisation seems to be limiting factor of potential use of the pressurised cooking. The effectiveness of the procedure was assessed using the analyses of indole and skatole distribution within the tripe's tissue (surface, muscle, fat, rumen, reticulum and omasum) and balancing of volatile compounds content during the pressurized and atmospheric cooking under the different conditions (pressure, time, vent).

The substances responsible for the scent were analysed by headspace solid-phase microextraction and gas chromatography-mass spectrometry (SPME-GC-MS). Studies on pressurised cooking of tripe showed that due to the low solubility, relatively high boiling point of skatole and restricted access of water to blocks of frozen tripe the removal of unpleasant aroma within the industrial processing is difficult. As the result of the project the optimal processing conditions were suggested and the affecting factors were evaluated. The highest average concentration of skatole and indole were found in the omasum (0.044 mg/kg) and the in the rumen (0.05 mg/kg), respectively. The skatole and indole content was reduced in half by traditional cooking and to third by pressure cooking.

G-19
ACRYLAMIDE RISK IN FOOD PRODUCTS: THE SHORTBREAD CASE STUDY

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The risk assessment of foods includes the evaluation of microbiological and chemical safety. The recent discovery of acrylamide in cooked foods has raised public concern about food safety because, on the basis of numerous studies, the International Agency for Research on Cancer (IARC) has classified acrylamide as “probably carcinogenic to humans”.

Among advanced hypotheses concerning acrylamide formation in cooked food, the Maillard browning reaction of a sugar and amino acid had received much attention as the most likely pathway. Several factors, such as the reactants concentration and ratio, temperature and time of cooking, and pH and water activity, have been shown to influence the formation levels of acrylamide in heat-processed foods.

Bakery products such as biscuits represent a class of food in which the effect of ingredients and processing promote the acrylamide formation. The aims of this research were the set up of an analytical method to determine acrylamide concentration in shortbread biscuits and the study of the technological parameters influencing the acrylamide formation during shortbread processing.

After centrifugation, filtration and SPE purification the shortbread extract samples were analysed by the combination of reverse-phase high-performance liquid chromatography coupled with Diode Array Detector (DAD). The chromatographic separation was achieved with isocratic analysis in a 10-min run. The method was reliable and sensitive. The coefficient of determination of the acrylamide standard calibration curve is 0.999; the limit of detection was 15 µg/kg and the quantification limit was 30 µg/kg. To establish the efficiency of the proposed method, the recovery test was conducted adding three different concentrations of acrylamide standard solution to the blank sample. The recovery of acrylamide ranged from 90% to 99%.

Five technological tests were conducted to produce five different experimental shortbreads. The experimental shortbreads were obtained using different amount of baking agents (sodium bicarbonate and ammonium bicarbonate) and applying different cooking temperatures and different times.

The results showed that particular conditions of ammonium bicarbonate concentrations in shortbreads and cooking temperatures influenced the acrylamide formation during the processing highlighting that to avoid the presence of a dangerous concentration of acrylamide, the processing parameters should be taken under control.

G-20

EXPOSURE TO ANTIMONY FROM THE CONSUMPTION OF READY-TO-EAT MEALS PREPARED IN POLYETHYLENE TEREPHTHALATE (PET) TRAYS

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Ready-to-eat meals and other products packed in ovenable PET (polyethylene terephthalate) trays were analyzed for antimony. As a result of the use of antimony trioxide (Sb2O3) as polycondensation catalyst in the polymer production, commercial resins contain residues between 150 - 300 mg/kg. The toxicity of antimony raised concerns about consumer safety. The migration of small fractions of these residues into ready meals and foods as an effect of cooking in PET trays was studied. Retail samples were measured to obtain accurate exposure data. In addition, the antimony background concentration was determined separately from a series of reference meals, which ranged from not detectable to 3.4 µg/kg. Microwave and conventional oven cooking caused a distinct increase in the concentration of antimony in food and ready meals. Depending on industrial preparations antimony concentrations between 20 and 35 µg/kg were found. Meat or vegetables prepared in PET roasting bags and ready made dough baked in PET dishes showed even higher antimony concentrations that ranged up to 240 µg/kg in the final product. About half of the ready meals prepared at a temperature of 180°C exceeded the specific migration limit set for the food contact material by the European Commission. However, even if the most contaminated ready-meals analysed in this study are consumed regularly the expected exposure would not be problematic from a toxicological point of view. Nevertheless, the antimony contribution from the use of such products clearly exceeds the usual dietary intake of this element.

G-21
DETERMINATION OF ACRYLAMIDE IN FOOD BY ON-LINE SAMPLE CLEAN UP LC-MS/MS

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Acrylamide is considered probably carcinogenic to humans. Its finding in food has initiated many research activities on, e.g. mechanisms of formation, or monitoring of its content in food. A number of analytical methods has been proposed so far for that purpose. Currently applied sample preparation methods include several steps such as extraction, centrifugation, solid phase extraction, and pre-concentration. These are laborious, time consuming, and expensive. A higher degree of automation could save in that respect time, money, and could improve the sample throughput. This study aimed to develop an on-line sample clean up liquid chromatographic tandem mass spectrometric analysis method for the determination of acrylamide in various types of food. Different types of commercially available clean up columns were tested. Best results were gained with a cartridge containing a hydrophilic lipophilic balanced copolymer (Oasis® HLB). The poster will present the instrumental set up as well as method performance data.

G-22
STUDY OF "MASKED" ACRYLAMIDE ORIGIN IN CHEMICAL MODEL SYSTEMS

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Recently, alkaline extraction of acrylamide from heat-processed foodstuffs was shown to give significantly higher results as compared to common procedures using water for target analyte isolation. Our analyses of various foodstuffs employing alkaline extraction showed increase of acrylamide levels up to 200%, as compared to levels found in neutral extracts. The follow-up study documented, that not improved extractibility of acrylamide, but decomposition of some water-soluble Maillard intermediates seem to be responsible for this phenomenon.

In model experiments, formation of „masked" acrylamide in various model systems was monitored, moreover, we attempted to identify precursors from which it is released under alkaline conditions. Both neutral and alkaline aqueous extracts obtained from pyrolysates of binary mixtures (asparagine with sugars, low molecular carbonyls and furane derivatives) and some known acrylamide precursors (N-glycosides, 3-APA) were analyzed for acrylamide content using LC-MS/MS method. Comparing amount of acrylamide extracted at pH 12 and 7, the ratio as high as 31 was determined in model pyrolysate prepared from asparagine and fructose heated 3 minutes at 140°C. For glucose and fructose this value was 10 and 1.5, respectively. The yield of „masked" acrylamide in extracts from pyrolysates prepared at 180°C was lower. Regardless pyrolysis temperature and sugar used, decrease of alkaline extractable acrylamide was observed with prolonged heating time.

Considering various Maillard reaction pathways and reaction intermediates studied, imines of asparagine with methylglyoxal and low molecular hydroxycarbonyls were proposed as the most potent precursors of „masked" acrylamide. Liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS) was used to investigate the pyrolysates for presence of such compounds.

This study was carried out with support from the Ministry of Education, Youth and Sports, Czech Republic - partly from the project MSM 6046137305 and project FRVS G4 2113, partly within the project COST OC 927.

G-23
STUDY OF ACRYLAMIDE LEVELS IN MALTS

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Alike many others heat processed food products prepared from raw materials rich in starch/reducing sugars, malts may contain acrylamide, a toxic processing contaminant originated from precursors contained in barley. Acrylamide levels depend on the temperature and the time employed for treatment of respective produtcs. In our study, we analysed two sets of malts that are commonly used as additives (colorants, aroma donors etc.) in bakery and/or brewing industry. The levels of acrylamide as high as 900 µg/kg were found in caramel rye malt used for the production of the speciality beers. Generally, the higher was the temperature used for roasting (dark, caramel malts), the higher acrylamide levels were found. No acrylamide was detected (LOD of LC-MS/MS method) in pale wheat malts. In any case considering high solubility of acrylamide in water, beer has to be considered as one of potential source of dietary acrylamide intake.

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G-24

A CAPILLARY ELECTROPHORETIC ANALYSIS OF ACRYLAMIDE IN FOOD BY DERIVATIZATION OF GLUTATHIONE

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Acrylamide is a useful industrial chemical that was labeled as a probable human carcinogen by the International Agency for Research on Cancer. Recent studies have shown that acrylamide was formed during the Maillard reaction. Food processed or cooked at high temperature like potatoes chips, French fried potatoes contains considerable levels of acrylamide according to World Health Organization (WHO).

In the present study, a capillary electrophoretic method was introduced to analysis of acrylamide in food. Acrylamide reacts easily and quickly with nucleophiles, especially α,β-unsaturated amides, imides and nitriles and many of the biological important proteins contain glutathione which is the princial thiol. With the benefit of this information, we used glutathione derivative to gain mobility to acrylamide for the capillary electrophoretic separation of this analyte. Derivatisation of acrylamide was achieved under nitrogen media to remove oxygen, and the reaction mixture stayed in the dark for 3 hour. The optimal glutathion-acrylamide ratio was selected as 1:4 and the concentration of the separation electrolyte was 10mmol/l phosphate at pH 7 for optimal capillary electrophoretic separation of acrylamide.

AUTHENTICITY, TRACEABILITY, FRAUD

(H1 – H23)

H-1
VERIFICATION OF THE GEOGRAPHICAL ORIGIN OF OILS AND FATS BY PTR-MS

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Consumers' interest in the origins of food has significantly increased over the last few years. Protected Designated Origin (PDO) regulations permit the labeling of some European products with the names of the areas in which they are produced. This certification increases the commercial value of the product. From a fair trade and a regulatory perspective there is a need for analytical procedures which are able to confirm the validity of the labeling claim. One of the reasons to purchase PDO products is the expected sensory properties. Volatile compounds are responsible for the characteristic aromas of oils and fats. In the present study Proton Transfer Reaction Mass Spectrometry (PTR-MS) was evaluated for verification of the geographical origin two model products: European olive oils and European butter samples. 182 Olive oils were assessed. They originated from Cyprus, Greece, Italy, and Spain. These oils all had 'declared geographical origins' (PDO). Samples were obtained courtesy of the EU-funded TRACE project (trace.eu.org). 83 Commercial butters from the south of Europe (France, Italy, Portugal, Spain, Switzerland), the north/west of Europe (Belgium, Denmark, Germany, Sweden, Ireland) and the east of Europe (Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Slovakia, Slovenia) were collected by EU-JRC IRMM in Geel, Belgium. The headspace of the fats and oils was analysed for their volatile compositions by Proton Transfer Reaction Mass Spectrometry. The headspace of the samples was equilibrated for 45 min and without further pre-treatment directed to the inlet of a PTR-MS system. The mass spectra (m/e 20-150) were acquired rapidly: collection of one full mass spectrum within 30 s. The average fingerprint mass spectra of two or three replicates were used for further calculations after background and transmission corrections. The mass spectral data were subjected to PLS-DA in order to estimate a classification model for the samples. The origin of the olive oil and butter samples were predicted using separate models. The performance of the fitted model was evaluated by cross-validation using a leave-10%-out procedure. Two random permutations of the class labels were carried out to verify the model. Cross-validation provided that 90% of the olive oil samples were correctly identified by their country of origin. The prediction of the origin of the butter samples was successful for 76% of the samples. The present study showed interesting correlations between the volatile compositions of oils and fats and their origins. It also showed the potential of PTR-MS as a rapid tool for origin verification.

H-2
INORGANIC PATTERN OF PUMPKIN SEED OIL – COMPARISON AUSTRIA AND CROATIA

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Oils extracted from plants have been used since ancient time for culinary purposes in many cultures. One reason for the application of olive or pumpkin seed oil in cooking is their flavour.

Pumpkin seed oil (Kürbiskernöl) is made by pressing the roasted skinless seeds of a special type of pumpkins, the "Styrian Oil Pumpkin" (lat. Cucurbita pepo var. styriaca, also known as var. oleifera). It is not only a culinary specialty of Styria, Austria, but also widely used and produced in the northwest parts of Croatia. Pumpkin seed oil is viscous and its colour ranges from dark green to dark brown. Its taste depends on the colour, i.e. brown oil bitter while green pumpkin seed oil tastes nutty. Pumpkin seed oil is very healthy due to its richness in polyunsaturated fatty acids.

Trace elemental oil characterization, i.e. their inorganic pattern, is the basis for further nutritional and food technological investigations such as adulteration detection. Furthermore the trace elemental composition of the oil depends on the geographical origin.

In the presented study pumpkin seed oils from Croatia were analyzed for their trace elemental content. The samples underwent an acidic microwave assisted digestion step prior to analysis by ICP-AES. The concentration of the following minor and trace elements were determined: Ca, Fe, K, Mg, Na, and Zn as well as Al, Cd, Cu, Co, Cr, Ni, Mn, Mo, Pb, and V. The results found for Croatian oils were compared with data for Austrian pumpkin seed oils obtained previously.

Statistically significant differences in the inorganic pattern of the oils were found for the minor as well as for the trace elements. For example Croatian pumpkin seed oils contain 5 – 10 times more Ca, but five times less Na and two times less Mg. Regarding the trace elements higher amounts of Cd, Cr, and Cu, but less Al and Ni were found in Austrian pumpkin seed oils. Pb could not be detected in pumpkin seed oils from both countries.

Based on these findings a geographical characterization of pumpkin seed oils using their inorganic pattern might be possible.

H-3
DETECTION OF MILK ADULTERATION ON PDO CHEESES BY PRINCIPAL COMPONENT ANALYSIS OF FATTY ACID DATA

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The total fatty acid composition has not previously been reported to be used for assuring the authenticity of milk or cheeses with respect to the milk origin, although fatty acid ratios have been used with success to discriminate between milk fat from bovine, ovine and caprine species (Ulberth, 2003, In: Food authenticity and traceability, 357-377, (Lees, M., Ed.)). Some studies based on fatty acid composition determined by gas chromatography have revealed that certain fatty acids could be potential indicators of the origin of cream and also of the Protected Designated Origin (PDO) "mountain" cheeses (Collomb et al., 1999 *Sciences Des Aliments*, 19, 97-110; Collomb et al., 2002, *International Dairy Journal*, 12, 649-659).

The three different milks that can be used for the manufacture of Portuguese PDO cheeses have different fatty acid composition. The research presented here is focussed on analysis of the lipid fractions of bovine, goat and ovine milk to evaluate their potential for milk authentication. Afterwards, the same methodology was applied to fat extracted from the thirteen PDO cheeses.

Instead of using a univariate approach, the information content of the total fatty acid profile can be more efficiently exploited by multivariate analysis. A pattern classification procedure, principal component analysis (PCA), was applied to the dataset to compare similarities or differences between the milk samples and cheese samples using fatty acid composition. In addition the ratio between some fatty acids led to some interesting conclusions regarding the bovine cheese samples, which always proved to be outliers on the graphical analysis. The calculation of the fatty acid ratios and the use of chemometrics to analyse the data set highlighted a possible future application of this approach to identify the geographical origin of a product and at the same time the authenticity of the milk origin. This approach could be applied to samples of PDO cheeses where the process is controlled from “farm to the fork”. A database with the typical fatty acid ratios of the cheeses, at the point of the sale, could be constructed and constitute a robust model for origin assignment.

H-4

PROFILING OF FOOD USING EXACT MASS TIME-OF-FLIGHT MASS SPECTROMETRY: APPLICATION OF CHEMOMETRICS FOR THE IDENTIFICATION OF POTENTIAL MARKERS

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A number of challenges exist for scientists in quality control laboratories for the food industry; identifying process changes that may lead to a change in quality, identifying the geographical origin of raw materials and detecting economic fraud due to product substitution and adulteration, as well as health risks from possible contamination. Food quality, authenticity and traceability are becoming increasingly important as globalisation continues. In 2004, about 4.4 million items of fake foodstuffs and drinks were seized at EU borders, a growth of about 200% since 1998[1].

QC issues are traditionally assessed by experts but this approach requires intensive training and also can still be subjective. Instrumental analysis would be advantageous but quality is a difficult parameter to quantify and the scientist is forced to measure indirect parameters that, individually, may be only weakly correlated to the properties of interest. Indirect parameters can be thought of as multivariate data. Patterns emerge that are related to quality and can be recognized. Chemometrics is a statistical approach to the interpretation of patterns in multivariate data.

Food commodities such as beer, coffee, tea and wine were studied using either ultra performance liquid chromatography or gas chromatography exact mass time-of-flight mass spectrometry (UPLC- or GC-TOF-MS). The separation methods developed to differentiate these commodities were fast and general, and can be applied equally well to the analysis of various other foodstuffs. The elevated resolution of TOF-MS with exact mass capabilities facilitates the deconvolution of the data without the need for complete chromatographic separation.

A chemometric approach based on principal component analysis (PCA) using the MarkerLynx application manager was used to mine exact mass TOF data from beer, coffee, tea and wine. Potential markers were successfully identified from each commodity using a combination of library searching and exact mass.

[1] http://www.foodqualitynews.com/news/ng.asp?n=68595-counterfeiting-intellectual-property-ciaa

H-5

MULTI-PARAMETRIC MEASUREMENT BY IRMS AND ICP-MS FOR THE TRACEABILITY OF THE ITALIAN PDO OLIVE OILS

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Isotopic ratios of stable bioelements (carbon, oxygen, sulphur, hydrogen, nitrogen) have been applied for more than 20 years in food authenticity control for several commodities (fruit juices, honey, wine, spirits and flavours). In this work we tested the suitability of $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$ as possible markers of geographical provenance of olive oil. About five hundreds of authentic Italian samples were collected up to 2000's since 2005' crop and submitted to analysis of $d^{13}C$ of bulk olive oil, $\delta^{13}C$ of glycerol and $\delta^{18}O$ of dried glycerol by Isotopic Ratio Mass Spectrometry (IRMS). The data were evaluated by crop and region and the isotopic values changed according to the latitude for all the years considered, but also according to the altitude and the distance from the sea even if the crop may play a significant role due to particular climatic conditions.

In about a hundred of samples of 2005' crop also $\delta^{18}O$ and $\delta^{2}H$ of bulk were determined to evaluate their relationship with $\delta^{18}O$ of glycerol which extraction is very time consuming. The correlation between $\delta^{18}O$ of glycerol bulk resulted significative, while $\delta^{2}H$ presented clear capabilities of regional differentiation.

Further, the analysis of mineral content by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after a microwave digestion was validated on olive oil and here it's presented a survey of the data obtained on some authentic samples collected during 2005' crop.

H-6
FATTY ACID PROFILE AND TOTAL LIPID CONTENT IN CHIONOCETES OPILIO SAMPLES

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Marine species are the main sources of ω3 fatty acids. In the last years, the utilization of the by-products from fisheries as feed in fish farms has attracted the attention of the aquaculture industry.

Several procedures for total lipid extraction have been described in the literature; however there is not unanimous agreement on the best.

In the present study, three methods (Soxhlet, Bligh and Dyer and Supercritical Fluid Extraction) to determine total fat content from *Chionoecetes opilio* shells were evaluated and compared. The fatty acid profile was determined by gas chromatography-flame ionization detection using a one-step-extraction-methylation method to obtain fatty acid methyl esters. GC-MS was used as a confirmatory technique.

The Soxhlet extraction showed the highest efficiency.

Twenty-one fatty acids were identified, as it was expected, a high content of ω-3 polyunsaturated fatty acids were found, (35.89% of the total fatty acid content), among them C20:4 ω3 (11.63%) was the predominant. Monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) were in identical percentage, 39.22% of the total fatty acid content and 39.80% respectively. The major saturated fatty acid was palmitic acid (C16:0).

H-7
CHARACTERIZATION OF POLISH RAPE AND HONEYDEW HONEY ACCORDING TO THEIR MINERAL CONTENTS USING ICP-MS

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Poland is significant manufacturer of honey, particularly high quality of honeys. The considerable quantities of bee's product are exported to European markets. In recent years the authentication is a wide-ranging issue and has come to prominence. It is the important thing for a honey consumer, which means the agreement with declared botanical origin and also it is a great importance for commercial. Honey specification consistent with its declared botanical origin is an important thing for consumer as well as its nutritious, prophylactic properties and unique flavor. This is why the estimation of quality parameters of honey is so significant. The Council Directive including general and specific honey compositional characteristics for testing the authenticity of botanical origin but there is nothing about trace element anlysis [1].

The determination of trace elements such as Ca, Mg, Zn and Sr or Mn is widely used in food authenticity studies. There are some studies in which authors made the botanical classification of honeys based on the estimation of mineral content. The elemental content of honey has been determined by many authors all over the world and many various methods was used [2,3]. The aim of these studies was differentiation of Polish honeys from different botanical origins according to their mineral content. In this work, seventeen elements (K, Na, Ca, Mg, Zn, Fe, Al, Mn, Cr, Ni, Sr, Co, B, Ba, Cu, Cd, Pb) were determined in 21 honey samples from different places of Poland and two different types of honey- rape and honeydew. Trace elements (Ni, Sr, Co, B, Ba, Al, Mn, Cr) and toxic elements (Cd, Pb) were determined by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) and major element (K, Na, Ca, Mg) and Fe, Zn, Cu were determined by Atomic Absorption Spectrometry (AAS).

Chemometric techniques are used to classify honeys according to their origin on the chemical data. Cluster analysis of honeys data revealed that the origin of honey samples correlated with their chemical composition. It is shown that rape honey include lower contents of manganese than honeydew honeys. Also honeydew honey include much higher concentrations of K, Fe, Cu, Al, Ni, Co in comparison with the rape honey. It shows that honeydew honey have more mineral content, what is connected with sources which is made honey. Trace element analysis showed that the differences in the values found in honey samples could be used as an evidence of quality of honey samples.

[1] Official Journal of the European Communities, Council Directive 2001/110/EC
[2] Fernandez-Torres R., Perez-Bernal J. L., Bello-Lopez M. A., Callejon-Mochon M., Jimenez-Sanchez J. C., Guiraum-Perez A. „ Mineral content and botanical origin of Spanish honeys" Talanta 65 (2005) 686-691
[3] Hernandez O. M., Fraga J. M. G., Jimenez A. I., Jimenez F., Arias J. J.
[4] „ Characterization of honey from the Canary Islands: determination of the mineral content by atomic absorption spectrophotometry" Food Chemistry 93 (2005) 449-458

H-8

ORIGIN CONTROL OF OLIVE OILS: MULTIVARIATE ANALYSIS OF 1H-NMR PROFILES OF THE UNSAPONIFIABLE FRACTION

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Globalization of the food market has lead to an increasingly broad range of foodstuffs on the market. This, along with increased economic wealth, means that the European consumer is faced with the often difficulty of choosing among the available products. Another inherent factor of such global trade lies in the opportunity that arises for economic fraud on account of the possible financial gains. Such fraud may involve the adulteration of a foodstuff or the falsification of added-value claims, e.g. geographical origin, as can occur in the olive oil sector, making it crucial that its production is controlled and protected.

Moreover Protected Denomination of Origin (PDO) olive oil, being a high-value foodstuff, is often a target for adulteration either through the addition of lower quality olive oil, which does not fulfil the PDO requirements, or the false labelling of oil from a different area of production. The need for validated methods which permit rapid, non-destructive analysis, to guarantee the authenticity and traceability of PDO olive oils is therefore evident. The scope of this study is to evaluate the applicability of NMR and multivariate analysis to verify the nature and origin of such a high-value product, in order to protect both the producer and the consumer.

The authentication of olive oils, considering their geographical and botanical origin, has been previously studied using various analytical approaches, such as NMR (^{1}H, ^{13}C, ^{31}P), FT-raman and FT-MIR spectroscopy, IRMS, LC-MS, GC-MS [1, 2, 3, 4, 5]. However, the number of samples and geographical areas covered so far in the literature has been limited. The present study reports recent investigations carried out on the unsaponifiable fraction of 94 extra-virgin olive oils samples from different geographical origins. In all 6 countries, namely Spain, Italy, Greece, Tunisia, Turkey and Syria were represented; covering most of the Mediterranean basin where 79% of the global production of olive oil takes place. Of each of these oils the unsaponifiable fraction was obtained by a standard procedure, prior to dissolving it in deuterated chloroform and then analysed by a high-throughput ^{1}H-NMR method. The multivariate techniques applied enabled us to filter out the most relevant information from the NMR spectrum, e.g. for classification regarding the geographical characterization of olive oils according to their country of production.

The results achieved prove that this approach; the combination of ^{1}H-NMR analysis and multivariate (chemometric) techniques, demonstrates considerable potential to act as a screening method for confirming the authenticity of a high-value foodstuff such as olive oil.

H-9

LB. BUCHNERI S2 - AS A BLIS-PRODUCING STRAIN ISOLATED FROM TRADITIONAL AZERBAIJANI CHEESE

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Lactic acid bacteria (LAB) are traditionally used as “natural” or “selected” starters in the manufacture of cheeses and fermented milk products because of their functions in preservation and for their contributions to flavors and aromas.

The purpose of current study was the isolation of the bacteriocin-like inhibitory substances (BLIS)-producing strains of LAB from native Azerbaijani cheeses (4 var. cheese) and to partially characterize the inhibitory activities of these BLIS’s.

Unidentified Gram-positive and catalase-negative 4 rods isolated from white cheese and showing positive results after the well diffusion assay against *L. bulgaricus 340*, were subjected to phenotypic identification by API 50 CHL System (bioMerieux, Lyon, France). The fermentation pattern of the isolated strains determined by API 50 CHL System and the rest of properties suggested, that these strains could be identified as *Lb. buchneri* S1, S2, Q2 and M1

The most active of these strains was *Lb. buchneri* S2. The growths of 4 from 6 used reporter strains (*L. bulgaricus 340, E.coli HB 101, E. durans, L. innocua*) were inhibited by the BLIS of this strain, which was inactive against *S. cerevisiae and C. pseudotropicalis*.

The activity of the inhibitory agents produced by the selected strains was tested by the well diffusion method. It was preserved under conditions, which eliminate the possible effect of organic acids by adjusting the pH of cell–free supernatant to pH 6.5 with 6 M NaOH and of hydrogen peroxide by incubation of supernatant for 2 h with catalase (1 mg/ml, in 20 mM in phosphate buffer) at 37^{o}C. The proteic nature of the antimicrobial agent was checked by treatment with the following proteases: pronase (11.4 U/mg), proteinase K (45 U/mg), and trypsin (10.6 U/mg) at final concentration of 1 mg/ml in 20 mM phosphate buffer (pH 7.0). The supernatant was incubated with these enzymes at 30°C for 2h. Complete inactivation or significant reduction in antibacterial activity of the agents produced by the selected strain was observed after treatment of cell–free supernatant with proteinases, what indicated the proteinaceous nature of the active agent. The activities of the strain studied were stable over a wide pH range from 3 to 12.

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H-10

TRACE ELEMENTAL CHARACTERIZATION OF OLIVE OILS DERIVED FROM OBLICA VARIETY

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Olive oil is widely used fruit oil, especially in the Mediterranean region. Its flavour as well as its specific chemical composition including high content of monosaturated fatty acids makes it attractive for the use in cooking. Furthermore olive oil is used for producing cosmetics, pharmaceuticals, and soaps, and as fuel for traditional oil lamps.

More than 1000 varieties of the olive trees used for olive oil production are grown worldwide. The main variety at the Adriatic coast is Oblica, an autochthonous Dalmatian variety, the most frequently variety in Dalmatian olive-groves.

The determination of organic parameters, such as acidity, peroxide value and specific absorptions in the UV region is the basis for the definition of oil quality, and thus widely performed. Although the inorganic analysis of edible oils was neglected, the content of trace and ultra trace elements provides a lot of information on the oil. The inorganic patterns of edible oils can serve as a basis for geographical characterization of oils as well as for adulteration detection.

Since the importance of Oblica variety in Croatia is very high, the objective of the present research work was the elemental characterization of olive oils derived from Oblica variety. All oils analyzed were confirmed as extra-virgin oils according to their free fatty acids, the peroxide value as well as the K-numbers (K232, K270, Δ K). They were produced in two different ways, pressing and centrifugation.

The concentration of trace and ultra trace elements were determined by inductively coupled plasma – optical emission spectrometry (ICP-OES) after acidic microwave assisted digestion. The investigation was focused on 20 elements, namely Al, As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Se, Si, Sn, V, and Zn.

In all oils analyzed the concentrations of As, Cd, Co, Cr, Mn, Mo, Pb, and V were below the limit of detection (LOD). The mode of production is refelected in the inroganic composition of olive oils. Oils produced in the classical way of pressing exhibit higher concentrations of Cu and Fe. Si (approx. 7 µg/g) was found only in these samples, whereas in oils derived by centrifugation the Si contents were below the LOD. Whilst Ni was found in the range of 0.3 up to 0.4 µg/g in oils from centrifugation, no Ni could be detected in pressed oil samples. Since these four elements (Cu, Fe, Ni, Si) depend on the way of production, their concentrations can not be used for characterizing the Oblica variety. The contents of Al, Ca, K, Mg, Na, Se, Sn, and Zn were in the ranges from 0.02 – 0.5, 0.8 – 11, 0.1 – 2.5, < LOD – 0.2, 2.7 – 4.2, 0.6 – 2.9, < LOD – 4.8, and 2.5 – 4.0 µg/g oil, respectively.

H-11

HPLC/MS PROFILING OF TRIACYLGLYCEROLS IN PLANT OILS IMPORTANT IN FOOD INDUSTRY

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Edible plant oils are complex mixtures of various compound classes with the content of triacylglycerols (TGs) from several per cents up to nearly 90%. In human body, TGs form a protective layer surrounding important organs they are also a source of energy, essential fatty acid (linoleic and linolenic acid), lipophilic vitamins (A, D, E, K) and other non-polar compounds. TGs may differ in acyl chain lengths and their stereochemical positions sn-1, 2 or 3 on the glycerol skeleton, and in the number, positions and cis/trans isomerism of double bonds in the acyl chains. Non-aqueous reversed-phase HPLC method was optimized to achieve the highest selectivity for TGs in complex natural mixtures [1-4] using two Nova-Pak C18 chromatographic columns connected in series and acetonitrile – 2-propanol gradient elution for the separation and positive identification of more than 300 TGs containing 35 fatty acids with acyl chain length from 6 to 28 carbon atoms and from 0 to 4 double bonds including TGs containing unusual fatty acids with an odd number of carbon atoms [1] or unusual double bond position [3]. Our quantitation method with the response factors approach [4] was developed for the characterization of complex triacylglycerol mixtures in more than 100 different plant oils. The statistical methods (Principal Component Analysis, Partial Least Squares, etc.) can be used for the division of plant oils into different groups depending on their composition. To verify the precision and accuracy of developed HPLC/APCI-MS method, plant oils were transesterified and analyzed by validated GC/FID method for the analysis of fatty acid methyl esters and the results were compared with HPLC/MS results for TGs. The comparison of average parameters (i.e. carbon number, equivalent carbon number and double bond number) calculated on the basis of GC/FID results on FAMEs and of HPLC/MS results on TGs show a good mutual agreement which confirms the applicability of our quantitation approach. Developed methods for the analysis of TGs are applied for the analysis of wide range of plant oils used in food industry including the positive identification of adulteration or the identification of oil sources based on our database of analyzed plant oils. Our method can be also applied for more polar lipid classes, such as diacylglycerols, monoacylglycerols, transesterfied methyl esters of fatty acids, etc.

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[1] M. Holčapek, P. Jandera, P. Zderadička, L. Hrubá, J. Chromatogr. A 1010 (2003) 195.
[2] M. Holčapek, P. Jandera, J. Fischer, B. Prokeš, J. Chromatogr. A 858 (1999) 13.
[3] M. Lísa, M. Holčapek, T. Řezanka, N. Kabátová, J. Chromatogr. A 1146 (2007) 67.
[4] M. Holčapek, M. Lísa, P. Jandera, N. Kabátová, J. Sep. Sci. 28 (2005) 1315.

H-12

DETERMINATION OF MINOR COMPONENTS OF OLIVE OIL BY FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

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Virgin olive oil is obtained from the olive, the fruit of the olive tree, using solely mechanical extraction methods in conditions, particularly thermal conditions, which do not alter the oil in any way. It has not to undergo any treatment other than washing, decanting, centrifuging and filtering. It excludes oils obtained by the use of solvents or re-esterification methods, and mixing with oils from other sources. Virgin olive oil has a free acidity, expressed as oleic acid, of not more than 0.8g/100g of oil (0.8%), and the other characteristics of which correspond to those fixed for this category. Virgin olive oils are classified depending on their organoleptic (taste and aroma) and analytical characteristics (the degree of acidity refers to the proportion of free fatty acids, not to the taste). It can be qualified as a natural product, and virgin olive oil can have a designation of origin when it meets the specific characteristics associated with a particular region.

Most current methods for detecting oil composition and classification are based on chromatographic analysis. Traditionally, for the determination of fatty acid composition is used the gas-liquid chromatography (GLC), for triglycerides' composition is used the high-performance liquid chromatography (HPLC). Chromatographic methods are currently widely used for the qualitative and quantitative analysis of sterols, which comprise a major portion of the unsaponifiable matter. GC-electronic impact ionisation mass spectrometry and on-line LC-GC-flame ionisation is used to detect different sterols in vegetable oils. HPLC coupled with UV detection can analyse tocopherols after saponification; GC-isotope ratio MS can reportedly identify volatile compounds in vegetable oils. The above-listed techniques typically require complicated and time-consuming isolation procedures. For example, GLC analysis requires that fatty acids first be converted to methyl esters; techniques based on quantitative analysis of particular chemical fractions require prior chromatographic separation to isolate triglycerides, sterols, tocopherols, etc.

Actually it is possible to characterize the olive oil using the FT ICR MS (Fourier Transform Ion Cyclotron Resonance Mass Spectrometer). This technique combines electro spray ionisation (ESI), a low-fragmentation ionisation technique for polar compounds, with Fourier transform ion cyclotron resonance mass spectrometry, a mass spectrometric technique with ultra-high resolution and mass accuracy capabilities. Applications are surveyed in fields such as proteomics, metabolomics, natural product analysis and non-covalent complexes.

This paper focuses on the application of this technique to the direct characterisation of tocopherols and other components in olive oil. The extra virgin oil (Circiriello variety), typical of Apulia region and extracted by decanter on stoned paste, was used for the experimentation.

Analysis was performed using a TermoElecton LTQ FT ICR 7 tesla mass spectrometer.

H-13
CHARACTERIZATION OF SOME ROMANIAN WINES BY ISOTOPE RATIO MASS SPECTROMETRY

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The quality and geographical origin of some wines produced in Romania were investigates by IRMS methods. We have measured $^{13}C/^{12}C$ and $^{18}O/^{16}O$ in wine ethanol and water from wine. The determination of $^{13}C/^{12}C$ of wine ethanol was performed in three steps: (i) 500 ml of a wine sample was distilled with a distillation apparatus with a constant reflux ratio of 10. The alcoholic distillate was collected with an ethanol content of about 93 % mass without isotope fractionation. (ii) The ethanol was converted off line into CO_2 by combustion in excess of oxygen. (iii) The carbon isotope ratio of the CO_2 obtained was analyzed by isotope ratio mass spectrometry (IRMS) using a Delta V Advantage mass spectrometer. The $^{18}O/^{16}O$ isotopic ratio of the water from wine was determined by IRMS using CO_2 which was obtained after equilibration of wine with carbon dioxide. The 1σ SD for the procedure was 0.02‰ for the ^{13}C (n=8), and 0.04‰ for the ^{18}O (n=8). The samples of wine from the 2002, 2003 and 2004 vintages and from six different wine-growing regions of Romania were analyzed. The varieties that were studied were Cabernet Sauvignon and Feteasca Regala. Isotopic content of ^{18}O has varied from -5.87‰ and 5.35‰ and depends on the geographical origin of the wines and the precipitation on the harvest area. For the same region and the same variety of wine there were differences between years of harvest. Meteorological conditions and precipitations are changing from year to year and are responsible for these differences. Isotopic composition of the ^{13}C has varied from -24.57‰ to -29‰. These variations were correlated with the temperature and the precipitation in the area of the harvest. Cabernet Sauvignon from Tg Bujor, Murfatlar and Tohani showed very different $\delta^{13}C$ values, indicating significant variability of the stomatal control in Vitis vinifera species.

H-14

COMPARISON OF VOLATILES FROM POLISH HONEYS OF DIFFERENT ORIGIN BY HEADSPACE SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY MASS SPECTROMETRY

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The floral origin of honeys is a very important characteristic of the quality of these food products. Honeys quality control in Poland as well as in the whole Europe is faced with many difficulties. Currently recommended physicochemical parameters are not sufficient to authenticate floral origin of honey appropriated for direct consumption, mainly due to not very adequate methods of chemical analysis. The lack of precisely defined criteria of honey differentiation is adverse for the producers as well as the consumers, thus there is a great need for enhancement of knowledge on honeys quality and elaboration of reliable methods for their differentiation.

Unambiguous determination of honey botanical origin and its authentication is difficult. Classical approach to the diagnosis of unifloral honeys including sensory, physicochemical and rarely used - melissopalynological methods is quite labor-intensive and needs specialized personnel for carrying out pollen and sensory analysis. Physicochemical methods such as pH, acidity, ash or sugar content determination are not sufficiently specific and do not allow to assess adulteration. Taking the above into consideration, the aim of this work was to identify and to compare volatile components present in the headspace fraction of honeys of different origin and also to find the relations between the volatile fraction composition and the quality of the product.

The determination of volatiles from honey was conducted by means of headspace solid-phase microextraction and gas chromatography with mass spectrometry detection (HS-SPME/GC-MS). The extraction of the volatile compounds was performed in accordance with earlier developed method [1], where all the conditions, such as suitable stationary phase of the fiber, temperature and time of extraction, sample volume were optimized to enable high efficiency of the volatile components isolation. The compounds were identified by comparing their mass spectra with those obtained in the NIST Mass Spectral Database.

The developed procedure was applied for the comparison of volatiles from several popular Polish honeys, namely: multiflorous, lime, acacia, buckwheat, rape, heather and honeydew.

[1] Plutowska, B., Gromadzka, J., Wardencki, W. *Zastosowanie techniki SPME-GC do oznaczania lotnych skladnikow miodow.* IV Krajowe Sympozjum "Naturalne i syntetyczne produkty zapachowe i kosmetyczne, Lodz, 2007, p. 21.

H-15
TERPENIC AND PHENOLIC COMPOUNDS AS MARKERS OF FLORAL ORIGIN OF HONEYS

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Honey is produced by honeybees from nectar of plants, as well as from honeydew, and is one of the most complex mixtures of sugars and other minor components in nature.

Assessment of the botanical (floral) and/or geographical origin of honey is of great concern in food analysis, since authenticity guarantees the quality of the product, prevents overpayment and helps to identify frauds.

In presented work, the honeys of four different floral origins, namely buckwheat, lime, rape and heather have been characterised in respect to their volatile and phenolic fractions by means of GC/MS and HPLC technique. The aim of this study is to obtain a “fingerprint” of the terpenic and/or phenolic compounds of certain branches honeys as markers of their quality. These results might be also used for the identification of the floral source of honeys purchased from various sources.

H-16
EVALUATION OF KETCHUP AUTHENTICITY

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Ketchup is a preserved vegetable product made with ripened tomatoes (tomato purees), vinegar, sugar, salt, spices and vegetable. The use of the word "ketchup" on product labels is allowed only when the product conforms to a set of strict guidelines. Czech food law (Decree No. 157/2003 of the law No. 110/1997) specifies the minimum of natural tomato content (NIST) in ketchup being 7 or 10% tomato solids in total refractive solid content, and 25 or 30 total refractive solid content for tomato ketchup and tomato ketchup labelled Prima, Extra, Special, respectively.

The procedure allowing the estimation of NIST based on the determination of several chemical markers was verified. Data validation was done on the calibration set of 15 samples of authentic tomato purees and verification set of 20 samples of tomato purees. Mean values of qualitative parameters for analysed tomato puree samples were: refractive index 26.9±2.1, lycopene 31±7 mg/100g, pyroglutamic acid 742±121 mg/100g, glucose 7±1 g/100g, fructose 10±2 g/100g, sucrose <0.1 g/100g, K 1111±343 mg/100g, Ca 50±11 mg/100g, Mg 64±20 mg/100g, formol number 179±26, tomato colour as a/b 1.8±0,1. The method for the estimation of natural tomato content in ketchup was proposed and compared with the available databases and literature sources. Effect of the origin, processing and storage conditions on the variability of individual chemical markers was discussed.

H-17
QUALITY EVALUATION OF TEA AND TEA BASED PRODUCT

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The aim of this study was to select and determine the qualitative parameters of tea and tea based products and to compare various chemical markers which could be used for the quality an authenticity evaluation of such products.

The chosen chemical markers of tea and tea based product quality and authenticity were: caffeine a theobromine content (by HPLC), total polyphenol content (by spectrophotometry) and characteristic amino acid theanine (by CE). The analyses was carried on the 57 samples of tea and tea product (22 iced tea, 4 instant tea drinks and 31 authentic samples of dry black, green and decaffeinated tea).

Due to the high variability of the tea leaves composition and a possible effect of processing, the analyses of several chemical markers of tea composition provide a very useful tool for the estimation of quality and quantity of tea extract used in tea based products. Labeling and composition of almost 20% iced tea and instant tea drink samples did not fulfill the requirements of recent Czech legislation.

H-18

DEVELOPMENT OF A NEW METHODOLOGY USING PRESSURIZED LIQUID EXTRACTION AND GAS CHROMATOGRAPHIC ANALYSIS FOR THE DETECTION OF HONEY ADULTERATIONS

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Honey is a natural food highly appreciated by consumers. Due to its relatively high price, honey can be the target of adulterations with cheaper commodities such as high fructose corn syrups (HFCS) and invert syrups (IS). Since their carbohydrate composition is similar to that of honey, the detection of these adulterations becomes a difficult task. Calorimetric, isotopic or spectroscopic methods have been proposed to determine syrup additions, although the use of chromatographic techniques is more extended. Despite all these attempts, there is still a need for an appropriate indicator which allows the detection of low levels of adulteration.

We have recently found the presence of difructose anhydrides (DFAs) in HFCS and IS by first time (Ruiz-Matute et al., in press). These compounds were not present in honeys, not even after heating or storing for long periods of time. Nevertheless, DFAs were found in adulterated samples and 5% of syrup addition could be detected. Therefore, DFAs can be useful markers for the detection of HFCS and IS adulterations. However, the analytical method previously suggested involves the removal of monosaccharides from honey by yeast treatment before GC analysis. This method although effective was time-consuming and laborious. In this work, a new methodology using pressurized liquid extraction (PLE) has been developed for the fractionation of honey carbohydrates which implies a concentration of DFAs fraction previous GC analysis.

A home made PLE system (Ramos et al., 2006) was used for the carbohydrate fractionation. Sugars were previously adsorbed onto activated charcoal and inserted into the extraction cell. A two step extraction procedure was used for the separation of carbohydrates after optimization of experimental conditions: monosaccharides were removed using ethanol:water 1:99 (v/v) for 5 min, whereas the extraction of the remaining carbohydrates were carried out using ethanol:water 50:50 (v/v) for 10 min. Pressure and temperature were kept constant at 1500 psi and 40ºC for all assays. Carbohydrates were converted onto their trimethylsilyl oximes pervious to their analysis by GC.

This method was evaluated for the detection of adulterations of honey with 5, 10 and 20% of HFCS and IS and compared to other methods previously reported in the literature for the fractionation of honey carbohydrates (yeast treatment and extraction using activated charcoal). Reproducibility was higher for the PLE extraction, this method being also more automatic and less time consuming.

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[1] Ruiz Matute AI, Soria AC, Martínez-Castro I, Sanz ML *J. Agric. Food Chem.* (2007) In press
[2] Ramos JJ, Diez Ch, Camara C, Ramos L. PTC-ES 2006/000311.

H-19

DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF EPOXIDISED SOYBEAN OIL IN FOODS BY GAS CHROMATOGRAPHY - MASS SPECTROMETRY

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A simple GC-MS method for the determination of epoxidised soybean oil (ESBO), migrating into foodstuffs from PVC gaskets of the metal lids of glass jars, was developed and validated. ESBO was transesterified into volatile methyl esters with a sodium methoxide solution in methanol. Methyl diepoxy eicosanoate was used as internal standard. Apart for the retention times, identification of ESBO was based on two more criteria: the mean relative abundances of three ions (m/z 55, 69 and 169) and the ratio of the peak areas of both the diastereomers of methyl diepoxy linoleate (18:2E). Concentrations of ESBO in oily foods (olive paste, olives in oil, tuna in oil and mayonnaise) and sunflower oil (migration stimulant) were determined using either the first eluted diastereomer (18:2E1) or the second one (18:2E2), depending on the matrix effect of the sample. Recoveries of ESBO from olive paste ranged from 85% to 107%, at three levels of ESBO (10, 60 and 125 mg/kg). Intra-day precision (as RSDs, n=6) ranged from 5.8% (at 10 mg/kg) to 1.2% (at 60 mg/kg). The limit of detection was 3.5 mg/kg. The method was applied to a wide range of food samples (sauces, vegetables in oil, fishes in oil, baby foods), which were packaged in glass jars with lids having PVC gaskets. ESBO was determined in 12 out of 26 samples at concentrations ranged from 13 to 322 mg/kg.

H-20

AROMA ANALYSIS OF SPANISH HONEYS FROM DIFFERENT FLORAL ORIGIN BY SPE AND CG/MS AND EVALUATION OF THEIR SENSORY CHARACTERISTICS BY QDA

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The volatile fraction of honey aroma is highly complex, comprising low concentrations of numerous compounds whose chemical nature varies considerably. Over 300 volatile compounds have been identified as honey aroma components, including acids, alcohols, ketones, aldehydes, terpenes and esters. Although honey volatile compounds may arise from various sources, any search for compounds that might be useful for differentiating between floral origins must focus on those deriving from plants, or their metabolites. The aim of this study was to identify volatile compounds and to investigate the influence of the floral origin on the volatile composition and sensory characteristics of Spanish honeys from different geographical provenances.

The study was carried out on Spanish honeys samples from different floral and geographical origins. Samples were acquired at the market. The isolation of volatile compounds of honeys was carried out using solid phase extraction (SPE) technique. Ten grams of each honey were dissolved in 50 mL of distilled water. Volatile compounds of interest were eluted with 30mL of dichloromethane. The organic phase was collected and concentrated in a Vigreux column and analysed by gas chromatography-mass spectrometry (CG-MS).

A total of 77 volatile compounds were detected by GC-MS. Certain compounds were found only in honey samples from specific floral sources and could thus be of interest for use as markers. Some terpenes, linalool derivatives; lilac aldehyde, lilac alcohol and terpineal isomers, and sinensal are proposed as chemical markers for citrus honeys. 1-hexanol and organic acids appeared to be characteristic of rosemary honeys. (levo), (meso) 2,3-butanediol, hidroxiketones (3-hydroxy-2-butanone) and norisoprenoids (3-oxo-α-ionona, 3-oxo-α-ionol and vomifoliol) are the floral markers of eucalyptus honeys. The different geographical origin of the samples does not have a big influence on the typical volatile composition of every floral source.

It was made a quantitative deive sensory analysis (QDA) by an expert panel of honey tasters and several attributes would be used as markers for each type of honey as the similar form that the volatile compounds. These aroma deors of honey are directly correlated with their volatile composition and the final characteristics of honey from each floral origin can be justified according with the presence or absence of the several volatile compounds.

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H-21

GAS GROMATOGRAPHY – OLFACTOMETRIC PROFILE OF RED WINES MADE FROM MINORITY GRAPE VARIETIES CULTIVATED IN LA MANCHA REGION

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The aroma of wine is extremely complex from a chemical point of view and this complexity itself on its aroma profile. The wine contains a higher number of odorants; some of them are present in all types of wine at concentrations not very different. On the other hands, there are several volatile compounds which can be considered as impact odorants in some wines, while can be completely absent in others. The present work has two complementary objectives: first the study of aroma composition of red wines made from different grape varieties cultivated in La Mancha region; and second to determine which odorants can be responsible for the aroma profile of the wines.

The isolation of volatile compounds of wines was carried out using solid phase extraction (SPE) technique and analysis by gas chromatography-mass spectrometry (CG-MS). The major volatile compounds were analyzed by direct injection on a GC with a FID detector. The organic extracts obtained by SPE were used in the GC-O analysis. It was made a quantitative deive sensory analysis by an expert panel of wine tasters.

The GC-O study revealed the presence of 102 aromatic notes in which 88 has been identified. Aroma compounds were classified according to their aroma deor similarity and summed in seven distinct aromatic series consisting of fruity, floral, sweet/toasted/caramel, spice, green/fresh/herbaceous, pungent/acid/chemical/dry and others (liquorice, leather, tobacco and cooked vegetable). Although there were distinct quantitative differences among the wines relative aromatic series profile of the seven wines were similar. Forty-five compounds of the components identified in the olfactometric analysis were quantified, 19 of which were found at concentrations higher than their corresponding odor thresholds. The strongest odorants in the CG-O experiments were similar in all cases, although significant differences in the intensity between the samples were noted. The components with the greatest capacity to introduce differences between these wines were *beta*-damascenone, ethyl dihydrocinnamate, *(E)*-4-allylsyringol, *gamma*-butyrolactone, 4-ethyl guaiacol, 4-vinylguaiacol, isoeugenol, syringol, guaiacol, ethyl caprate and eugenol. The correlation between the olfactometric intensities and the quantitative data is, in general, satisfactory if the olfactometric differences between the samples are high.

Keywords: red wine, aroma compounds, GC-MS, CG-O, sensory deive analysis.

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H-22

C, N AND O STABLE ISOTOPE RATIOS AS A TOOL TO DISCRIMINATE THE BEEF ORIGIN

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Stable isotope ratios of animal tissues reflect native habitats. Carbon and nitrogen isotope ratios ($\delta^{13}C$ and$\delta^{15}N$) reflect feed pattern, and oxygen isotope ratio ($\delta^{18}O$) reflects drinking water. $\delta^{18}O$ of water vary by geographical factors such as latitude, altitude and inland effects. In this study, we attempted to discriminate the origin of the beef distributed in Japan, where the Japanese, Australian (The State of NSW) and American (Middle America) beef are widely distributed to the retail outlets, by analyzing carbon, nitrogen and oxygen stable isotope ratios of the beef. The results showed no significant difference among the $\delta^{15}N$ values of the three origins. $\delta^{13}C$ values of the American beef (-12.1±1.1‰) (mean±sd) showed higher than the Japanese (-18.5±1.1‰) and the Australian (-22.1±1.5‰). This result implies the rate of corn included in the feed is relatively high in the beef industry of the U.S. $\delta^{18}O$ values were the highest in the Australian beef (17.2±1.1‰), and significant difference was not shown between the Japanese (11.6±2.0‰) and the American (10.8±1.1‰). This result corresponds well with the weighted annual $\delta^{18}O$ of precipitating water presented by IAEA. From the results shown above, we found it possible to discriminate domestic and imported beef distributed in Japan by the carbon and oxygen stable isotope ratios.

Moreover, we examined a possibility of discriminating the origin of the Japanese domestic beef products in Japan even among four geographically different regions (Ishigaki, Matsuzaka, Yamagata and Hokkaido; shown in order from the south to the north). Significant differences were not shown among the four districts for $\delta^{13}C$ and $\delta^{15}N$ values. The result implies that there are almost no differences in feeding contents for beef fattened in Japan. On the other hand, $\delta^{18}O$ values were the highest in the beef from Ishigaki, the most southerly among the four regions, while they decreased as the region moves toward the north. This shows the same tendency as that of $\delta^{18}O$ values of water in these regions, from the south to the north. The results above suggest a possibility of discriminating the origin of the Japanese domestic beef within Japan by the oxygen stable isotope ratio.

H-23
DETECTION OF SUGAR ADULTERATION IN APPLE JUICES USING CAPILLARY ELECTROPHORESIS

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Adulteration of fruit juices is a common problem in food industry. The main abundant components of fruit juices are carbohydrates. Unfortunately producers of fruit-based products use some additives such as cheaper fruits, commercial sweeteners, sugar syrup and etc. The sugar composition of apple juice provides to detect sugar adulteration since the concentration of fructose in apples is much higher than that of glucose, and even the sugar content of apples varies from location to location, literature studies show no significant difference on F/G ratio between locations. Consequently, F/G ratio is a good indicator for sugar adulteration in commercial apple juices.

In recent years the application of capillary electrophoresis (CE) for the separation of chemicals has become popular diligently because of its shorter analysis time, minimum consumption of sample and also reagent and offering effective separations. Capillary electrophoresis is a well-established method for the analysis of carbohydrates.

In this study, F/G ratios of different commercial fruit juices obtained from supermarkets in Turkey were detected using CE. With the use of 50 mmol L^{-1} glycylglycine electrolyte at pH 12, the fast separation and indirect detection of sugars were obtained. F/G ratios of the all-commercial pure apple juices are found similar that found in pure authentic homemade juices. However, F/G ratios of apple-concentrated juices were outside the range of authentic apple juices.

ALLERGENS

(I1 – I6)

I-1
RIDASCREEN® GLIADIN COMPETITIVE - NEW APPROACH TO GLUTEN ANALYSIS IN HYDROLYZED FOOD SAMPLES

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Some foods contain highly processed cereal proteins, e.g. beer, starches or syrups. Hydrolyzed proteins do not allow the use of classical sandwich ELISA methods for determination of gliadins. R-Biopharm developed a competitive enzyme immunoassay for the detection of gliadin from those samples, the RIDASCREEN® Gliadin competitive. A 33 amino acid peptide from gliadin with the sequence LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF which is resistant to gastric and pancreatic hydrolysis acts as a strong stimulator to intestinal T cells is discussed to be the toxic sequence. This peptide, respectively sub-sequences of it were used to check for their reactivity with the R5 antibody. This antibody is internationally recognized as the most fitting for determination of the gliadin content in foodstuffs. A small peptide – QQPFP – was selected for the development of the competitive gliadin ELISA. In house studies using hydrolyzed starches, syrups and beer showed in many cases notably higher “gliadin concentrations”, compared to analysis using the classical sandwich ELISA. The ELISA detects the intact molecule as well as fragments down to one epitope.

I-2
RIDASCREEN® GLIADIN AOAC RESEARCH INSTITUTE PERFORMANCE TESTED METHOD SM 120601

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RIDASCREEN® Gliadin, a sandwich enzyme immunoassay produced by R-Biopharm AG, Germany. The next generation ELISA for gluten analysis detects prolamines from wheat (gliadin), barley (hordein) and rye (secalin) equally down to a level of 2.5 ppm from liquid and soft samples as well as from meat products and sausages. For preparation of all types of matrices, the "cocktail" extraction-solution is recommended. The standard included in the test kit is calibrated against a reference standard recommended by the Working Group on Prolamin Analysis and Toxicity.

RIDASCREEN® Gliadin was tested in an international ring trial organized by the Working Group on Prolamin Analysis and Toxicity in 2002. In 2006 the Codex Alimentarius set up the method as "Type 1 Method". The test kit shows excellent stability, provides acceptable accuracy and precision data. It has been granted AOAC Research Institute *Performance Tested Methods*^{SM} status and assigned certification number 120601 in December 2006.

I-3
RAPID ISOLATION AND DETECTION OF ANISAKIS SIMPLEX ALLERGEN ANI S 1

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Eating fish infested by *Anisakis simplex* larvae can cause an inflammatory reaction when they penetrate into the digestive mucosa. In addition to the anisakiasis, the larvae or their secretory/excretory products can sensitize humans and induce immunoglobulin E (IgE)-mediated allergic reaction. Symptoms range from a mild urticarial reaction to an anaphylactic episode with hypotension and shock.

Diagnostic methods for the study of allergic reactions to *A. simplex* have usually been performed based on whole-body extracts of larvae. These extracts contain a large number of allergens which could lead to a significant variability between patients in their immunological response. In order to improve the specificity and sensitivity of the diagnosis of *A. simplex* allergy, the diagnostic methods should be performed for each allergen separately.

This work describes the rapid isolation of the main allergen, Ani s 1, following its extraction from parasites with sodium phosphate buffer and the subsequent analysis of the supernatant by gel permeation chromatography using a HiLoad 16/60 Superdex 75 column. The detection of Ani s 1 protein in isolated fraction has been carried out through skin prick test and basophil activation test assays. The prick test was positive with the presence of a wheal ≥ 3 mm in diameter or larger than that elicited with a histamine control. Percentage of activated basophils, evaluated with flow–cytometric analysis, was calculated by subtracting spontaneous CD63 expression (negative control) from the data obtained with *A. simplex* stimulation. Both tests were carried out with patients diagnosed with allergy to *A. simplex* and with a healthy patient (negative control) after patients gave their informed consent. Results obtained from both assays indicated that patients sensitive to *A. simplex* had a positive response to whole-body extracts of larvae and fractions containing allergen Ani s 1. The lack of response in the healthy patient used as negative control showed that the positive response is specific.

In our knowledge, the *Anisakis simplex*-induced basophil activation evaluated by flow cytometry has been previously reported only once, where whole-body extracts of larvae were tested. In our work, flow cytometry has been also used to evaluate Ani s 1-induced basophil activation and to prove the serum specific IgE response.

The described methods could be also applied to the rest of identified allergens. Besides improving the specificity and sensitivity of the diagnosis of *A. simplex* allergy, this approach should allow to specify the culinary/food treatments required to minimize the reactivity of each allergen.

I-4

DUPLEX REAL-TIME PCR METHOD TO DETERMINE POTENTIALLY ALLERGENIC SESAME AND HAZELNUT IN FOOD

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Food allergies pose an increasing health problem, particularly in industrialized countries. Both sesame seeds and hazelnut are known to be very potent allergens causing particularly severe reactions with a high risk of life threatening anaphylaxis in sensitised persons. The only preventive strategy for allergic patients is to strictly avoid food containing the certain allergens. In order to facilitate the identification of allergenic ingredients in foodstuffs, the European Commission established a list containing twelve specified allergens which must be indicated in the list of ingredients, including sesame seeds and hazelnut and products thereof (Directive 2003/89/EC).

Highly selective and sensitive analytical methods are necessary to verify if allergen containing products are labelled in compliance with the regulations and to enable the detection of hidden allergens. The methods developed so far are based on either protein or DNA detection. Several papers have already demonstrated the applicability of real-time PCR to detect peanut (1,2), hazelnut (3,4) or walnut (5) in food.

In the present paper a duplex real-time PCR method is presented enabling the simultaneous determination of sesame and hazelnut in food. To detect sesame and hazelnut, the genes coding for the two major allergenic proteins Ses i 1 and Cor a 1 were selected. The assay is specific for sesame and hazelnut and does not show any cross-reactivity with common food ingredients.

[1] Stephan, O.; Vieths, S. Development of a real-time PCR and a sandwich ELISA for detection of potentially allergenic trace amounts of peanut (Arachis hypogaea) in processed foods. J. Agric. Food Chem. 2004, 52, 3754-3760.
[2] Hird, H.; Lloyd, J.; Goodier, R.; Brown, J.; Reece, P. Detection of peanut using real-time polymerase chain reaction. Eur. Food Res. Technol. 2003, 217, 265-268.
[3] Holzhauser, T.; Wangorsch, A.; Vieths, S. Polymerase chain reaction (PCR) for detection of potentially allergenic hazelnut residues in complex food matrixes. Eur. Food Res. Technol. 2000, 211, 360-365.
[4] Arlorio, M.; Cereti, E.; Coisson, J. D.; Travaglia, F.; Martelli, A. Detection of hazelnut (Corylus spp.) in processed foods using real-time PCR. Food Control 2007, 18, 140-148.
[5] Brezna, B.; Hudecova, L.; Kuchta, T. A novel real-time polymerase chain reaction (PCR) method for the detection of walnuts in food. Eur. Food Res. Technol. 2006, 223, 373-377.

I-5
QUANTITATIVE DETERMINATION OF POTENTIALLY ALLERGENIC SESAME IN FOOD BY REAL-TIME PCR

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The prevalence of food allergy has been estimated to be 1-3% in adults and 4-6% in children. Allergenic foods can elicit rather mild reactions on the skin, in the gastrointestinal tract (e.g. oral allergy syndrome), the respiratory tract (e.g. asthma), the eyes and/or the central nervous system. Very potent allergenic foods, however, can cause life threatening anaphylaxis. Among all food allergens, particularly peanut, tree nuts and sesame are associated with particularly severe reactions with a high risk of anaphylaxis. Sesame allergy is very common in Eastern countries like Israel. However, sesame allergy is also becoming frequent in European countries, probably because of an increasing consumption of sesame seeds and sesame oil in these countries.

Allergic patients must strictly avoid the certain food allergen. In order to facilitate the identification of allergenic ingredients in foodstuffs, according to the European Union legislation twelve food allergens must be indicated in the list of food ingredients, including sesame seeds and products thereof. Sensitive analytical methods are necessary in order to verify if allergen containing products are labelled in compliance with the regulations. Enzyme immunoassays (ELISAs) are most frequently used to quantify food allergens. However, several PCR methods have already been published for semiquantitative determination of celery (1), hazelnut (2), walnut (3) and peanut (4) in food.

The present paper presents a real time PCR method for detecting and quantifying sesame in food. For detecting sesame the gene coding for the major allergenic protein Ses i 1 was selected. An artificial sequence from lambda bacteriophage DNA was used as internal standard in order to correct for influences from the food matrices on the efficiency of the PCR reaction. The method was evaluated by analyzing food samples (cookies, crackers) which were spiked with different amounts of sesame seeds.

[1] Hupfer, C.; Waiblinger, H.U.; Busch, U. Development and validation of a real-time PCR detection method for celery in food. Eur Food Res Technol (2007) 225:329–335.
[2] Arlorio, M.; Cereti, E.; Coisson, J. D.; Travaglia, F.; Martelli, A. Detection of hazelnut (Corylus spp.) in processed foods using real-time PCR. Food Control 2007, 18, 140-148.
[3] Brezna, B.; Hudecova, L.; Kuchta, T. A novel real-time polymerase chain reaction (PCR) method for the detection of walnuts in food. Eur. Food Res. Technol. 2006, 223, 373-377.
[4] Stephan, O.; Vieths, S. Development of a real-time PCR and a sandwich ELISA for detection of potentially allergenic trace amounts of peanut (Arachis hypogaea) in processed foods. J. Agric. Food Chem. 2004, 52, 3754-3760.

I-6

APPLICABILITY OF A COMMERCIAL ELISA TO DETECT POTENTIALLY ALLERGENIC SESAME IN FOOD

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In the past, sesame (*Sesamum indicum)* allergy was only common in Eastern countries. Recent papers, however, indicate that allergic reactions to sesame have become more frequent in European countries. Numerous papers report on the severity of allergic reactions caused by the consumption of sesame seeds, including life threatening anaphylaxis.

Sesame is one of 12 food allergens which have to be given in the list of food ingredients according to European Union legislation. Analytical measurements have to be carried out to verify if sesame containing food products are labelled in accordance with the regulations and if non-labelled food products are actually free of sesame.

The detection of food allergens is, however, a very difficult task since they are often present in minute amounts in very complex food matrices. The analytical method should be specific for the food allergen and sensitive enough to enable the detection of allergen amounts which are sufficient to elicit allergic reactions in highly sensitized patients. It is generally agreed that the LOD should be in the range of 1 to 100 ppm (1).

Although enzyme linked immunosorbent assays (ELISAs) are frequently used to determine allergens in foods, up to now no study has been published dealing with the development of a sesame specific ELISA. However, some test kits for detecting sesame in food are commercially available. In general, the commercial food allergen tests suffer from providing very little information with regard to the applicability to different food matrices.

The present paper therefore investigates the applicability of a commercial test kit to detect sesame in different food products purchased in Austrian food stores. We analysed crisp bread, cereals and crackers. Some of the samples were labelled with “contains sesame”, some were labelled with “may contain sesame” and others were non-labelled.

[1] R. Krska, E. Welzig, S. Baumgartner. Immunoanalytical detection of allergenic proteins in food. Anal. Bioanal. Chem. 378 (2004) 63-65.

FLAVOURS AND ODOURS

(J1 – J17)

J-1

„ELECTRONIC NOSE" TECHNOLOGY AND GAS CHROMATOGRAPHY FOR INVESTIGATING FOOD QUALITY

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The quality of food product can be determined by its odour, a gaseous complex mixture, that could contain up to hundreds individual chemical compounds. The chemical techniques used to determine the food quality are: molecular spectroscopy, chromatographic and other sophisticated separations in addition to hyphenated techniques such as gas chromatograph - mass spectrometry, but they are expensive, time consuming and require high qualified personnel. For the fast, in-situ, automated analysis it could be used chemical sensors, having many advantages: various matrix samples (liquid, solid), low reagent volumes consuming, it does not need previous sample processing.

This study compare two methods to determine the freshness in animal originated food products: gas sensor system "electronic nose" and GC-MS. Identification and quantification of characteristics compounds in headspace of food samples were determined by GC-MS analysis, after air pump sampling and pre-concentration on sorbent material.

J-2
THE APPLICATION OF HS-SPME TECHNIQUE FOR ANALYSIS OF VOLATILE COMPOUNDS IN VEGETABLE OILS

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The vegetable oils, like other food products, have a characteristic profile of volatile flavor substances, which create oil aroma. In the creation of aroma bouquet have part both natural volatile compounds and derivative substances which came from extraction or press of oil and which are dissolved in oil phase. The next group of compounds which also take part in smell formation are substances that are created during oil changes, e.g. in the lipooxygenase pathway or autooxidation process. Short chain hydrocarbons, ketons, aldehydes, alcohols, epoxides, esters and lactones may be fomred gaving a smell and taste of rancidity to food products.

The presence of many polyunsaturated acids in the oils is the most endangered for lipids changes in oxidation process caused by atmospheric oxygen leading to the changes in volatile substances composition.

The best indicator of the oxidation process can be the level of hexanal in sample or the hexanal / nonanal ratio. Nonanal is formed only in auto-oxidation process of oleic acid and shows the highest rate of increase during the oxidation process and therefore it can be the appropriate oxidation marker.

The aim of thepresent work is to develope a fast, easy and reproducible method to determine the degree of rancidity of oil. The use of such method can also allow to detect aduleration of goodqualityoils by cheaper ones.

The headspace solid phase microextraction (HS-SPME), as extraction technique and capillary gas chromatography (GC) with flame ionization detector (FID) and mass spectrometry, as determination technique was used in this research.

Over 70 different volatile compounds, responsible for the smell of the investigated oil were identified using this approach. The identification was done by comparing the received mass spectra with those presented in the NIST mass spectra library.

J-3

IDENTIFICATION OF VOLATILE CONSTITUENTS OF TUNISIAN FENUGREEK SEEDS: COMPARISON OF SOLVENT EXTRACTION AND STATIC HEADSPACE SOLID-PHASE MICROEXTRACTION

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Fenugreek (*Trigonella foenum-graceum L.*) is an annual herb from the family of leguminous. The plant is widely cultivated in Mediterranean countries and Asia, as it is a popular food, consumed in various ways. Moreover, this plant is frequently used as a medicinal plant. However, its use remains limited due its characteristic strong aroma, which is in part responsible for the subsequent strong odour of sweat in humans consuming this plant. In addition, fenugreek aroma may vary greatly depending on the degree of seeds maturation, as well as the geographic origin of the plant. So, we intended to characterize the volatile compounds implicated in the aroma of Tunisian fenugreek seeds, especially as some compounds may be responsible for the strong sweat odour.

Different extraction conditions were tested, with a view of both identifying the volatile extracted and obtaining the more representative extract (i.e. with the odour as close as possible as that of fenugreek seeds). We compared solvent extraction (using different solvents and extraction times) and static headspace solid phase microextraction (SHS-SPME) with several fibers (polydimethylsiloxane (PDMS), carboxen/polydimethylsiloxane (CAR/PDMS), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), and polyacrylate (PA)). All extracts were analysed by gas chromatography-ion trap mass spectrometry (GC-IT-MS). The identification of volatile compounds was carried out by comparing their mass spectrum to those given in NIST database. For confirmation retention indices (RI) were calculated using a modified Kovats method and compared to the values given in the literature (two GC columns of different polarity were used). The sensory quality of all extracts was measured by direct gas chromatography-olfactometry (D-GC-O), a novel instrument tool for evaluating odors representativeness of headspace extracts.

Numerous volatile compounds were identified, some of them being reported for the first time in fenugreek, such as cubenol. With regards to solvent extraction, best results were obtained with methanol as this solvent extracts a higher level of compounds with a wide range of polarity. For SHS-SPME, the DVB/CAR/PDMS fiber 2cm length was found the more efficient fiber in extracting compounds with different polarities, such as 2-heptanone, 2-octenal-2-butyl, and octadecanoic acid. In addition, this fiber afforded a solventless extract with an overall odour very similar to that of genuine fenugreek seeds.

Keywords: fenugreek; aroma compounds; odor representativeness; headspace solid-phase microextraction.

J-4

IDENTIFICATION OF AROMA GENERATING SUBSTANCES OF DRY WINES BY METHOD OF COLUMN CHROMATOGRAPHY WITH SUBSEQUENT DETERMINATION ON CHROMATO-MASS SPECTROMETER

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Nowadays numerous investigations are carried out in order to study and determine aromatic components of wine both to improve technological processes and to identify falsification.

Objects of the present investigation were white dry wine Chardonnay and red dry wine Cabernet from homogeneous sorts of grape, as the most frequently used in wine-making technology.

Aromatic substances of wines were exposed to extraction consequently by hexane, diethyl ether, dichloromethane and butyl acetate to study the entire composition of wines.

The comparison of results for extraction with different solvents showed that the greatest yield of components was reached using diethyl ether and dichloromethane and it came to 41.2% and 42.3% respectively for Chardonnay or 44.2% (using diethyl ether) and 42.3% (using dichloromethane) for Cabernet.

The received fractions of wines were concentrated, and then they were separated by the method of column chromatography on silica gel.

The glass column (600x30 mm) was used for the separation; it was filled up with silica gel (sort L, granulation – 60-100 mesh). The following solvents were used as eluents: hexane, hexane-diethyl ether (1:1), diethyl ether and acetonitrile. The speed of elution was 2 ml/min. The received fractions were concentrated in an atmosphere of nitrogen.

The results of column separation of extracts showed that a considerable amount of wine extract spreads between fractions of hexane-ether (1:1) and ether. This fact indicated predominant content of polar compounds.

Analysis of extracts was realized by means of chromato-mass spectrometer “Hewlett Packard 5960B” using glass capillary column HP – 5MS (30 m x 0.25 mm) with motionless fluid phase OV-101. Temperature changed from 70 to 250°C at a speed 10°C/min. Chromatograms were recorded over a full ion current. Mass-spectra were recorded at energy of ionizing electrons 70 eV.

Before putting the samples into chromato-mass spectrometer syrupy masses were converted to derivatives of trimethylsilyl, except fraction of hexane, by dint of treatment with N,O-bis-(trimethylsilyl)trifluorineacetamide.

J-5
THE APPLICATION OF HS-SPME TECHNIQUE FOR ANALYSIS OF VOLATILE COMPOUNDS IN VEGETABLE OILS

Justyna Gromadzka[1*], Marta Lores[2], Waldemar Wardencki[3], Katarzyna Lipińska[4], Maria Fernandez-Alvarez[5]

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The vegetable oils, like other food products, have a characteristic profile of volatile flavor substances, which create oil aroma. In the creation of aroma bouquet have part both natural volatile compounds and derivative substances which came from extraction or press of oil and which are dissolved in oil phase. The next group of compounds which also take part in smell formation are substances that are created during oil changes, e.g. in the lipooxygenase pathway or autooxidation process. Short chain hydrocarbons, ketons, aldehydes, alcohols, epoxides, esters and lactones may be fomred gaving a smell and taste of rancidity to food products.

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The aim of thepresent work is to develope a fast, easy and reproducible method to determine the degree of rancidity of oil. The use of such method can also allow to detect aduleration of goodqualityoils by cheaper ones.

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Over 70 different volatile compounds, responsible for the smell of the investigated oil were identified using this approach. The identification was done by comparing the received mass spectra with those presented in the NIST mass spectra library.

J-6

RAPID HS-SPME-GC-TOFMS METHOD FOR QUALITATIVE PROFILING OF ICE WINE VOLATILE FRACTION, RELATIVE CHARACTERIZATION OF CANADIAN AND CZECH ICE WINES

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An analytical method for the determination of volatile and semi-volatile compounds representing various chemical groups in ice wines was developed and optimized. A combination of the fully automated solid-phase microextraction (SPME) sample preparation technique and gas chromatographic–mass spectrometric (GC–MS) system to perform the final chromatographic separation and identification of the analytes of interest was utilized. Full spectral information in the range of *m*/*z* 35–450 was collected across the short GC run (less than 5 min) utilizing the high-speed time-of-flight (TOF) analyzer without compromising in the detection sensitivity, as compared to other scanning mass analyzers operated in selected ion monitoring or MS^n mode to achieve similar sensitivity. The MS data acquisition rate of 50 spectra/s was selected as optimal for the rapid analysis of this relatively complex matrix. The optimized analytical method did not exceed 20 min per sample, including both the isolation and pre-concentration of the analytes of interest, the final GC–TOF-MS analysis and the fiber bake-out. The repeatability of the developed and optimized HS-SPME-GC–TOF-MS method for ice wine analysis, expressed as relative standard deviation (RSD, %, n = 7), ranged from 3.2 to 9.0%.

The method was used to characterize and classify a large set of ice wines according to their origin, grape variety and oak or stainless steel fermentation/ageing conditions, based on a statistical evaluation (principal component analysis, PCA) of the measured data. More than 130 ice wine samples collected directly from Canadian and Czech wine producers were analyzed. The SPME step was beneficially carried out utilizing the new-generation super elastic Divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber assembly to isolate compounds from ice wine samples. The identification of analytes was performed by a combination of the linear temperature-programmed retention index (LTPRI) approach using C_8 – C_{20} alkanes loaded onto the fiber with the comparison of the obtained spectra with three libraries included in the ChromaTOF software. A total of 201 peaks were tentatively assigned as ice wine aroma components and 58 of those compounds were evaluated in all of the examined samples. The results were submitted to critical interpretation by using a self-organizing map (SOM) technique and commented in terms of relative characterization of samples according to their geographical origin, grape varieties, and vintage years. When clear clustering was obtained, the most determinant compounds responsible for the observed differentiations were identified.

The authors would like to acknowledge the assistance of LECO (St. Joseph, MI, USA), Leap Technologies (Carrboro, NC, USA) and the Natural Sciences and Engineering Research Council of Canada (NSERC). The cooperation of the Canadian and Czech wineries involved in the study is also highly appreciated.

J-7

IMPROVED SENSITIVITY FOR THE ANALYSIS OF VOLATILE ORGANIC CONTAMINANTS (VOC) IN DRINKING WATER WITH A HEADSPACE/GC/MSD SYSTEM

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Introduction:

Without careful monitoring, many chemical by-products of industrial production can be released into the environment; often contaminating ground and surface water bodies upon most of our drinking water is depending. In many parts of the world, public drinking water is chlorinated to kill bacteria. By-products of this process are halogenated methanes which must be also routinely monitored.

Methods and Instrumentation:

Purge-and-Tap sample concentration followed by gas chromatography with mass spectral detection is the most widely used technique for the analysis of volatile compounds in drinking water. This combination delivers the right sensitivity but Purge and Trap concentration somehow is tedious to handle. Static Headspace concentration on the other hand is very easy to handle but lacks often in the required sensitivity for regulated drinking water analyses. Optimizing the sensitivity of a Headspace GC/MSD system to comply with the requirements for drinking water regulations is the focus of this paper.

Transferring the vapour from a headspace sampler to a capillary column is mostly performed in split mode to get reasonable peak width especially for the early eluting compounds. Splitless sample transfer would result in very broad peaks. Normal cryo-focusing of the volatile organic compounds in a glass liner or the beginning of the capillary column would require very low (around -150°C) temperature to effectively focus them. By using a Tenax TA filled glass liner, even the most volatile compounds are focused already at -40°C. This allows the usage of liquid CO2 for the inlet cooling.

The instrument configuration for the ultra sensitive VOC analysis includes a headspace sampler with a 3ml sample loop. The content of the loop is transferred to a PTV inlet containing the Tenax filled liner in pulsed splitless mode for fast sample transfer. The inlet is kept at -40°C during the sample transfer and then heated very fast with 720°C/minute to 280°C for effective transfer of the compounds to the capillary column. Separation of the VOC's is performed on a 20m x 0.18mm x 1.0µm DB-624 column. Detection occurs with a quadrupole MSD in simultaneous Scan/SIM mode.

Preliminary Data:

With this setup, quantification of the VOC's in 10ml salt saturated water using the SIM trace of the analysis was possible below 0.01 µg/l and identification using the Scan trace below 0.1 µg/l.

J-8

ANALYSIS OF ALDEHYDES AS O-(2,3,4,5,6-PENTAFLUOROBENZYL) OXIME DERIVATIVES USING MULTIDIMENSIONAL GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC TECHNIQUES

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Carbonyl compounds, particularly aldehydes, are widely found in biological systems, often as products of lipid-oxidation processes (1) or as fermentation metabolites. Some of them have extraordinary odor qualities with extremely low odor thresholds (2) as e.g. (E)-alkenals or (E,E)-alkadienals. The knowledge of both, qualitative and quantitative composition of these compounds is of importance for a large variety of food samples and biological systems.

In biological applications, measurement of aldehydes is often performed with photometric assays (3), such as the commonly applied thiobarbituric acid test, yielding a fundamental parameter for the assessment of oxidative stress in tissues. Yet, for the characterization of aldehyde-based off-flavors, analysis of individual compounds is necessary. A well-known example is (E)-2-nonenal in beer or other foodstuffs (4, 5). Furthermore, some aldehydes are described as biomarkers for diseases, e. g. hexanal and heptanal in the case of lung cancer (6). Determination of aldehyde compounds can be performed by direct headspace gas chromatography (HS-GC) or liquid injection of extracts obtained from the corresponding matrices. Unfortunately, classical headspace analysis shows low sensitivity for higher molecular weight aldehydes and analysis of liquid extracts is often hindered by co-elution due to the hundreds of compounds found in complex extracts, such as wine aroma. Sample pre-fractionation, usually done by chromatography on silica gel or other sorbents, is advantageous; however, multidimensional gas chromatographic techniques are occasionally needed for an unambiguous assignment of compounds. Due to the reactivity of the carbonyl group, derivatization methods have widely been described (1, 7), which in many cases enable a selective detection of the corresponding aldehydes. In this respect, oxime derivatives formed by reaction with O-(2,3,4,5,6-pentafluorobenzyl)hydroxyl amine hydrochloride (PFBHA) exhibit excellent chromatographic properties and allow selective detection with either electron capture, thermionic or mass-selective detectors.

An improvement of a recently presented method (8) for the analysis of aldehydes as their oxime derivatives will be described using combined modern multidimensional chromatographic and mass spectrometric techniques. Headspace analysis and direct derivatization on a solid phase microextraction (SPME) fiber allows fast sample preparation and sensitive analysis, also minimizing critical contamination risks throughout lengthy sample preparation steps. The potential of combining on-fiber derivatization SPME with either GC-MS or comprehensive two-dimensional GC-MS (GCxGC-MS) will be presented as adequate techniques for reliable analysis of aldehydes in complex food matrices such as wine or vegetable oils. GC coupled to an ion-trap mass spectrometric detector allows specific analysis via selective MSn experiments. Alternatively, the combination of the comprehensive 2D chromatographic technique coupled to a quadrupol mass spectrometer in selective ion monitoring mode will be shown to be an efficient tool for the analysis of aldehyde classes in complex matrices as well. The latter HS-SPME-GCxGC-qMS installation combines the advantages of high chromatographic separation efficiency and selectivity obtained by recording specific ion traces with a fast scanning quadrupol mass spectrometer.

[1] Spiteller, G.; Kern, W.; Spiteller, P., Investigation of aldehydic lipid peroxidation products by gas chromatography-mass spectrometry. Journal of Chromatography A 1999, 843, (1 + 2), 29-98.
[2] Ullrich, F.; Grosch, W., Identification of the most intense volatile flavor compounds formed during autoxidation of linoleic acid. Zeitschrift für Lebensmittel-Untersuchung und -Forschung 1987, 184, (4), 277-82.
[3] Sawicki, E.; Sawicki, C. R., Aldehydes: Photometric Analysis, Vol. 1., Academic Press, London, New York, San Francisco, 1975.
[4] Vanderhaegen, B.; Neven, H.; Verachter, H.; Derdelinckx, G., The chemistry of beer aging – a critical review. Food Chemistry 2006, 95, (3), 357-381.
[5] Widder, S.; Grosch, W., Precursors of 2-nonenals causing the cardboard off-flavor in butter oil. Nahrung 1997, 41, (1), 42-45.
[6] Deng, C.; Li, N.; Zhang, X., Development of headspace solid-phase microextraction with on-fiber derivatization for determination of hexanal and heptanal in human blood. Journal of Chromatography B 2004, 813, (1-2), 47-52.
[7] Blau, K.; Halket, J. M. (eds.), Handbook of Derivatives for Chromatography. Second Edition; John Wiley & Sons: Chicester, England, 1993.
[8] Schmarr, H.-G.; Ganß, S.; Sang, W.; Schröder, J.; Fischer, U., Analysis of carbonyl compounds in wine aroma using PFBHA derivatization, In Proceedings of the 29th International Symposium on Capillary Chromatography & 3rd GCxGC, Riva del Garda, Italy, May 29 - June 2, 2006; Sandra, T.; Sandra, P. (eds.) I.O.P.M.S. vzw, Kortrijk, Belgium.

J-9

EFFECT OF CONVENTIONAL AND MICROWAVE PREPARATION ON SENSORY PROPERTIES OF FRESH AND FROZEN BROCCOLI

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Background: Freezing is a highly efficient method to keep vegetables for long times, with the original characteristics of the product mostly undisturbed. It is common that many vegetables are cooked by a simple boiling process or in the microwave before use. These various cooking preparation would certainly bring about a number of changes in physical characteristics of the product, which are additionally affected during storage, due to development of off-odour, colour losses and change of the texture. Therefore the effect of conventional and microwave cooking on sensory properties of fresh (FrB) and frozen broccoli (FzB) immediately after preparation and after 1 and 2 days storage at 4 °C was evaluated.

Methods: The sensory evaluation was performed with an objective Quantitative Deive Analysis (QDA) and with hedonic ranking test of preference. The sensory profile in QDA consisted of the following deors: intensity of colour, taste, odour, off-taste and off-odour, sweetness, bitterness, juiciness and firmness of broccoli florets. The overall quality score (OQS) of the product included rating of the all investigated attributes.

Results: At the day of cooking (day 0) the conventional prepared FrB and the microwave prepared FzB were similar in their sensory properties and showed a high overall quality score (6 points of the 9 points scale). The microwave prepared FrB had the lowest OQS (3,8 points). At the 1st day of storage no relevant differences were observed in comparison to day 0. After 2 days storage the OQS of FrB and FzB, in both preparations were lower and averaged for microwave: FzB=4,3 points; FrB=3 points; for conventional cooking: FzB=5,1 points; FrB=5,7 points. However the OQS of conventional prepared FrB was the highest among the investigated samples.

Conclusion: The sensory evaluation revealed that because of the better preservation of the sensory attributes, especially firmness and juiciness, the conventional cooking is better for the preparation of fresh and microwave for the frozen broccoli. Due to a high distinct of taste and odour, typically for broccoli, the conventionally prepared FrB showed the highest OQS among the investigated samples after 2 days storage and was preferred by the consumers immediately after cooking as well as after 1 and 2 days of storage. A hard texture, low juiciness and additionally higher intensity of bitterness and colour losses after storage were shown to be responsible for the lowest OQS in the microwave prepared FrB during the whole period of the experiment.

J-10

CHARACTERISATION OF GLOBAL VOLATILE COMPOSITION OF WHITE AND RED GRAPE VARIETIES BY HS-SPME-GCxGC-TOFMS

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Several studies carried out on grapes characterization recognized a relationship between the wine varietal character and the volatiles and semi-volatiles of grapes. Previous studies concerning the grapes from the white variety Fernão-Pires and red variety Baga analyzed by Headspace-Solid Phase Microextraction one-dimensional Gas Chromatography coupled with quadrupole Mass Spectrometry detection (HS-SPME-GC-qMS) reported the presence of monoterpenoids, sesquiterpenoids, C_{13} norisoprenoids and aromatic alcohols [1, 2]. However, the demand of complex samples such as the grapes, quickly overwhelm GC-MS analysis techniques. The wide range of analyte concentrations within the sample, combined with the high degree of analyte co-elution, makes an accurate characterization of this matrix very difficult. Comprehensive two-dimensional gas chromatography (GCxGC), which employs two orthogonal mechanisms on two different columns to separate the constituents of the sample within a single analysis, allows the expansion of the chromatographic space in two dimensions in which more peaks can now be resolved. Time-of-Flight Mass Spectrometry (ToFMS) provides a full mass range spectral acquisition rates up to 500 Hz, which offers MS data with sufficient density to address the requirements of GCxGC separations. In a first approach the HS-SPME-GCxGC-ToFMS methodolody was successfully applied to the characterization of only one class of compounds, the monoterpenoids, of Fernão-Pires white grape variety [3] using the *m/z* 93, 121, and 136.

The aim of this study is the application of HS-SPME, using the carbowax divinylbenzene coating fibre (65 μm of thickness), coupled with GCxGC-ToFMS for the volatile global characterization of grapes from Fernão-Pires and Baga varieties. Several chemical classes were identified in total ion current (TIC) chromatogram: monoterpenoids, sesquiterpenoids, norisoprenoids, phenols, aliphatic alcohols, aliphatic aldehydes, aromatic alcohols, aromatic aldehydes, esters, acids, ketones, lactones, halogenated compounds, aliphatic hydrocarbons, aromatic hydrocarbons, furans, ethers, and sulfur compounds. The GCxGC-ToFMS total ion current chromatogram contour plots of the two grape varieties (Figure 1) show varietal clusters. One main difference is the monoterpenoid cluster that is more expanded in white grapes, which contains an higher amount of compounds. I the red grapes chromatogram, two small clusters, one with monoterpene hydrocarbons and other with monoterpenols, are observed. That result is in accordance with the monoterpenic varietal character of this white variety, which is characterized by floral and citric odors [2]. Figure 1 also allows to observe sesquiterpenoids in Fernão-Pires variety, a class of compounds never reported to occur in this variety when using GC-qMS. The sesquiterpenoids present in the white and red varieties are not exactly the same. In order to find diagnostic compounds for each variety, in deep analysis of these varietal compounds can be obtained by the extraction of characteristic ions of the observed chemical groups.

The information given by HS-SPME-GCxGC-ToFMS represents a valuable advance for future studies in the establishment of the volatile composition of grape samples and to identify potential varietal marker compounds in wine samples.

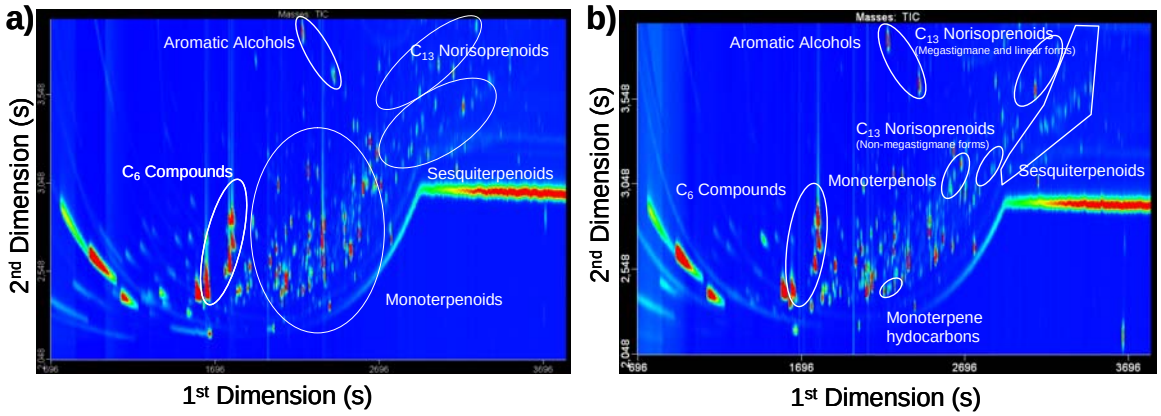

Figure 1. GCxGC-ToFMS total ion current chromatogram contour plot of grapes from a) Fernão-Pires white variety and from b) Baga red variety. Clusters formed by structurally related compounds are indicated.

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[1] E. Coelho, S.M. Rocha, I. Delgadillo, M.A. Coimbra, Headspace-SPME applied to varietal volatile components evolution during *Vitis vinifera* L. cv. 'Baga' ripening, Anal. Chim. Acta, 563 (2006) 204.
[2] E. Coelho, S.M. Rocha, A.S. Barros, I. Delgadillo, M.A. Coimbra, Screening of variety- and pre-fermentation-related volatile compounds during ripening of white grapes to define their evolution profile, Anal. Chim. Acta, 597 (2007) 257.
[3] S.M. Rocha, E. Coelho, J. Zrostlikova, I. Delgadillo, M.A. Coimbra, Comprehensive two-dimensional Gas Chromatography with Time-of-Flight Mass Spectrometry of monoterpenoids as a powerful tool for grape origin traceability, J. Chromatogr. A, 1161 (2007) 292.

J-11

A MEAT-FRESHNESS ANALYSIS SYSTEM WITH A BIOSENSOR FOR METHYL MERCAPTAN

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In the field of food industries, an evaluation of food freshness is important for quality control. For example, K value with complicated operation is widely used for the assessment of fish freshness. We have also reported trimethylamine level as a novel index of fish-freshness, and developed the TMA biosensor (as the convenient approach for evaluating fish freshness) using drug metabolizing enzymes for metabolizing TMA in human liver [1]. Similarly, methyl mercaptan (MM) has been reported a typical index of meat freshness because the concentration of MM increases by meat putrefaction. In this research, a meat-freshness sensor was developed using flavin containing monooxygenase type-3 (FMO3) as a one of the enzymes for metabolizing MM in human liver. The sensor was also applied for evaluating the meat freshness. The freshness sensor was constructed by attaching an FMO3 immobilized membrane onto a sensitive area of a Clark type dissolved oxygen electrode using nylon net and a silicon O-ring [2]. The enzyme was immobilized into a dialysis membrane using photocrosslinkable polymer. A flow injection system with standard MM solutions and meat extracts solution was used for evaluating the sensor behaviour. The sensor was possible to detect the oxygen consumptions induced by the oxidation of MM as FMO3 enzyme reaction. The calibration range of the biosensor was obtained against MM from 1.6 mmol/l. As the results of the experiment with the meat extract samples, the sensor output increased with progression of the putrefaction level.

[1] Dolphin C.T., et.al., Nat. Genet., 17, 491-494, 1997.
[2] Mitsubayashi K., Hashimoto Y., Electrochemistry, 68(11), 901-903, 2000.

J-12
DEVELOPMENT OF AN ELECTRONIC NOSE TO DETECT SPOILED FRUIT

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The perception of volatile compounds by the human nose is of great importance in everyday life, and is, along with vision, a powerful instrument in evaluating the quality of food. Spoiled food has a foul smell which is readily detected by someone entering a kitchen, a warehouse, or a marketplace.

Human fatigue and exposure to perfumes can affect nose sensitivity. Therefore, several attempts to mimic human nose have been made and instruments called electronic noses have been manufactured. They can be used in several fields, as in environmental monitoring, in medical or food industry applications. These devices employ various types of electronic chemical gas sensors devoted to detect volatile compounds.

In this work, an electronic nose for non-destructively monitoring of the fruit maturing stage is presented. The system used six bulk acoustic wave sensors, coated with different sensing membranes where analytes can be adsorbed. The resonance frequency decreases when volatile molecules are adsorbed onto membrane, and the frequency recovers after desorption. Frequency of the six sensors was stored in a computer.

Frequently the sensors are non-specific to the volatile compounds which need to be detected. This problem is solved with a range of sensors each one with different sensitivities and selectivities to odours or families of compounds, and the responses of all of them subjected to mathematical treatment. The present arrangement is no exception, although coatings sensitive to several classes of compounds have been selected.

The capability to identify fruit rotness for five different fruits was tested. Fruit tested included New Hall orange, Starking apple, Golden apple, William pear and Kiwi. Principal component analysis (PCA) was used for preliminary data analysis to reduce the dimensionality. The loadings allowed identifying the must important sensors.

Then, a pattern-recognition system, based on Bayes' theorem, was used to classify the fruit. Each type of fruit was separately classified using a linear driscriminanting function. Rotten fruit was sucessefully identified for all types of fruits, with a few misleading interpretatios just in the case of the Starking apple. The complete set of all fruits was also analysed by applying a quadratic discriminating function. Discrimination between edible and rotten fruit was achieved with 95% of success.

J-13
EFFECT OF NEW FUNGICIDE RESIDUES ON THE AROMA COMPOSITION OF MONASTRELL RED WINES

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The aroma composition of a wine depends on different factors: varietal, environmental, agronomic and technological. While there is a wealth of literature on wine aroma, there are few studies on the effects of pesticide residues found in this fraction. For this reason, and to get more information about this research topic, the effect of several fungicide residues (famoxadone, fenhexamid, fluquinconazole, kresoxim-methyl, quinoxyfen, trifloxystrobin) have been studied in relation to the aroma composition of Monastrell red wines. Two fungicides treatments were carried out with authorized formulates following the manufacturer doses. The first one was carried out under good agricultural practices (GAP), obeying the preharvest interval, and the second one under critical agricultural practices (CAP), applying at the day of harvesting. The trials were carried out in triplicate. The wines obtained in the thirteen trials (one control, six with treated grapes obeying the preharvest interval and six treated at the day of harvesting or at most unfavourable conditions) were analysed by stir bar sorptive extraction (SBSE)-GC-MS. Volatiles by SBSE extraction have been carried out at RT during 1 hour and thermally desorbed at 290 ºC for GC/MS analysis. The identified volatile wine compounds have been grouped as follows: ethyl esters *group* (ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, ethyl-9-decanoate, diethyl succinate); acetates *group* (3-methyl-1-butanol acetate, hexyl acetate, 2-phenylethanol acetate); C_6 compounds (1-hexanol); terpenoids (damascenone and nerolidol); acid *group* (hexanoic acid and octanoic acid); and others (3-methyl-1-butanol, 2-phenylethanol and benzaldehyde).

As results, it was observed that all fungicides treatments significantly affect the wine aroma composition, although it does not necessary indicate changes on the sensorial profile as the variation range do not overpass the olfactory threshold of each compound. The most affected group of volatiles, in terms of active principle or treatment, are the acetate and acids indicating that all fungicides may have some influence on the yeast activity while alcoholic fermentation takes place. It is important to point out that quinoxyfen and trifloxystrobin do not affect the terpenoid volatiles, aromas that come from the grape and not from the fermentation, when the GAP treatments are carried out but significantly change with the CAP treatment. Kresoxim-methyl and fenhexamid active principles have the lowest effect on the aroma composition, whether GAP or CAP treatments are used, while fluquinconazole and frifloxystrobin principles are the most reactive.

J-14

COMPARISON OF POTENTIALITIES OF STIR BAR SORPTIVE EXTRACTION AND SOLID-PHASE MICROEXTRACTION FOR QUANTIFICATION OF ETHYL ESTERS AND ACETATES IN WINES

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The production of ethyl esters and acetates by the yeast during fermentation can have a significant effect on the fruity flavours in wine. Basically, two factors are important for the rate of ester formation, i.e. the concentration of the two substrates, acyl-CoA and fuse1 alcohol, and the total activity of the enzymes involved in the formation and breakdown of the respective ester. The shorter chain fatty acids ethyl esters contribute more to wine aroma than the less volatile longer chain esters. The esters if present in concentrations higher that their sensorial perception limit can contribute individually to the wine aroma, furthermore the presence of different esters can also have a synergistic effect on the individual flavours, contributing to the wine overall aroma.

Quantification of volatile compounds in wines is challenging due to complexity of the matrix and low concentration of the volatiles. Solid-phase microextraction (SPME) has gained popularity for wine volatile analysis due to its simplicity and sensitivity. More recently, a stir bar sorptive extraction (SBSE) technique has been developed to study table and sweet wines (1,2). This technique uses a Twister™, a glass stir bar onto which is bonded a sorptive phase, often the polydimethylsiloxane (PDMS). As the SBSE exhibits much larger volume of polymeric coating than SPME, may open new opportunities to the volatile studies.

The aim of this study is to develop and optimise SPME-GC-qMS and SBSE-GC-qMS methodologies to isolate and quantify ethyl esters and acetates in Terras Madeirenses Portuguese table wines. The performance of these two methodologies was evaluated in terms of precision, accuracy, linearity, and detection and quantification limits. Several experimental parameters that influence in sorption processes were studied, namely extraction time and temperature, sample volume and dilution factor.

The SPME and SBSE methodologies showed good linearity over the concentration range tested, with correlation coefficients higher than 0.986 (SPME) and 0.982 (SBSE) for all the analytes. The repeatability, in terms of relative standard deviation (RSD) of the methods, was estimated between 5% and 16% for SPME and 8% and 19% for SBSE. Additionally low and similar detection and quantification limits were achieved for both methods.

[1] R.F. Alves, A.M.D. Nascimento, J.M.F. Nogueira, Anal. Chim. Acta, 546 (2005) 11.
[2] A. Zalacain, J. Marin, G.L. Alonso, M.R. Salinas, Talanta, 71 (2007) 1610.

J-15

DETECTION OF METHOXYPYRAZINES ADITION INTO MORAVIAN SAUVINGON BLANC WINES

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Methoxypyrazines are very important components of wine flavor contributing to the vegetative, herbaceous and bell pepper character of wines that are produced mainly from particular grape varieties (Cabernet Sauvignon, Sauvignon Blanc and Merlot). In the last several years several causes indicating falsification of wines with addition of artificial methoxypyrazines into wine were described. In this study the SPME GC/MS procedure for the analysis of volatile profiles and quantification of the methopxypyrazines was optimized. Aroma profiles of thirteen Moravian Sauvignon Blanc wines were analysed, as well as the profile of the artificial aroma mixture Fantasia S (Sauvignon), which is available in the region. The content of 2-methoxy-3-(1-methylpropyl)pyrazine in the analysed samples varied between 4.7-17.0 ng/l. The artificial flavouring mixture contained another isomer of similar taste 2-methoxy-3-(2-methylpropyl)-pyrazine, the addition of artificial aroma was not detected in any of analysed samples. An acceptable concentrations of methoxypyrazines in wine was estimated by hedonic evaluation of less distinct sort of wine Veltlinske zelene spiked with 1 to 50 ng/l of 2-methoxy-3-(2-methylpropyl)-pyrazine. The concentration varying between 5 to 10 ng/l was most acceptable for majority of assessors.

J-16

EVALUATION OF ODOUROUS COMPOUNDS IN A POLYOLEFIN FOOD PACKAGING MATERIAL BY SIMULTANEOUS WATER DISTILLATION/EXTRACTION (SDE) AND SOLID PHASE MICRO EXTRACTION (SPME)

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Food packaging should prevent damages during distribution and storage as well as from insects, micro organisms and chemical spoilage. Protection against loss of food ingredients and a specific gas and water permeability are required too.

Several materials are employed for food packaging but during the last decades plastic packaging materials became more and more important, not only as single materials but also as layers in composites together with metal and/or cardboard/paper layers [1,2].The used plastic packaging materials can be separated in two big groups: “bulk” materials such as polyolefins (polyethylene PE and polypropylene PP) and polyethylenetherephthalate (PET) and “functionalized” materials such as polyvinylidenedichloride (PVDC), polyacrylnitrile (PAN) and ethylvinylalcohol-copolymer (EVAL or EVOH) which are used e.g. as barrier layers [1,2].

Beside physical and chemical properties such as tear strength, chemical resistance and vapour permeability, odour and taste of packaging materials are an important issue due to safety reasons as well as increased consumer awareness.

Generation and structure of odourous substances in plastics are not yet clear completely. Nevertheless some information is available and in most cases oxidation products are the main odour drivers in plastic materials.

In the presented poster different extraction methods for identification of odourous components in a typical polyethylene based packaging material grade are compared. Beside Simultaneous Water Distillation/Extraction (SDE), Headspace Solid Phase Microextraction (HS-SPME) with two different fibre materials are used for subsequent capillary gas chromatography coupled with mass spectroscopy (GC-MS) or flame ionization detection (GC-FID) combined with olfactometry (GC-O) to detect and identify odourous components.

The investigation not only compares those methods in their ability to extract volatiles of the investigated polymeric material but also how selective and sensitive these methods are for identification of odourous components both by GC-O and GC-MS.

[1] A.L. Baner and O.-G. Piringer, in: O.-G. Piringer and A.L. Baner, Plastic Packaging Materials for Food, Wiley-VCH, Weinheim, 1993, p.1.
[2] P. Ackermann, Jägerstad M. and T. Ohlsson, Foods and Packaging Materials, The Royal Chemical Society, Cambridge, 1995

J-17
FAST GC-FID ANALYSIS OF MESIFURANE IN ARCTIC BRAMBLE (RUBUS ARCTICUS L.)

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Arctic bramble (Rubus Arcticus L.) is considered as a precious and high value berry due to its unique aroma and rarity. The main aromatic component in the arctic bramble is mesifurane (2,5-dimethyl-4-methoxy-2,3-dihydro-3-furanone). Concentration of the aromatic compounds can vary depending on several factors such as strain, growing environment, climate, harvesting and storage conditions. Quantitative analysis of aromatic components is needed to characterize and compare the aromatic properties of different strains for cultivation and foodstuff production. Due to the rareness of this berry, no data is available of the comparison of different arctic bramble strains. Presently, the aroma analysis of berries (generally using strawberry) has been performed mainly by sensory analysis, which is technically laborious and expensive for routine use. Published GC-MSD based methods have several sample treatment steps and therefore increased risk of losses of the volatile aromatic compounds. Mesifurane is very soluble in water and therefore also HPLC methods have been developed, but these analyses suffer from the lack of sensitivity. In this study a fast water extraction was combined with GC-MSD/FID analysis for rapid determination of mesifurane in arctic bramble. Suitable GC column (FFAP) was found which enables the efficient separation and detection of mesifurane in water phase. MSD (with authentic standard) was used for identification of the mesifurane from arctic bramble extract and flame ionization detector was used for quantitation.

BIOLOGICALLY ACTIVE, HEALTH PROMOTING FOOD COMPONENTS

(K1 – K38)

K-1

VITAMIN DETERMINATION WITH VITAFAST® IN INFANT FOOD AND ENTERAL CLINICAL NUTRITION

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Infant food and food for special medical purpose (FSMP) are highly complex food matrices. Vitamins are added as premixes to increase the nutritional value in order to fulfil nutritional requirements and regulations. The natural vitamin content of the raw materials has to be taken into account since the total vitamin content, native and added, must be labelled.

The innovative microbiological assays VitaFast® in microtiter plate format are able to measure the content of all water soluble B-vitamins in different types of vitamin enriched foods. For the determination of added vitamins, a hot water extraction is usually sufficient. For measuring the total vitamin content, including the native vitamins, the vitamins are extracted by specific enzymatic treatment.

In cooperation with the Central Laboratories Friedrichsdorf GmbH, Germany, four VitaFast® parameters, biotin, pantothenic acid, folic acid and vitamin B12, were validated for infant milk formula (IMF), milk cereals and enteral clinical nutrition (ECN).

K-2

PURIFICATION OF POLYPHENOLOXIDASE FROM WILD EDIBLE MUSHROOM LACTARIUS SALMONICOLOR BY AFFINITY CHROMATOGRAPHY

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Polyphenol oxidase (PPO) was purified from a wild edible mushroom *Lactarius salmonicolor* (LsPPO) using a Sepharose 4B-L-tyrosine-p-amino benzoic acid affinity column. At the optimum pH and temperature, the K_M and V_{Max} values of LsPPO towards catechol, 4-methyl catechol and pyrogallol were determined as 0,025 M ; 0,7481 EU/mL, 1,809 ×10 $^{-3}$ M : 0,723 EU/mL, 9,465 ×10^{-3} M: 0,722 EU/mL, resepectively.

The values V_{max} / K_m showed that LsPPO has the greatest reactivity towards pyrogallol among the substrates used. Optimum pH and temperature values of LsPPO for the used three substrates ranged between the pH 4.5–11.0 and 5–50^{o}C. Enzyme activity decreased due to heat denaturation with increasing temperature. Effects of some classical PPO inhibitors of Glutation, L-Cystein, p-aminobenzenesulfonamide, sulfosalicilik acid and L-tyrosin were investigated on the activity of LsPPO using as a catechol substrate. IC_{50} values for glutation p-aminobenzenesulfonamide and L-cystein and L-tyrosin on LsPPO respectively as 9.1x 10^{-4}, 2.3x 10^{-4} M, 1.5 x 10^{-4} M and 0.0038x10^{-4} as greatest inhibition values but sulfosalicilik acid behaved as an activator for LsPPO on this study.

Key Words: Lactarius salmonicolor, Affinity chromatography, Inhibition, Enzymatic browning

K-3
ANALYSIS OF ANTIOXIDANTS IN PLANT MATERIAL AND THEIR ANTIOXIDATION ACTIVITY

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The terms antioxidants and free radicals are popular for nutritionists and other health professionals. The last few years it has been published a lot of information about the role of oxidative stress causing, and the potential therapeutic role of antioxidants preventing, a number of serious diseases such certain cancers or cardiovascular diseases.

Free radicals are very reactive compounds, which are formed in human organism owing to external environment (smoking, exhaust gases, UV radiation, stress) and also internal environment (purine metabolism, adrenaline synthesis). Activity of these matters is tied by antioxidants. These decrease probability of free radicals creation or divert them to less reactive or non-reactive states.

Antioxidants can be divided to 2 groups, synthetic and natural. In almost all plants there can be discovered natural antioxidants. The most important group of natural antioxidants are vitamin E (tocopherols), flavonoids and coumarines.

Ultrasonic extractions in liquid and gas environment are prejudiced by cavitation factor and by microfluctuation or surface instability, which is formed on liquid-liquid or gas-liquid boundary-line. The crushed sample is mixed with suitable solution and it is placed into ultrasonic bath, where optimal temperature and optimal extraction time are set. Also ultrasonic probe can be used, it allows us to prejudice the extraction by amplitude choice selection. Obtained extracts could be measured by HPLC-UV method.

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K-4

POTENTIOMETRY IN RESEARCH ANTIOXIDANT ACTIVITY OF AGRICULTURAL RAW MATERIAL, FOOD STUFFS AND HERBS

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Free radicals play dual role in human organism. On one hand, free radicals participate and are being formed in biochemical reactions providing organism cells 'vital functions'. On the other hand, negative impact of various environmental factors may lead to excess free radicals formation and as a result – to oxidative stress rise. Oxidative stress state causes diseases and fastens organism ageing in general. The system protecting organism from free radical excess comprises enzymes with oxide reductive activity, non-enzyme proteins, polypeptides, water and oil soluble vitamins, SH-containing amino acids, flavonoids, carotinoids, etc. Most of these compounds prevent oxidative stress, interrupting chain oxidative reactions. That is why these substances are called substances with antiradical activity as well as antioxidants (AO). Foodstuff, nutrients and some drugs are sources of most antioxidants. The use of fruit, vegetables, berries, wine, herbal medicinal products raises a level antioxidant protection of an organism.

The existing analysis techniques of AOA investigation are based on interaction of AO with long living radicals or active oxygen compounds and following registration of analytical signal by spectrophotometric or electrochemical methods. Those methods are sufficiently complicated, expensive, time-consuming, and as a rule, cannot be used for continuous monitoring. Therefore elaboration of new express accessible methods is a problem of current importance.

In the present work it is offered new potentiometric method for antioxidant activity investigation. This method based on interaction of AO and mediator system consisting of two complex compounds with metal in different oxidation degree. Potentiometry with the use of mediator system allows to propose a very simple express-method for measuring antioxidant activity of biological liquids, nutrients, drugs and foodstuffs.

On the basis of the offered method the serial sample of portable multipurpose potentiometric set with function of antioxidant activity measurement is developed.

With the help of new potentiometric method AOA of some vegetative food stuffs (fresh juices from crush fruits and vegetables, juices and nectars of industrial manufacturing, some grades of tea, beer and wine) were investigated. High level of AOA observed in products which are rich with vitamin C or polyphenols. Comparative research of water and ethanol extracts obtained from some herbs, fruits and vegetables has been carried out. Antioxidant activity of ethanol extracts was higher than this in water extracts due to the fact that phenols have better solubility in ethanol. Hence, they are more full taken from a vegetative material by ethanol.

A number of samples of grape wine have been investigated by offered potentiometric method and capillary electrophoresis method for definition of authenticity of a drink. AOA of natural wines was much higher, then of samples identified as diluted by capillary electrophoresis method. Thus potentiometric method of antioxidant activity investigation allows to carry out express selection of natural grape wines.

The high degree of correlation of the results obtained by the offered method and

(1) method "RANDOX" while wine analysis (R=72%);

(2) chemiluminescent method for medicinal herbs extracts (R=91%);

(3) photometric method with use of 2,2-diphenyl-1-picrylhydrazyl stable radical at the analysis of water and ethanol extracts of herbs (R=99%) is obtained.

New potentiometric method of AOA investigation does not demand expensive chemical, it is quick and easy to carry out. It can be used for quality definition of foodstuffs and food raw material over the technological processing and over the storage. This method provides easy and express receiving of information about antioxidant activity of vegetative objects.

Special instrument MPA-1 is presented.

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K-5
STUDY ON THE CONCENTRATION OF QUERCETIN AND KAEMFEROL FROM JAM AND FRESH STRAWBERRIES

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Two selected phenolic aglycons (kaempferol and quercetin) in strawberries and their quantitative changes as influenced by jam processing have been evaluated using optimised HPLC with diode array detection. Fresh strawberry sapmles, and strawberry sampes after jam processing were analysed, and the total amounts of quercetine and kaempferol were identified and determined by acid hydrolysis. Their contents in fresh and jam samples are general indicators regarding the influence of jam processing on the bioavailability of phenolic compounds. The total phenolic content of each sample was also determined by the Folin-Ciocalteu method. The four samples – freash strawberry, jam and acid hydrolysate of the fresh and jam strawberries had similar total phenolic contents.

K-6
STUDY ON SOME PHENOLIC ACIDS AND FLAVONOIDS FROM CULTIVATED STRAWBERRIES

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It is known that the phenolic acids have beneficial effects on health as antioxidants and anticarcinogens. Some phenolic acids(gallic, protocatechuic and p-hydroxy benzoic acid) were analyzed in cultivated strawberries (*Fragaria moschata*) with and without alkaline and acid hydrolysis. All fractions were quantified by HPLC with DAD and ECD detection. The results of alkaline and acid hydrolysis were calculated as to represent total phenolic acids. Selected flavonoids (kaempherol, quercetin) were separated and analysed from strawberries too. The results showed a little amount of free phenolic acids in strawberries but, after hydrolysis of strawberry samples an increased content of phenolic acids was noticed. From all analysed phenolic acids, the one detected in the greatest quantity was p-hydroxy benzoic acid.

K-7

MONITORING THE PROCEDURE OF PROCESSING RADIX REHMANNIAE BY INFRARED SPECTROSCOPY USING CONTINUOUS WAVELET TRANSFORM AND KERNEL INDEPENDENT COMPONENT ANALYSIS

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Radix rehmanniae, the root tuber of *Rehmannia glutinosa Libosc*, is a kind of natural products with the consanguinity for drug and food in Chinese traditional medicines. It is usually classified into fresh, dried, and prepared rehmannia root according to its processed stage. The dried rehmanniae root is baked from the fresh rehmanniae root, and the prepared rehmanniae root is processed from the dried rehmanniae root by different steaming methods. The radix rehmanniae in different stage has different tastes, drug efficacy, color and luster because of the existing complex physical and chemical changes in the processing procedure. Therefore, it is important to propose a qualitative and quantitative method for monitoring the processing procedure instead of the traditionally visual inspection or sensorial estimation.

In this work, an approach that using infrared spectroscopy, continuous wavelet transform and kernel independent component analysis (IR-CWT-KICA) was proposed for monitoring the procedure of processing radix rehmanniae. In the proposed approach, diffuse reflection IR spectra of the sample at different steaming period were firstly measured. Then the raw IR spectra with different background and lower Signal-to-Noise (SNR), which led by the complexity of the sample, were pretreated by CWT for elimination of the background, the noise signal, and for enrichment of the spectra resolution with appropriate parameters set, so the CWT coefficients are highly corresponding to the raw IR spectra. Thirdly, the independent components (ICs), which can be considered as representation of the characteristic peaks of the source spectra profiles of the mixtures, were estimated from the CWT coefficients, and the variation of the relative intensities of the ICs were calculated for monitoring and understanding the processing procedure.

It was found that the four estimated ICs are chemically meaningful: IC1, with the number of characteristic peaks much less than that of other ICs and intensity variation behaving random fluctuation, can be reckoned as CO_2 or other interferents in the sample; IC2, with the main characteristic peaks at 3486, 3005, 1805, 1618, 1165, 1046, 792, and 657 (cm^{-1}), respectively, and the contents level relative higher in prepared rehmanniae root, can be reasonably considered as iridoid glycosides; IC3, with main characteristic peaks at 3555, 2984, 1736, 1649, 1183, 1130, 815, and 664 (cm^{-1}), respectively, and higher contents level in the fresh, dried and prepared rehmanniae root, can be considered as saccharide compounds; IC4, with similar profiles to that of IC2 and characteristic peaks at 3622, 2930, 1739, 1644, 1157, 1144, 797, and 643 (cm^{-1}), respectively, is reasonably corresponding to catalpol and catalposide. IC2~IC4 are corresponding to three groups of active components in the radix rehmanniae. It was also found that the relative intensity of IC2 gradually increase in the first 6h during the processing procedure and to be stable after 6h; whereas, the relative intensity of IC3 and IC4 gradually decrease in the first 6h during the procedure and to be stable after 6h. Therefore, the endpoint of the processing procedure can be obtained from the intensity variation of the estimated ICs. IR-CWT-KICA approach is a novel method that can be used for monitoring and understanding the procedure of processing radix rehmanniae.

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K-8
ANALYSIS OF SOY ISOFLAVONES USING UPLC-MS

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Soybeans were originally found in eastern Asia over 5000 years ago. The Chinese farmers were responsible for cultivating these to create an edible crop, which then spread throughout Asia to Korea, Japan, and Southeast Asia. Soy eventually arrived in Europe around the 1700s.

More recently the consumption of soy products has been linked to many health benefits, as they are contain isoflavones. Isoflavones are commonly known as phytoestrogens and the 12 main isoflavones found in soybeans are daidzein (De), glycitein (Gle) and genistein (Ge) and their respective malonyl, acetyl and glucosyl forms

Research studies have indicated that isoflavones and consumption of isoflavone-containing foods are associated with a wide variety of health benefits, including prevention of breast and prostate cancers, cardiovascular disease, and reduced symptoms of diabetes and postmenopausal bone loss.

Genistein in particular has been shown to interact with animal and human estrogen receptors, causing effects in the body similar to those caused by the hormone estrogen.

The approval by the US Food and Drug Administration in 1999 allowing the food industry to promote soy protein for heart health led to an escalation in sales of soyfoods, and these foods are also being promoted for their isoflave content.

So this analysis of these type of compounds is important within a food environment.

The soy isoflavones were analysed using UPLC to see if the current methods could be shortened as literature shows that run-times can take up to 1 hour for the analysis of these compounds. The following settings were used in the experiment and a run-time of 5.5 minutes could be achieved using a dietary supplement.

The results showed that good chromatography could be achieved by using UPLC where the run-time is only 5.5 minutes. All 12 compounds identified as the key components in this supplement could be identified, with relatively good resolution and quantification would be facile with use of the mass spectrometry data.

K-9
MONITORING OF SULFONAMIDES IN MANURE AND WATER SAMPLES BY ELISA

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Sulfonamides are chemotherapeutics widely used in veterinary and human medicine to prevent and treat bacterial and protozoa infections, as well as to promote animal growth in livestock. Sulfonamides are one of the most prevalent pharmaceuticals found in waterways and in the environment due to low soil sorption, and slow microbial and chemical degradation. Recently, sulfamethaxazol was frequently detected in river waters whereas other sulfonamides such as sulfamethazine were found in higher concentrations in pig manure. In this study, a highly sensitive and specific ELISA for detection of sulfamethazine [1] and Abraxis ELISA kit working with our generic (group specific) antibody [2] were utilised for the screening analysis of manure and water samples. Samples were collected from pig farms, agriculture localities and urban settlements. Prior to ELISA detection, samples were filtrated or centrifuged and when necessary diluted with assay buffer. Nine field pig manure samples were analyzed by specific and generic ELISA. One of the 9 samples was tested positive, showing the presence of 1.80 $mg \cdot kg^{-1}$ of sulfamethazine. LC-MS/MS determined the concentration of 0.57 $mg \cdot kg^{-1}$ sulfamethazine. Further, ELISA was utilised for the screening of 75 samples of surface waters. Samples showing positive responses (16 of 75) will confirmed be by LC-MS/MS. ELISA can be utilised as a cost-effective, portable and high throughput analytical method for sulfonamide analysis whereas LC-MS/MS will provide detailed composition of sulfonamide species in these samples.

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[1] Franek, M.; Kolar, V.; Deng, A. P.; Crooks, S. Food Agric. Immunol. 11, 339-349 1999.
[2] Franek, M.; Diblikova, I.; Cernoch, I.; Vass, M.; Hruska, K. Anal. Chem. 78, 1559-1567, 2006.

K-10
PHYTOESTROGEN DATABASES: DIFFERENT WAYS OF PRESENTING DATA

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Phytoestrogen analysis has been performed for decades and several phytoestrogen databases have been established in the last years. Most of these databases were created as tools to assess the dietary phytoestrogen intake in epidemiological studies. However, the number of foods covered in the individual databases, the data sources, the amount of information presented and the quality control of the data are highly variable which has implications on the outcome of intake assessment.

In this poster, different approaches of constructing phytoestrogen databases will be presented and the reasons why these databases and databases in general should be used with caution will be pointed out. In addition, recommendations will be given how the obtained data should be presented in scientific articles so that they can be included in phytoestrogen databases.

In short, the natural variability of the contents of phytoestrogens in plant foods, the variability introduced by different processing conditions, the limited comparability of values obtained by different analytical methods and lacking information for some of the values reported in the literature pose great problems in the establishment of phytoestrogen databases. Authors publishing information on the phytoestrogen contents in foodstuffs can greatly facilitate the inclusion of their values into a database by accurately describing the samples analyzed, giving details about the sampling procedure, sample handling, the analytical method used and its validation and by expressing the data in aglucone equivalents.

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K-11
HOP (HUMULUS LUPULUS L.): A NEW SOURCE OF PROVITAMIN D2 AND VITAMIN D2?

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Ergocalciferol (vitamin D_2) is the synthetic form of vitamin D that can be formed from the plant steroid, ergosterol (vitamin D_2) by UV irradiation. In this work it was investigated the presence of provitamin D_2 and vitamin D_2 in hop plant. To our knowledge the presence of these compounds in hop has never been reported. In addition, a simple and reliable analytical methodology for analysis of these compounds in different commercial forms of hop is presented. The identification and quantification of provitamin D_2 and vitamin D_2 in hop was achieved by using high-performance liquid chromatography with diode array detection (HPLC-DAD), after sample extraction and pre-treatment. The identity of the compounds was confirmed by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry in positive ion mode (HPLC-ESI/MS/MS). The performance of the method was assessed by the evaluation of some parameters such as absolute recovery (higher than 70%), repeatability (lower than 3%), linearity (r^2 higher than 0.9988) and limits of detection (ranging from 0.034 for vitamin D_2 to 0.058 mg/L for provitamin D_2) and quantification (ranging from 0.113 for vitamin D_2 to 0.195 mg/L for provitamin D_2).

On the basis of standard additions applied with the optimized extraction procedure, it appears that *Nugget* hop plant (crop 2006) contains 1.84 ± 0.09 micrograms/gram of provitamin D_2 and 1.95 ± 0.05 micrograms/gram of vitamin D_2. As the presence of provitamin D_2 is generally associated with a fungus contamination of the sample, the results obtained suggest that the hop plant (variety *Nugget*) analyzed in this work was infected with a fungus. The presence of provitamin D_2 here reported should have great potential for the assessment of hop products as related to the contamination proportion and hence the quality of this raw material.

K-12

QUANTITATIVE ANALYSIS OF ALPHA LIPOIC ACID IN EGG YOLK USING HPLC WITH CEAD AND ESI-MS DETECTION MODES

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Alpha lipoic acid, 1,2-dithiolane-3-pentanoic acid, is an universal antioxidant present in all prokaryotic and eukaryotic cells. In the human body, it is linked to lysine residues and acts as a cofactor in different multi-enzyme complexes [1,2]. The present paper deals with the development of a rapid and reliable method for the quantitative determination of this valuable compound in food and biological samples using high performance liquid chromatography with two different detection modes i.e. coulometric electrode array detector (CEAD) and an electrospray ionization mass spectrometer (ESI-MS). At present, the method has been successfully employed for the quantitative determination of α-LA in egg yolk. First, α-LA was extracted by sonication in acidified methanol for one hour at room temperature followed by the Polyamide extraction. Chromatographic separation was then achieved by isocratic elution [acetonitrile/methanol/50 mM potassium dihydrogen phosphate 305:65:630, v/v/v, adapted to pH 3 with phosphoric acid] using an ACE 3-C-18 column (150×3.0 mm, particle size 3 µm) at a flow rate of 0.45 ml/min. The potentials in the CEAD system were set at +300, +400, +450, +500, +550, +600, +650, +700 mV against palladium reference electrodes. For ESI-MS detection (negative mode), the composition of the mobile phase was changed to 0.1% acetic acid in water/acetonitrile 55:45, v/v using an ACE 3-C-18 column (150×2.1 mm, particle size 3 µm) applying a flow rate of 0.2 ml/min. The intra- and inter-day repeatability was less then 5%. Both chromatographic methods were validated and the results were in good correlation. Simple sample preparation with no tedious derivatization steps make this method useful for analyzing lipoic acid in food and biological samples with similar matrices.

[1] F. Navari-Izzo, M.F.Quartacci, C. Sgherri, Plant Physiol. Biochem. 40 (2002) 463-470.
[2] L. Packer, E.H. Witt, H.J. Tritschler, Free Radical Biol. Med. 19 (2) (1995) 227-250.

K-13

DETERMINATION OF VITAMIN E AND CAROTENOID PIGMENTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN SHELL OF CHIONOECETES OPILIO

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This study determines the presence of vitamin E and carotenoids pigments by HPLC, in shells of Chionoecetes opilio. Two extraction methods were compared, a saponification and a simple extraction with acetone. Two extraction methods were compared, a saponification and a simple extraction with acetone. The last method was chosen because allowed to identify and to quantify the astaxanthin and astaxanthin derivatives, while in the saponification method these compounds were degraded by the heat.

A HPLC method with Fluorescence and UV-VIS detectors in series has been optimized by the determination of vitamin E and carotenoids. The vitamin E was determined by means of fluorescence detector (288-331 nm), while β-carotene, free astaxanthin, and astaxanthin derivatives were identified in the diode-array detector and determined at 450 nm. HPLC-MS have been used for the confirmation.

Thirty shell crab samples have been analyzed. The mean value higher correspond to vitamin E (23.3 mg 100 g^{-1} dry weight), followed of astaxanthin total (9.49 mg 100 g^{-1} dry weight) and the lower to β-carotene (0.2 mg 100 g^{-1} dry weight).

A rapid expansion of fisheries is demanding an adequate supply of efficient, nutritious and inexpensive fish feed. The presence of vitamin E, astaxanthin and β-carotene indicate the convenience of the use of this resource, up to now waste, with economical advantages and a high value nutritional, for the elaboration of organic feed in aquaculture.

K-14

SEPARATION OF POLYPHENOLIC COMPOUNDS IN WINE SAMPLES BY HPLC AND DETRMINATION OF THEIR ANTIOXIDANT ACTIVITY BY RADICAL SCAVENGING ASSAY

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Wine is an important source of phenolic compounds. In recent years numerous studies have reported their beneficial health effects such as anti-inflammatory and cardioprotective. Furthermore polyphenols play an important role in sensory properties.

In this study a method for the determination of polyphenols in white wines samples by on line HPLC coupled with UV and fluorescence detectors was optimized and validated.

Eleven phenolic compounds including, flavanols and flavanols dimmers (procyanidins), hydroxycinnamates, flavonols and stilbene derivatives were identified and quantified. The separation was performed on a TEKNOKROMA, Tr-015605 TRACER EXTRASIL ODS2 (25cm X 0.4 cm, i.d; 5 μm) column. Samples were injected into the chromatograph directly without previous treatment.

Precisions, linearity, recoveries and limits of detection achieved for all analytes were satisfactory.

In the second part of the work the antioxidant activity of the wine samples was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The results were expressed as Trolox equivalents. The contribution of individual polyphenols in antioxidant properties was also investigated.

K-15

PRESSURIZED FLUID EXTRACTION – NEW PROGRESSIVE ALTERNATIVE OF EXTRACTION OF BITTER ACIDS FROM HOPS AND HOP PRODUCTS AND PHENOLIC COMPOUNDS FROM MALT AND HOPS

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The EBC method 7.7 (Analytica of the European Brewery Convention) currently used in analysis of bitter acids from hops and hop products includes time–consuming and laborious extraction method. Our aim was to propose a new extraction method based on Pressurized Fluid Extraction (PFE). Compared to conventional extractions, PFE offers a number of important benefits.

PFE on OnePSE automated extractor was used for extraction of α–and β–acids from hops and hop products. The parameters influencing extraction efficiency were studied as well as the influence of the sample preparation method. The quantitative determination of α- and β-acids in extracts was accomplished using an HPLC equipped with diode array detector. The experimental results were compared with those obtained by standard EBC method 7.7. PFE method presents an adequate but quicker alternative to the EBC method 7.7.

A role of polyphenols and phenolic compounds in malt, hops and beers is very important and therefore new rapid extraction techniques are asked. Modern Pressurized Fluid Extraction technique coupled with HPLC and high sensitivity CoulArray detection was used for analysing of some important phenolic compounds in malt and hops.

Qualitative and quantitative dependence of extracted polyphenols on extraction temperature was found under optimized extraction conditions of PSE technique. Recoveries within temperature interval 40–60 °C were constant with the exception of ferulic acid. Outside of this interval concentrations of some polyphenols depend on the temperature significantly.

Obtained results were compared with results obtained by currently used warm water extraction methods (e. g. congress mashing).

This PSE method together with HPLC fitted with a high sensitivity CoulArray detector could be a basis for standard analytical methods which could be independent on any enzymatic activities.

K-16
PURIFICATION OF POLYPHENOLOXIDASE FROM WILD EDIBLE MUSHROOM

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Polyphenol oxidase (PPO) was purified from a wild edible mushroom Lactarius salmonicolor (LsPPO) using a Sepharose 4B-L-tyrosine-p-amino benzoic acid affinity column. At the optimum pH and temperature, the KM and VMax values of LsPPO towards catechol, 4-methyl catechol and pyrogallol were determined by Lineweaver–Burk method. The values Vmaz/ Km showed that LsPPO has the greatest reactivity towards pyrogallol among the substrates used. Optimum pH and temperature values of LsPPO for the used three substrates ranged between the pH 4.5–11.0 and 5–50oC. Enzyme activity decreased due to heat denaturation with increasing temperature. Effects of some classical PPO inhibitors of Glutation, L-Cystein, p-aminobenzenesulfonamide, sulfosalicilik acid and L-tyrosin were investigated on the activity of LsPPO using as a catechol substrate. IC50 values for glutation p-aminobenzenesulfonamide and L-cystein and L-tyrosin on LsPPO respectively as 9.1x 10-4, 2.3x 10-4 M, 1.5 x 10-4 M and 2.310-4 M as greatest inhibition values but sulfosalicilik acid behaved as an activator for LsPPO on this study.

Key Words: Lactarius salmonicolor, Affinity chromatography, Inhibition, Enzymatic browning

K-17

CARBON PASTE ELECTRODES INCORPORATING SYNTHETIC ZEOLITES MODIFIED WITH METHYLENE BLUE FOR AMPEROMETRIC DETERMINATION OF ASCORBIC ACID IN FRUIT JUICES

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L-Ascorbic acid (AA), also known as Vitamin C, is an organic compound playing a key role in living bodies, being essential for the formation of bone and connective tissue, helping iron absorption and helping burns and wounds heal. Vitamin C is an antioxidant, protecting cells against damage by free radicals, which are reactive by-products of normal cell activity. This is the reason why L-Ascorbic acid is widely used as a dietary supplement and is also added to manufacture foods as an antioxidant for preservation [1]. Consequently, measuring ascorbic acid content is very important for assessing food product quality.

Satisfactory techniques for the determination of AA must ideally be specific, reproducible, rapid, simple and sensitive, and that is why electrochemical methods have been proposed for its determination [2]. These methods have clear advantages, especially for the analysis of samples containing fine particles or of deep color and high viscosity.

In this context, two new electrodes were obtained by incorporating two synthetic zeolites modified with Methyene Blue (MB) in carbon paste, in order to be used as amperometric sensors for AA. The electrochemical behavior of the modified electrodes (MB-13X-CPEs and MB-4A-CPEs) was investigated using cyclic voltammetry and the independence of formal standard potential on the pH of the surrounding solution was put on evidence. The modified electrodes were successfully tested for electrocatalytic oxidation of AA in phosphate buffer (pH 7.0), at an applied potential with more than 500 mV lower than that used on unmodified carbon paste electrodes. The amperometric sensors for ascorbic acid have a linear concentration range of 10^{-5} M-10^{-3} M AA for MB-13X-CPEs and 10^{-5} M-10^{-4} M AA for MB-4A-CPEs. The theoretical detection limits, calculated from the slope of the regression equation and standard deviation of the calibration curve, were $4.7 \cdot 10^{-5}$ M for MB-13X-CPEs and $1.7 \cdot 10^{-5}$ M for MB-4A-CPEs.

These studies are expected to be constructive in the development of an AA sensor for real samples. Thus, several fruit juices containing AA were analyzed by the amperometric method and the results were compared with those obtained by spectrophotometric and standard addition methods [3].

[1] J. C. Bauernfeind, D. M. Pinkert, "Ascorbic Acid as an Added Nutrient to Beverage", Hoffmann-La Roche, Basel, **1970**, 24–28.
[2] M. Arvand, Sh. Sohrabnezhad, M. F. Mousavi, M. Shamsipur, M. A. Zanjanchi, Anal. Chim. Acta, 491, **2003**, 193-201.
[3] U. S. Pharmacopoeia XX, Mack Co., Easton, PA, **1980**.

K-18

DETECTION OF CAFFEINE AND ASCORBIC ACID FROM AQUEOUS MEDIA AND SOFT DRINKS USING A COMMERCIAL AVAILABLE BORON-DOPED DIAMOND ELECTRODE

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The presence of caffeine (CAF) and ascorbic acid (AA), well-known antioxidants, in foods and soft drinks demands an easily accessible evaluation method for qualitative and quantitative characterization. The possibility of electrochemical detection (voltammetric/ amperometric) represents a very promising solution for practical purposes. The aim of our study was to test the utility of a commercial boron-doped diamond electrode (BDDE) for the individual determination of CAF and the simultaneous determination of AA and CAF in both mixed synthetic aqueous solutions and real samples, i.e. CAF from Coca-Cola products such as Cola Zero or CAF and AA from Ice-Tea soft drinks. The use of BDDE provides several advantages regarding the experimental conditions, i.e., large potential window, low background current, low fouling effect and high stability of the electrode material, making it potentially useful for a large number of various practical applications. The electrochemical measurements were carried out in a Metrohm-three electrode cell equipped with a BDDE, working electrode - 3 mm-diameter stationary disc embedded in Teflon rod, counter-electrode - platinum foil, reference - saturated calomel electrode (SCE). The diamond electrode (Windsor Scientific Ltd.) for electroanalytical use was a mirror - polished doped polycrystalline industrial diamond (microcrystalline; doping degree ~ 0.1% boron). The cyclic voltammograms (CVs) and differential pulse voltammograms (DPVs) were collected using an Autolab PGStat 20 EcoChemie system (GPES Software version 4.8). The supporting electrolyte was 0.1 M Britton-Robinson buffer, pH 2. The potential ranges corresponding to the anodic limiting currents and the peaks of the oxidation of AA to dehydro-ascorbic acid (DHAA) and the oxidation of caffeine, respectively, involving the corresponding N-methyl-derivative intermediates, were determined from CVs and DPVs in both individual and simultaneous investigation tests. The linear calibration plots of the useful amperometric signal vs. concentration with very good correlation degree (R^2 around of 0.99) even in mixed systems, together with the high sensitivity, reproducibility and the low limits of detection, allowed an efficient application of DPV at BDDE for analytical purpose in individual or mixed solutions and real samples, either as standard addition or as calibration plots variants. In conclusion, both the detection of CAF and the simultaneous detection of AA and CAF were directly and easily assessed by the anodic oxidation of the target compounds on a commercial BDDE sensor without major effects of the matrix system, thus suggesting that the method presented here could be used for both analytical and practical purposes, e.g., for rapid estimation of the stability of system finger pattern quality, in food and soft-drinks industry.

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K-19

DETERMINATION OF FLAVONOIDS IN ORGANICALLY AND CONVENTIONALLY GROWN CARROT

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Flavonoids are one of the most important groups of compounds from polyphenols effective in plants' defence system. They are mostly involved in the UV light resistance and protection against herbivorous [1]. The main difference between organically and conventionally grown plants is in the protection measures allowed to be applied by the farmer. In conventional agricultural systems pesticides are generally used while in organic agriculture these compounds are prohibited. This could be one source of different levels of stress between organically and conventionally grown plants. Our aim is to assess if this is also reflected in the content of flavonoids. Organically and conventionally grown carrot from the variety Nerac was selected as the model plant for this target. Polyphenols were already reported as possible marker for discrimination between agricultural systems [2, 3].

In this work, several procedures for the extraction of some flavonoids in carrot samples were tested and a fast RP-HPLC method was developed. The flavonoids were extracted from freeze-dried carrots with aqueous methanol in an ultrasonic bath and subsequently pre-concentrated by evaporation. After filtration through nylon membrane microfilters they were separated by a binary gradient elution and identified with a diode array detector within analysis time less than 20 min. An external standard procedure was used for the determination of the selected flavonoids. The obtained limits of detection were in the range between 0.7 and 7.3 mg/L. This fitted for purpose procedure was applied for the analysis of carrots organically and conventionally cultivated in order to determine whether there is any statistically significant correlation between the content of selected flavonoids and the method of production (i. e. organic and conventional agricultural systems).

The homogeneity of the fields and the system of cultivation were evaluated by ANOVA single factor analysis and differences were considered significant at $p<0.05$. Results from the statistical treatment showed differences in the content of Epigallocatechin between carrots organically and conventionally grown and collected in 2006.

[1] Santos I.; Fidalgo F.; Almeida J. M.; Salema R. Plant Science 167(4) (2004), 925-935.
[2] Winkel-Shirley B. Current Opinion in Plant Biology 5(3) (2002), 218-223.
[3] Dixon R. A.; Paiva N. L. The Plant Cell 7(7) (1995), 1085-1097.

K-20

A NEW METHOD FOR SIMULTANEOUS DETERMINATION OF SUGARS AND CYCLITOLS IN OAK WOOD BY PRESSURIZED LIQUIDS EXTRACTION

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Alcoholic beverages (wines, vinegars, and distillates) aged in wood casks or alternatively, using oak chips are considered to be value-added products. The reason relies on the increased sensorial features that aged beverages demonstrate as a consequence of extraction and the degradation of many compounds from the wood's matrix.

It is well known that when distillates and wines are stored in oak barrels there has been an increase in the content of sugars during the ageing. This effect is usually attributed to the acid hydrolysis of the wood hemicellulose with the subsequent release of sugars. There are several deions in the literature of the transference and evolution of the monosaccharides content in wines and distillates during maturation process. On the other hand, the presence of cyclitols in wines also has been investigated, exploring their evolutions during the industrial manufacture and ageing of wines in oak wood under different conditions.

However, no references were found on the determination of these non-volatile compounds in oak wood samples. This fact encouraged us to set up a method which allows to evaluate the occurrence of monosaccharides and cyclitols in oak wood, establishing special emphasis on quercitol, a specific oak cyclitol used as marker of wine ageing in oak wood.

As a consequence, pressurized liquid extraction has been proven to be capable of extracting these compounds. To optimize the extraction process, an experimental design (three-level factorial design) was developed, testing different solvents. Two main factors were considered, the extraction temperature (40, 70 and 100ºC) and the extraction time (3, 9 and 15 min). As response variable, the extraction yield was used. The parameters of the model were estimated by multiple linear regression. The best yields were obtained with methanol at 94ºC and 15min.

The chemical characterization of the extracts was performed by gas chromatography coupled to mass spectrometry previous two-step derivatization procedure. Results pointed out that the method developed is suitable to carry out the simultaneous determination of monosaccharides and cyclitols presented in wood.

K-21
HYDROLYSABLE TANNINS: CHEMISTRY AND ANTIMYCOTIC ACTIVITY

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Hydrolysable tannins are secondary metabolites that generally are found in nature as multiple esters of gallic acid with glucose. They represent a biologically relevant group of polyphenols that encounter a growing interest to consumers, food manufacturers and pharmaceutical industries. That's because they influence the characteristics of food quality and the importance that foodstuffs have in human health. Based upon their structural characteristics, they are classified into simple esters, depside metabolites, hexahydroxydiphenoyl esters (HHDP), or ellagitannins, and oligomers formed by oxidative coupling of monomers. The qualitative and quantitative determination of glucogalloyl derivatives in food matrices, and nature general, is a difficult task even for modern analytical techniques. The present work is focused through a) the stereo-defined synthesis of glucogalloyl compounds as simple esters, with the HHDP unit and the m-depsidic link; b) the extraction and isolation of hydrolysable tannins from natural matrices c) the study of their spectrometric and chromatographic properties in order to develop new analytical tools for the identification of hydrolysable tannins in food and natural extract; and finally d) the in vitro antimycotic activity of the combination of Amphotericin B with the synthetic and natural glucogalloyl derivatives.1,2

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[1] Arapitsas, P.; Menichetti, S.; Vincieri, F. F. and Romani, A. 'Hydrolysable Tannins with the Hexahydroxydiphenoyl Unit and the m-Depsidic Link: HPLC-DAD-MS Identification and Model Synthesis' J Agric Food Chem, 50 (2007) 48.
[2] Romani, A.; Menichetti, S.; Arapitsas, P.; Nativi, C.; Turchetti, B.and Buzzini, P 'O-Methylglucogalloyl esters: Synthesis and evaluation of their antimycotic activity' Bioorg Med Chem Lett 15 (2005) 4000.

K-22

DETERMINATION OF COUMARIN, CINNAMALDEHYDE, CINNAMIC ACID, CINNAMYL ALCOHOL AND CINNAMYL ACETATE IN CINNAMON AND CINNAMON-CONTAINING BEVERAGES

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Cinnamon bark is widely used as spice for many different dishes. It is available in dried form as sticks and in grounded form as powder. The cinnamon of commerce derived mainly from two species of the genus cinnamon: Cinnamonum zeylanicum (Ceylon cinnamon, synonym C. verum) and Cinnamomum cassia (synonym C. aromaticum). The geographical origins are Sri Lanka, China, India, Vietnam, Indonesia and Brazil. They differ characteristically in flavour and fragrance properties and also in chemical composition. For example Cassia cinnamon contains often high levels of the toxicological relevant substance coumarin and low levels of eugenol, whereas in Ceylon cinnamon only traces of coumarin but higher levels of eugenol are detected.

Other main chemical compounds in cinnamon are cinnamaldehyde, cinnamic acid, cinnamyl alcohol and cinnamyl acetate.

In a first trial, the concentrations of these components in different cinnamon products, from different manufacturers, powder and quills and from different geographical origin both Ceylon and Cassia cinnamon were determined by HPLC-UV.

These constituents were also quantified in different cinnamon containing beverages under special consideration of their preparation: different cinnamon-based herbal teas, different black teas with cinnamon, commercially available and home-made mulled wine were tested. The influence of different preparation like infusion time and temperature (cold, pouring boiling water with and without simmering) and also the amount and type of added cinnamon were varied. For risk assessment the transfer of toxicological ingredients like coumarin into the beverage during preparation is of particular interest.

Coumarin concentrations ranged from 1700 mg to 5600 mg/kg in cassia powder and from 150 mg to 7900 mg/kg in sticks. In contrast in Ceylon cinnamon coumarin was detected only in traces both in powder and quills.

The large concentration range of coumarin detected in cassia cinnamon is possibly caused by different growing regions or differences in harvesting (inner, middle or outer bark). For powder another explanation is the mixing of cinnamon from different sources.

Before preparing the beverages, an aliquot of the solid material was homogenised, minced and the concentrations of the interesting compounds determined. In all tested beverages coumarin was released quickly into the liquid. Preparing herbal teas according to the instructions of the manufacturer, coumarin migrated nearly completely into the liquid. Even by steeping herbal teas in cold water, 70% of coumarin was detected in the liquid.

K-23

USING OFF-LINE AND ON-LINE COMPLEX FORMING PROCESSES FOR ELECTROPHORETICAL DETERMINATION OF BIOACTIVE COMPOUNDS IN FOOD

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The possibilities of capillary electrophoresis method (capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC)) with UV-detection for determination of biologilally active compounds (antioxidants, sugars, hydroxyacids etc.) in food are discussed. It was established that using off-line (liquid and solid phase extraction) and on-line (stacking, sweeping) complex forming processes with hard metal ions (Cu2+, Ni2+, Fe3+ etc.) had allowed to detect compounds nonabsorbing at UV-light, decreased its detection limits and also simplified electrophoretical profile of real object due to bonding of interfering matrix compounds. The determination of sugars which don't absorb at UV-light in different beverages is the important problem of analytical chemistry. It was noticed that the addition of solution containing ions Cu2+ into ammoniac running buffer in CZE mode allows to detect sugars with the high selectivity. It was shown that separation of mixture of the main biologically active tea components (polyphenols and alkaloid caffeine) is possible in MEKC conditions. The content of these compounds in tea may be the criterion of different falsifications of tea production. The influence of additives (milk, lemon) into tea, quality of drinking water and brewing conditions on polyphenols concentrations in tea beverage were studied. It was shown that the addition of milk to tea had resulted in considerably decrease of free polyphenols concentrations due to their interaction with milk protein caseine. The forming of complex was confirmed by spectrophotometry. On the contrary, the additive of lemon prevents the decomposition of thermally unstable polyphenolic compounds. Organic acids of lemon create acid medium (pH ~ 4), so protect polyphenols from the oxidation. The changing of tea polyphenols content was shown to occur due to its interaction with hard metals ions that are present at drinking water. The study of the complex forming processes of polyphenols with ions Fe^{3+} has allowed to simplify electrophoretical profile of biologically active components of green tea. Only caffeine peak is observed at electrophoregram of tea extract after adding of salt $FeCl_3$ into analyzed sample. It was verified independently that caffeine doesn't interact with Fe^{3+}. So, the additive of $FeCl_3$ into real object containing caffeine together with polyphenolic compounds (for example, different sorts of green and black tea) makes possible to determine caffeine with the high selectivity even when the resolution is not enough. This work is supported by grant of Russian Foundation for basic research 06-03-32580 a

K-24
PROANTHOCYANIDIN ANALYSIS IN ANTIOXIDANT DIETARY FIBRE FROM GRAPE

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The antioxidant activity of grape pomace has lead to the development of a new concept of *antioxidant dietary fibre (ADF)* [1], much more attractive than conventional dietary fibre due to the potential prevention effects provided by polyphenolic species. Proanthocyanidins (PAs), anthocyanins, flavonols, and phenolic acids are the main polyphenolic constituents of grape pomace. PAs are mixtures of oligomers and polymers composed of flavan-3-ols units, differing in the degree of polymerization (DP) and galloylation (G). The biological activity of PAs depends strongly on their DP. Whereas small PAs are absorbed through the gut barrier (up to trimers), larger PAs are not adsorbed, reaching the colon in its original form [2]. Therefore, polyphenols, and especially polymeric PAs, might play an important role in the prevention of cancer of colon.

In this work, the polyphenolic constituents of grape pomace ADF were determined. Due to the high number of compounds present, the soluble part of the fibre was fractionated. Each fraction was analyzed by LC-UV-ESI-MS/MS, using different modalities to identify and obtain structural information of the compounds. Accurate masses of the main components were also determined by HPLC-ESI-TOF. The polymeric fraction was separately analyzed, first by thiolytic degradation which provided an estimation of the mean size of the PAs, and then by ESI-MS-TOF and MALDI-TOF, so that the polymeric distribution was also determined. The data obtained can be used to establish composition-activity relationships on dietary fibre designed to prevent diseases such as colon cancer.

[1] Saura-Calixto, F., J. Agric. Food Chem. 1998, 46:4303.
[2] Terrill T.H., Waghorn G.C., Woolley D.J., McNabb W.C., Barry T.N., Br. J. Nutr. 1994, 72:467.

K-25

EFFECT OF PROBIOTIC YOGHURT ON OXIDANT PARAMETERS AND FLAVOPROTEINS IN HEALTHY WOMEN

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Introduction: Considering that increased oxidative stress caused by enhanced immune functions leads to alterations in the enzymatic defends system and thereby to an increase of the flavoprotein turnover in human body we tried to examine to which extent daily consumption of probiotic or conventional yoghurt (as a good source of vitamin B_2) has an influence on oxidant parameters and concentrations of flavoproteins (FAD, FMN) in plasma of healthy humans.

Subjects and design: After a preadjustment phase of 7d, female volunteers consumed 100g/d of a probiotic (probiotic group=PG: n=17) or conventional fermented yoghurt (conventional group=CG: n=16) for 14d (T1-T2) and 200g/d for another 14d (T2-T3). A wash-out phase lasting 14d followed.

Methods: Plasma concentrations of B_2 vitamers (FAD, FMN), conjugated dienes (CD) and malondialdehyde (MDA) were analyzed using HPLC.

Results: During the period T1-T3 the depletion of several plasma antioxidants led to a significant increase of the average plasma levels of oxidant parameters as MDA and CD in the PG (MDA: $p<0.01$; CD: $p<0.001$) and the CG (CD: $p<0.01$).

Simultaneously FAD concentrations were reduced significantly (T1>T3: $p<0.01$) in both groups. In contrast, plasma FMN concentrations increased significantly (PG: $p<0.01$; CG: $p<0.001$) in the period T1-T2 (100g yoghurt/d) and remained elevated throughout the whole interval of daily yoghurt intake. Due to the coherence of FAD and FMN in many biochemical reactions significant negative correlations between these two B_2-vitamers (FAD/FMN: PG: T2: $r=-0.687$; $p<0.01$; T3: $r=-0.801$; $p<0.001$; CG: T2: $r=-0.562$; $p<0.05$; T3: $r=-0.678$; $p<0.01$) and also between the B_2-vitamers and a marker of oxidative stress (FAD/CD: PG: T3: $r= -0.493$; $p<0.05$; CG: T3: $r= -0.529$; $p<0.05$; FMN/CD: PG: T2: $r= 0.353$; $p<0.05$; T3: $r= 0.500$; $p<0.01$; CG: T2: $r= 0.558$; $p<0.05$; T3: $r= 0.534$; $p<0.05$) were observed.

Although several analysed parameters changed during the study partly more distinct in the PG than in the CG, no significant differences between the groups were observed.

Conclusion: The outcome of this study indicates that the alterations of plasma FAD and FMN found throughout the four weeks lasting period of daily yoghurt intake could be the result of increased flavoprotein turnover caused by their involvement in several biochemical, immunological and antioxidant reactions which might be enhanced because of oxidative stress due to the stimulation of the immune system induced by both, probiotic and conventional yoghurt strains. This suggestion was also supported by the significant correlations found between FAD/FMN and between these B_2-vitamers and a marker of oxidative stress.

K-26

QUANTIFICATION OF APPETITE SUPPRESSING STEROID GLYCOSIDES FROM HOODIA GORDONII IN PLANT MATERIAL, PURIFIED EXTRACTS AND FOOD PRODUCTS USING LC-UV AND LC-MS METHODS

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Hoodia gordonii (Masson) Sweet ex Decne. is a succulent plant native to arid areas of southern Africa. Extracts of *Hoodia gordonii* can reduce food intake in rats and humans and are for that reason attractive as natural ingredients in food products for the management of body weight. Through bioassay-guided fractionation, one of the main active ingredients was identified to be the compound shown in Figure 1: 3-O-[β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl]-12–O-tigloyl-3β, 12β, 14β-trihydroxy-pregn-5-en-20-one [1].

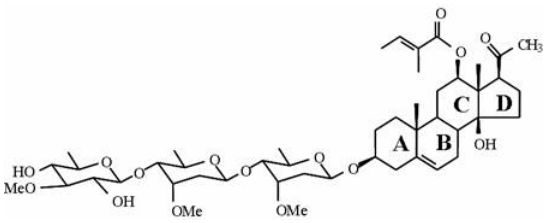

Fig. 1: Structure of one of the main active ingredients of Hoodia gordonii. This family of steroidal glycosides with food-intake reducing activity consists of a steroid core (A-D rings), a tiglate group attached to the steroidal C-ring and a chain of (deoxy-)sugars.

Extracts of the *Hoodia gordonii* plant contain a range of compounds with structures similar to that of the compound shown in Fig. 1, but with small differences in the sugar chain, *viz*. number and type of deoxy-sugars [2, 3].

Avula has recently developed a liquid-chromatography (LC) method for the specific analysis of the compound shown in Figure 1 [4]. In our study LC methods were developed that allow the quantitative analysis of the family of *Hoodia gordonii* actives in raw plant material, in purified and enriched extracts and in various prototype food products fortified with *Hoodia gordonii* extracts. Mass spectrometry was used to confirm that the compounds contained the characteristic steroidal core, the (deoxy-)sugar chain and the tiglate group.

The first step in the analytical protocol is an extraction. For solid materials as *e.g*. dried plants or for non-fatty foods, this is performed using methanol. For products where the steroid glycosides are present in an oil matrix, direct injection of the oil after dilution in tetrahydrofuran is applied. In the latter case special precautions have to be taken to avoid rapid column deterioration. The LC separation is performed on an octyl-modified reversed-phase column in the gradient mode with UV (λ = 220 nm) detection. Quantification is performed against an external calibration line prepared using the compound from Fig. 1. The pure standard material was isolated from an enriched extract using preparative liquid chromatography. Correction factors based on the molar mass of the respective compounds are applied to determine correct quantitative levels using the assumption that the molar extinction coefficients for all the glycosides are equivalent.

[1] F.R. van Heerden, R.M. Horak, V.J. Maharaj, R. Vleggaar, J.V. Senabe,
[2] P.J. Gunning, Phytochemistry (2007), doi:10.1016/j.phytochem.2007.05.022
[3] R.S. Pawar, Y.J. Shukla, S.I. Khan, B. Avula, I.A. Khan, Steroids 72 (2007) 524-534.
[4] S. Dall'Acqua, G. Innocenti, Steroids 72 (2007) 559-568.
[5] B. Avula, Y.H. Wang, R.S. Pawar, Y.J. Shukla, B. Schaneberg, I.A. Khan, Journal of
[6] AOAC International 89 (2006) 606-611.

K-27

ALOIN DETERMINATION BY 2D-LC WITH TANDEM MASS SPECTROMETRY DETECTION IN VARIOUS FOODSTUFFS CONTAINING ALOE VERA

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Since several years, various foodstuffs containing *Aloe vera* are commercialized. The structure of the *Aloe vera* sheets includes/understands a central part containing the juice or jelly and an external layer containing anthracene derivatives, of which aloin. Aloin possess the laxative and irritating effects of anthracene derivatives, which limits their use as well in the food or pharmaceutical field. For these reasons, UE and Switzerland legislation give a limiting value for the maximum content of aloïne of the foodstuffs. This value is fixed at 0,1 mg/kg. In order to satisfy these requirements, the mode of preparation of the aloe juice is particularly important if one wants to avoid contaminating the edible part of the sheets by the anthracene derivatives. In practice, it is the stage of separation between the freezing and the peripheral part of the sheets which is important. This operation is carried out manually. This stage is often neglected and some commercialized foods can contain high concentration of aloin. Then it is necessary to food control authority to have reliable analytical method for the determination of aloin in various foodstuffs. Aloin analyses were carried out by chromatography in two-dimensional liquid phase coupled with tandem mass spectrometry detection (HPLC-MS/MS). The first dimension is done with a Nucleodur 100-3 C_{18}-ec 30mm x 2 mm LC column and the second one with a Synergi 4 µ POLAR-RP 80A 150mm x 2mm column. Tandem masse spectrometry detection is performed with an electrospay ionisation interface and in MRM mode with three transitions. This approach allows to reach an excellent sensitivity and specificity and can be carried out on all products of the market announcing aloe like ingredient such as dairy products, juices of aloe or drinks containing of aloe juice, herb tea, liquid extracts in concentrated form and products presented in the form of pills.

Method was fully validated and results of a control plan is presented. It shows that if the value limit was generally respected in the dairy products based on *Aloe vera,* the case of the juices was very different. Indeed, some products exceeded the limiting value up to 350 times !

K-28
A NOVEL ANTICANCER AGENT, AC3, INDUCES G1 ARREST AND APOPTOSIS IN HUMAN CANCER CELLS

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The cell cycle is an evolutionary conserved process used by all eukaryotic cells to control growth and division. Proliferative disorders, such as cancer, are recognized as diseases of the cell cycle. Accordingly, it is likely that manipulating the activity of cell cycle regulatory proteins, such as CDKs, in diseased states will provide an important route for treating proliferative disorders, along with the opportunity to develop a novel class of future medicines. In the course of screening for a novel cell cycle inhibitor, a potent CDK inhibitor, AC3, was isolated from the root of *Aralia continentalis*. The root of *A. continentalis* has long been used as folk medicine in Korea for the treatment of various diseases. The molecular ion of AC3 was identified at m/z 285 with a molecular formula of $C_{17}H_{19}O_4$, and the chemical structure of AC3 was 3-propyol-2-vinyl-1,2,3,3a,3b,6,7,7a,8,8a-decahydrocyclopenta[a]indene-3,3a,7a,8a-tetraol. The current study reports on the biological properties of AC3 as a selective cell cycle inhibitor and apoptosis inducer in HeLa cells.

The antiproliferative effects of AC3 were assessed in four human cancer cell lines and normal lymphocytes as the control. The growth of all of the cancer cells tested was inhibited in a concentration-dependent manner by AC3. Interestingly, AC3 demonstrated no antiproliferative effect on the normal lymphocyte cells used as the control. The cell cycle analysis was performed on HeLa cells, after exposure to AC3 at 103 μM for 0, 12, 24, and 48 h. Flow cytometric analysis revealed an appreciable arrest of cells in the G1 phase of the cell cycle and apoptosis after treatment with AC3. Furthermore, Western blot demonstrated that the treatment of HeLa cells with AC3 resulted in an accumulation of hypophosphorylated pRb, decreases the levels of cyclin D, and increase the levels of CKIs, including p21, p27, and p53. To investigate the apoptotic induction in cancer cells by AC3, the DNA fragmentation of HeLa cells due to apoptosis was measured directly by a TUNEL assay. When HeLa cells were incubated with 103 μM of AC3 for 48 h, an apoptotic DNA fragmentation was observed in the HeLa cells. Furthermore, AC3 induced proteolytic cleavage of procaspase-3 and procaspase-9 into its active form, and subsequent cleavage of PARP. The activation of caspase-9 is likely to occur via the mitochondria pathway which leads to cytochrome c release, followed by cleavage of procaspase-9. This work was supported by 'Seoul R&BD Program(10508)'.

K-29
THE USE OF ELECTROCHEMICAL METHODS FOR THE DETERMINATION OF QUERCETIN AND ITS GLUCOSIDES IN ONION

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Onion, a worldwide-consumed vegetable, is as a rich source of dietary quercetin known as strong antioxidant. Quercetin concentration in onion is from traces in white to 2.5-3 mmol/kg in red varieties where occurs as various O-beta-glycosides with D-glucose as the main sugar residue. For this reason, an accurately and simple methods for determination of quercetin and its glucosides are highly requested.

The aim of this study was to determine quercetin and its glucosides in 80% methanol extract from lyophilized onion bulbs (*Allium cepa*) by use of HPLC with amperometric detection and cyclic voltammetry (CV) methods. A HPLC-UV recorded at 360 nm was used as a reference method.

Flavonoids were separated and determined by HPLC with amperometric detection after selection of the optimal potential applied to the glassy carbon (GC) working electrode. The selection was based on the analysis of the hydrodynamic voltammograms of flavonoids obtained within the range of applied potential from 0.05 to 1.0 V. The optimal conditions for the flavonoids separation has been established.

The CV method was applied for the qualitative assessment of onion flavonoids followed by the determination of anodic peak potential (E_a) of the standards. The quantitative determination of onion flavonoids was based on the anodic peak current (I_a) of the extracts after external standards addition.

The data provided by HPLC-UV showed presence of quercetin (Q) and its glucosides: quercetin 3,4'-O-glucoside (Q3,4'G), quercetin 3-O-glucoside (Q3G) and quercetin 4'-O-glucoside (Q4'G) in 80% methanol extracts of lyophilized onion.

The hydrodynamic voltammetric profiles of flavonoids showed a peak current of Q; Q3,4'G; Q3G and Q4'G increased rapidly, when applied potential exceeds +0.45 V. The high sensitivity and low background current was observed at applied potential of +0.95 V (versus Ag/AgCl). Therefore, the HPLC-amperometric detection chromatograms were recorded at +0.95 V. The lower limits of detection (LOD) were determined at signal-to-noise ratio of 3 and showed the following values: $8.05 \cdot 10^{-8}$ mol/L Q, $1.08 \cdot 10^{-7}$ mol/L Q3G, $1.22 \cdot 10^{-7}$ mol/L Q4'G and $2.6 \cdot 10^{-7}$ mol/L Q3,4'G. In this case, the detection limits were lower when compared to those derived from HPLC-UV detection.

The recordered cyclic voltammograms of above flavonoid standards provided the following E_a values: 123 mV, 238 mV, 346 mV and 650 mV for Q, Q3G, Q4'G and Q3,4'G respectively. The difference in values of E_a allowed for the identification of flavonoids in onion extracts. In this study it was possible to determine Q4'G and Q3,4'G by CV method however an additional steps in the extraction procedure are required in order to determine Q and Q3G, which are present in low concentration in the investigated onion extract.

K-30
VOLTAMMETRIC ANALYSIS OF ANTIOXIDANTS

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Nowadays problem of safety and quality of food is very important. Especially it is associated with food and food additives. The problems of effective concentrations, stability, methods of treatment, composition of biological active substances (BAS) in food additives still are not solved. This work reports a method and new analyzer "Antioxidant" (fig.1) for total antioxidant activity determination of food additives, enzymes, biological objects for monitor of their quality.

Fig.1 New voltammetric analyzer "Antioxidant" for the total antioxidant activity determination of food additives, enzymes and BAS.

This work offers to use effective and convenient voltammetric method for determination of the total antioxidant activity of food, investigation of the mechanisms of the reactive oxygen species (ROS) interaction with antioxidants by recording the current of the electrochemical oxygen reduction (ER O_2) at indicator electrode. The latter process has been treated as a "model" reaction because of similar processes of ER O_2 and the oxygen reduction in tissues:

$$O_2 + e^- \leftrightarrow O_2^{\cdot -} \quad (1)$$

$$O_2^{\cdot -} + H^+ \leftrightarrow HO_2^{\cdot} \quad (2)$$

Comparison of antioxidant activity determination in vivo and by this method showed identical results [1]. Applying the above approach, we offered the K as total antioxidant activity (TAA) coefficient of substances, which reflects the quantity of the ROS reacting with antioxidant at μmol l^{-1} min^{-1}. Moreover new coefficient of TAA (K^*) relatively standard antioxidant (dihydroquercetin) could be used for routine analysis.

This coefficient is comparable to the international sings ORAC (oxygen radicals absorbance) for evaluation of the total antioxidant activity of BAS [2]. The total antioxidant activity coefficients of some antioxidants are presented in table1.

Table 1 Total antioxidant activity coefficients of some antioxidants

Substance name	K, μmol l^{-1} min^{-1}	K^*, *ORAC eq.*
Dihydroquercetin	40.94	1.00
Bittner Balsam	6.64	0.16
Valerian extract	19.50	0.47
White wine (Vino da tavola, Italia)	20.98	0.51
Fir extract	37.45	0.91
Ionol	47.84	1.17
Green tea extract	109.10	2.66
Eucalyptus extract	122.00	2.98
	125.02	3.05

This method could be used for evaluation of effective concentrations, composition, stability of BAS, food and food additives [3].

Advantages of new analyzer "Antioxidant" are: 1. Analysis of different samples (0.01g or 0.01ml) without destruction; 2. Very cheap analysis because of the use of dissolved oxygen in solvent as reagent. 3. Sensitive, comfortable, automotive analysis. 4. Time of analysis: 10 min.

[1] E.I. Korotkova, O.A. Avramchik, Y.A. Karbainov., T.V. Kagiya, N.V. Tcherdyntseva // Talanta, v.63, N3, 729-734 (2004).
[2] L.Campanella; A.Bonanni; D. Bellantoni; G. Favero; M. Tomassetti // Journal of Pharmaceutical and Biomedical Analysis, v. 36, N1, 91-99 (2004).
[3] O.A. Avramchik, E.I. Korotkova, E.V. Plotnikov, A.N. Lukina, Y.A. Karbainov..// J. Pharmaceutical and biomedical analysis, v.37, N1, 1149-1154 (2005).

K-31
PHOTOCHEMIC TRANSFORMATION OF RESVERATROL AND ITS ANALOGUES

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The phenolic compound *trans*-resveratrol (3,4´, 5-trihydroxystilbene) belongs to main antioxidants of the wine origin. It is intensively studied during recent several years, because proving a range of positive effects on human health.

The presented work deals with photochemical transformation of *trans*-resveratrol, producing its *cis* isomer and also its dehydro-derivative 3,5,7-trihydroxyphenantrene, as well as couple of dimers, trimers and further oligomers. Products obtained in this way should afford compounds readily available from botrytic wines, formed during the biotransformation caused by the action of noble rot *Botrytis cinerea*. It should facilitate obtaining these rare natural compounds by a more accessible synthetic way.

Detection and identification of the compounds and all phototransformation products was performed by HPLC with UV detection and by the HPLC-MS method. Some of the isolated pure compounds were identified by NMR spectrometry.

It was found, that during the phototransformation process is formed a large scale of dimmers, trimers and also tetramers. However, according to our actual results obtained in our laboratory conditions, there were not found identical compounds with those occurring in current or botrytic wines. In majority, the obtained transformed substances are structural analogues of the natural compounds suitable for further biological assays and for mutual confrontation of their biological properties.

K-32

LC/ESI-MS2 AND MALDI-TOF ANALYSIS OF DIGESTED B-LACTOGLOBULIN GLYCATED WITH PREBIOTIC GALACTOOLIGOSACCHARIDES

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Prebiotics are non-digestible carbohydrates which are selectively fermented by the gut microflora increasing the number of beneficial bacteria. Recent researches in prebiotic area are focussed on the production of new persistent compounds which could reach intact the more distal parts of the large intestine where most of the intestinal affections (ulcerative colitis, colon cancer, etc.) take place. Some studies have demonstrated that Amadori compounds produced from the reaction between carbohydrates and free amino groups from amino acids, peptides and proteins, can reach the colon and be available for gut microflora fermentation (1). Therefore, the conjugation between a prebiotic carbohydrate and a protein could potentially allow carbohydrates to reach the distal parts of the colon and be fermented distally.

Recently, with the aim of obtaining a multifunctional ingredient (2), we have glycated β-lactoglobulin (β-LG) with prebiotic carbohydrates, galacto-oligosaccharides (GOS), via Maillard reaction. Both native and glycated β-LG were submitted to an in vitro gastrointestinal digestion (pepsin, trypsin and chymotrypsin) giving rise to a complex peptide profile. In this work, the chemical structures of glycated and non-glycated peptides were determined by MS techniques (MALDI-TOF and LC-ESI-MS2) to further compare their behaviour on gut microflora.

MALDI-TOF profiles of both native and glycated β-LG after enzymatic digestion showed marked differences mainly related to the lower intensity of glycated β-LG ions. The assignment of observed ions to the corresponding amino acid sequences was based on the known sequence of β-LG by using the tools available at the protein database Swiss-Prot and TrEMBL. This allowed the identification of 32 and 23 peptides covering 88% and 76% of the mature β-LG sequence in the digests of native and glycated samples, respectively. The further LC/ESI-MS2 analyses corroborated the MALDI-TOF results and they also enabled an extensive investigation into the glycated peptides produced by the in vitro gastrointestinal digestion. Thus, a total of 15 peptides glycated with GOS of different degrees of polymerization (from 2 to 7) could be identified. In conclusion, from these results could be inferred that Lys residues located at positions 47, 75, 77, 83, 91, 100, 135, and 138 are GOS glycation-sites. The characterization of these glycated peptides is needed to understand their effects in gut microbiota.

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[1] Erbersdobler and Faist, *Nahrung* **2001**, 45, 177.
[2] Sanz et al., *J. Agric. Food Chem.* **2007**, 55, In press.

K-33

OMEGA 3 HEALTH PRODUCTS; THE HEALTH BENEFICIAL EFFECTS OF CERTAIN OILS MAY BE COMPROMISED BY CONTAMINATING CHEMICAL POLLUTANTS

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An increasing body of evidence indicates that omega 3 fatty acids are beneficial for the health. Consumption of omega 3 fatty acids, either from eating fatty fish or omega 3 health products, could prevent a wide range of medical problems, including cardiovascular disease, depression, asthma, and rheumatoid arthritis. Although the health beneficial effects of omega 3 fatty acids are recognized, depending on the source of the omega 3 oil, these products may contain significant amounts of dioxins, PCBs and pesticides which may compromise the positive effects of these oils. In order to address this issue, we set out to analyze a hand full of omega 3 health products in Sweden. Different brands of omega 3 products and from different sources were bought at the local health store and at the supermarket; 1 vegetable oil (flaxseed), 5 fish oils (1 containing canola oil), and 1 seal oil. The 7 samples were processed by specific clean up steps and further quantified for PCDD/F, PCB and pesticides on a HRGC/HRMS. The tetra- to octachloro- isomers of PCDD/F, tri- to hepta-isomers of PCB and organochloropesticides (OCP) were identified and quantified in pg/g (fat). All fish oil samples showed low or non detectable values regarding the analysed compounds (Table 1). Negligible PCDD/F, PCB and OCP concentrations were found in the vegetable oil sample. However, in the seal oil we detected PCDD/F concentrations of 2.3 pg WHO-TEQ/g (fat), the highest concentration measured in any of the samples and critical with respect to the limit of 2 pg WHO-TEQ/g (fat) established by EU.

Table 1.PCDD/F, PCB, α-HCH, β-HCH, γ-HCH, 4,4'-DDT, 4,4'-DDD, 4,4'-DDE and Endosulfan-I concentrations (pg/g (fat)) of vegetable oil (a), fish oil (b-f) and seal oil (g)

sample	PCDD/F (WHO-TEQ)	PCB (WHO-TEQ)	α-HCH	β-HCH	γ-HCH	4,4'-DDT	4,4'-DDD	4,4'-DDE	Endo sulfan-I
a	0.003	0.001	24.8	n.d.	219	26.4	n.d.	9.8	169
b	0.68	0.02	10.4	6.9	n.d.	n.d.	69.4	54	246
c	0.09	0.15	n.d.	n.d.	n.d.	43	45.1	89.9	173
d	0.57	1.1	221	1255	51.4	2090	4893	22754	265
e	0.24	0.18	n.d.	43.1	n.d.	n.d.	326	243	30.8
f	0.86	0.17	22.8	26.4	n.d.	n.d.	379	229	n.d.
g	2.3	4.9	36736	14241	3490	6503	9294	135580	552

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K-34

MIXED-LINKED (1-3),(1-4)-B-D-GLUCAN AND ANTHOCYANIN CONTENT OF COLORED BARLEY IN KOREA

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Barley is recommended for healthy diets due to its high fiber content and antioxidant substances. In this study, the amount of fibers (mixed-linked (1-3), (1-4)-β-D-glucan) and antioxidant (anthocyanin) were determined in ten colored barley lines including four lines of hulled purple barley, four lines of unhulled purple barley and two lines of unhulled blue barley. Mixed-linked (1-3), (1-4)-β-D-glucan contents of colored barley were assayed by an enzymatic method and anthocyanins were analyzed using high-performance liquid chromatography (HP! LC). Results showed that mixed-linked (1-3), (1-4)-β-D-glucan contents of colored barley ranged from 4.26% for E 384/7 to 6.80% for Iksan 416, with a mean of 5.59%. The concentration of anthocyanin varied from 102.1 to 1478.8 μg g-1. Among the colored barley, the unhulled purple barley group had the highest average content of mixed-linked (1-3), (1-4)-β-D-glucan and anthocyanin. Results further indicate that colored barley were very good source of high fiber and antioxidant required for healthy foods.

K-35
QUERCETIN AS HEALTH PROMOTING FOOD COMPONENT

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The most important class of polyphenolic secondary metabolites in plants is flavonoids. It has been found that flavonoids in agricultural products have various physiological functions, such as antioxidaive, antitumor, antiviral, anti-inflammatory activity and a UV protective effect. Flavonoids function in plants as a defense filter of ultraviolet radiation. The absorption of UV-Vis radiation by some hydroxyflavones that are not stable against irradiation leads to their chemical changes followed by their eventual degradation.

Quercetin (3,5,7,3,'4'-pentahydroxyflavone) is the most abundant of the flavonoids and is also a building block for other flavonoids. Quercetin is found in many common foods including apple, tea, onion, nuts, berries, cauliflower and cabbage. It is well known that quercetin undergoes autoxidation the non-enzymatic reaction with atmospheric oxygen in organic solutions and aqueous media above pH 8.00. The autoxidation can also take place under physiological conditions.

We examined the effects of UV radiation on quercetin at pH 7.50 and pH 10.00. Our results showed that UV radiation accelerated quercetin autoxidation even at pH 7.50 *via* the formation of the oxidation product. The stability of quercetin and oxidation product was investigated as a function of irradiation time by using spectrophotometric and HPLC techniques. The apparent pseudo-first-order rate constants for quercetin degradation and oxidation product formation were calculated.

K-36
ANTIOXIDANT POTENTIAL OF PORTUGUESE HONEYS FROM LOUSÃ REGION

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Antioxidants play an important role in human health and food preservation by delaying or preventing the damage of auto-oxidation processes.

In the last years, studies were carried out on the antioxidant properties of different foods and some sources of natural antioxidants were also investigated in an attempt to answer the today's consumer trends, looking for food products away from the addition of synthetic preservatives [1,2,3].

Honey is used traditionally as a sweetening agent. This natural product contains a variety of preservative substances such as flavonoids and other phenolics compounds, enzymes and amino acids [3]. Many of these substances have antioxidant properties so, the determination of the total phenolic and flavonoid contents, the antioxidant capacity and the radical scavenging activity of honeys from different floral sources may deserve consideration.

The purposes of the present work were the quantification of antioxidant potential of three portuguese multifloral honeys from the Lousã region in comparison with the antioxidant properties of unifloral honeys of different botanic origin (*Eucalyptus*, *Trifolium*, *Pitosporum*, *Citrus* and *Erica*).

The Folin-Ciocalteau assay was used to determine the total phenolic content with a standard curve generated by galic acid [1,2]. The quantification of the flavonoid compounds was performed by the Dowd method, using a standard curve of quercetin [1]. The antioxidant content and the scavenging activity of honey samples for the radical 1,1diphenyl-2-picrylhidrazyl (DPPH) was measured by a spectrophotometric method and standard curve of ascorbic acid was used [1,2].

The highest levels of phenolic compounds, antioxidant capacity and scavenging activity were found in the *Erica* honey followed by the multifloral honeys from Lousã region, .which also had the highest flavonoid content. The lowest antioxidant potential was observed in the *Citrus* honey.

Furthermore, a good correlation was found between the phenolic content, the antioxidant capacity, the radical scavenging activity and the color of the honey samples.

Finally, the data from this research may promote the use of Lousã multifloral honeys as dietary sources of antioxidants to consumers.

[1] Meda, A., Lamien, C., Romito, M., Millogo, J., Nacoulma, O., *Food Chemstry,* 91, 571(2005).
[2] Beretta, G., Granata, P., Ferrero, M., Orioli, M., Facino, M., *Analytica Chimica Acta*, 523,185 (2005).
[3] McKibben, J., Engeneth, N. J., *Journal of Agricultural and Food Chemistry*, 50, 592 (2002).

K-37
ANALYSIS OF SOY ISOFLAVONES USING UPLC-MS

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Soybeans were originally found in eastern Asia over 5000 years ago. The Chinese farmers were responsible for cultivating these to create an edible crop, which then spread throughout Asia to Korea, Japan, and Southeast Asia. Soy eventually arrived in Europe around the 1700s.

More recently the consumption of soy products has been linked to many health benefits, as they are contain isoflavones. Isoflavones are commonly known as phytoestrogens and the 12 main isoflavones found in soybeans are daidzein (De), glycitein (Gle) and genistein (Ge) and their respective malonyl, acetyl and glucosyl forms

Research studies have indicated that isoflavones and consumption of isoflavone-containing foods are associated with a wide variety of health benefits, including prevention of breast and prostate cancers, cardiovascular disease, and reduced symptoms of diabetes and postmenopausal bone loss.

Genistein in particular has been shown to interact with animal and human estrogen receptors, causing effects in the body similar to those caused by the hormone estrogen.

The approval by the US Food and Drug Administration in 1999 allowing the food industry to promote soy protein for heart health led to an escalation in sales of soyfoods, and these foods are also being promoted for their isoflave content.

So this analysis of these type of compounds is important within a food environment.

The soy isoflavones were analysed using UPLC to see if the current methods could be shortened as literature shows that run-times can take up to 1 hour for the analysis of these compounds. The following settings were used in the experiment and a run-time of 5.5 minutes could be achieved using a dietary supplement.

The results showed that good chromatography could be achieved by using UPLC where the run-time is only 5.5 minutes. All 12 compounds identified as the key components in this supplement could be identified, with relatively good resolution and quantification would be facile with use of the mass spectrometry data.

K-38

DETERMINATION OF PHASEOLAMIN FROM KIDNEY BEAN (PHASEOLUS VULGARIS): AN ALPHA-AMYLASE INHIBITOR IN DIETARY SUPPLEMENTS

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The potential dangers of obesity and its high prevalence in developed countries are well known: it has been indicated as a significant risk factor in a wide range of morbidities, from apnea, dyslipidemia, hypertension, diabetes mellitus to coronary artery disease (1). The varying remedies currently available to control excess body weight include pharmacological preparations and dietary supplements intended to restrict energy absorbance and promote weight loss. Some of dietary supplements, so-called 'starch-blockers', are based on the protein concentrate from *Phaseolus vulgaris* or kidney bean, known to contain high levels of the α-amylase inhibitor or phaseolamin, which may reduce adsorption and prevent the digestion of carbohydrates.

The aim of this study was to have a reliable analytical method to determine phaseolamin in raw materials and dietary supplements because information on the composition of the basic material and on the manufacturing processes of these products is not available.

Currently methods to determine the levels of α-amylase inhibitor are based on the measurement of α-amylase activity by the different iodine staining power in the presence or absence of inhibitor during the action of the enzyme on soluble starch (2) or by using an alkaline reactive whose brown reduction products were determined photometrically as reported by Bernfeld (3). Since these colorimetric methods cannot be applied to dietary supplements, which are complex mixtures of different ingredients that may interfere with the measurement, we propose to determine the level of phaseolamin in dietary supplements by measuring directly, by high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD), the amount of maltose resulting from the enzyme action of porcine α–amylase on soluble starch in the presence and absence of the inhibitor.

The assay described proved sensitive and accurate for use with both dietary supplements and raw materials, and we verified the linearity, repeatability and applicability of the proposed method.

[1] World Health Organization (WHO) (1998) Obesity: preventing and managing the global epidemic. Report of a WHO Consultation on Obesity. Geneva. Switzerland.
[2] Bird R, Hopkins RH (1954) The action of some alpha-amylases on amylose. Biochem J 56: 86-99
[3] Bernfeld P (1955) Amylase α and β. Meth Enzymol I: 149-153

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A-38

DETAILED CIS/TRANS FATTY ACID ANALYSIS OF EDIBLE OILS AND FATS USING COMPREHENSIVE GC×GC–FID

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The determination of the fatty acid composition of edible oils and fats (as their methyl esters or FAMEs) is routinely performed in numerous food industries and laboratories all over the world. As long as no detailed information on the trans levels is required the analysis is rather straightforward. If trans-level analysis is desired, however, the analytical task starts to become more complex and long, highly polar GC columns with lengthy separations are needed with the interpretation of the chromatogram even than being a tedious process.

The standard method for trans-FAME analysis uses a capillary GC separation on a 50 to 100 meter highly polar cyanopropyl column (e.g. CP-Sil 88). With this technique trans analysis is possible in relatively 'simple' samples such as untreated or mildly processed vegetable oils. For more complex samples, such as for example the analysis of fat blends incorporating fish oils, the task starts to become highly challenging and prone to (small) errors.

In this contribution we will demonstrate the powerful characteristics of comprehensive GC×GC with FID detection for trans fatty acid analysis of commercial fat blends, processed oil samples and fish oils. Various column combinations for comprehensive GC×GC system are evaluated. Optimisation of the method is done by use of an Excel-based spreadsheet for GC×GC. Special attention is also paid to the methods for group-type integration available in the LECO ChromaTOF software. The results obtained with the new comprehensive GCxGC method are compared with those from the current standard GC methods, not only in terms of quantitative performance, but also with regard to operator time, reliability of peak identification etc.

C-30

RESULTS OF INTERCOMPARISON STUDIES AND TRAINING AS PART OF THE UNEP CAPACITY BUILDING PILOT PROJECT FOR POP ANALYSIS

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Within the work of the United Nations Environmental Protection (UNEP) Capacity Building project for training of laboratory staff in developing countries on persistent organic pollutant (POP) analysis, two small-scale intercomparison exercises were organised. The target compounds in the project were polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). Seven laboratories from five countries (Ecuador, Uruguay, Kenya, Moldova, and Fiji) participated. The first exercise, a national sample comparison, was organised in November 2006, before a series of on-site training sessions, and comprised shadow analyses by IVM of samples that had been selected and analysed by the participating laboratories. The second exercise was organized in January 2007, after the training sessions and after installing new GC columns, and comprised a test solution, a sediment and a herring tissue. The results were discussed in February 2007, at a workshop in Amsterdam. This part was held together with the discussion of a dioxin intercomparison that was organized by the Örebro University (Sweden), and in which laboratories from China and Vietnam had participated. In addition to these discussions, a 4-days training on POP analysis was given.

The results of the intercomparison were not essentially different from those obtained in the 1980s and 1990s in European POP laboratories. Only occasionally results were within ±20% of the target values. Calibrations were generally OK, and better for PCBs than for OCPs. For most of the laboratories it was the very first time they analyzed PCB congeners in environmental samples. The canned herring sample was recently certified for PCBs (BCR718). This allowed for the use of certified values with 95%-confidence interval half-widths as target values. In herring, nine values for PCBs were within 20% of the target value. For the few OCPs with target values that are reasonably high (over 1 $\mu g.g^{-1}$ fresh weight: p,p'-DDE, HCB and dieldrin) seven cases of reported values within 20% of the target value can be discerned, four of them concern p,p'-DDE.

Creating an effective network of POP laboratories in different continents together with a series of interlaboratory studies is suggested to improve the measurements of POPs all through the world.

This study was carried out by UNEP Chemicals, Switzerland, with financial support through the medium-sized GEF-funded Project "Assessment of Existing Capacity and Capacity Building Needs to Analyse POPs in Developing Countries". Besides the GEF, the governments of Canada, Germany, and Japan contribute financially to this project.

E-26

DETERMINATION OF DAPSONE RESIDUES IN MILK USING LIQUID CHROMATOGRAPHY / TANDEM MASS SPECTROMETRY

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4,4'-diaminodiphenylsulfone (DDS), commonly named dapsone has been used in the past in veterinary medicine either alone or in combination with others drugs, for the intramammary treatment of bovine mastitis, for the oral treatment of bovine coccidiosis and the intra-uterine treatment of endometritis. Since 1994, this compound has been banned from use within the European Union for food producing animals. It is classed in Annex IV of Council Regulation no 2377/90/EC, therefore no residue of dapsone should be found in food products.

A simple and rapid method using liquid chromatography / tandem mass spectrometry is presented for the confirmation of dapsone in bovine milk. The extraction of the drug substance was based on a liquid/liquid extraction using acetonitrile. After evaporation, the reconstituted extract was analysed using LC/MSMS operated in electrospray positive mode using MRM detection. Determination of dapsone residues in milk was carried out by external calibration without internal standard. Validation of the method was performed according to the criteria mentioned in Commission Decision 2002/657/EC. Validation data will be presented. Trace determination of dapsone at level lower than 1 µg/l could be easily obtained.

F-50

COMPARATIVE STUDY CONCERNING THE LEVELS OF OCHRATOXIN A IN ENSILED MAIZE WITH DIFFERENT PROVENANCE

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Ochratoxin A (OTA), a toxin produced by *Aspergillus ochraceus* and *Penicillium verrucosum*, is one of the most abundant food-contaminating mycotoxins in the world. Human exposure occurs mainly through consumption of improperly stored food products, particularly contaminated cereals (mainly corn, wheat and oats) and pork products, as well as coffee and wine grapes. Exposure to ochratoxins through diet can have acute toxicity to mammalian kidneys and may be carcinogenic, so that the European Community established maximum levels for contamination in cereals (5 µg/kg – EC 1881/2006). Maize constitutes a great part from the dietary staple for both human and the majority of animals in some agricultural regions in Romania, therefore the estimation of the amount of OTA is more important.The aim of the present study was to evaluate and compare the content in OTA of maize harvested in 2006 and ensiled in different conditions. There were analyzed 6 samples of maize ensiled in big industrial silos and 22 samples provided by some peasant farms. The levels of OTA were determined using a HPLC method. Grounded maize kernels (10 grams) were extracted in 1% bicarbonate solution and filtered. The samples were cleaned-up using Ochraprep immunoaffinity columns. The elution of OTA was made with methanol-acetic acid (98:2 v/v), which was injected in HPLC system and than chromatographed on a reverse-phase symmetry C18 column with water-acetonitrile-acetic acid (47:51:2 v/v) as mobile phase. The resulted data were quantified using a fluorescence detector (333 nm excitation and 443 nm emission). Certified reference material with 16.8 µg/kg OTA (+/- 4.5 µg/kg) was used as control. The obtained results allow us to establish the safety of the maize storage in conditions typical for the peasant farm so that the amount of OTA in the contaminated maize samples to not exceed the safe limit legislated by the European Community.

F-51
PREVALENCE OF PYRROLIZIDINE ALKALOIDS IN BEE HONEY

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In 2005 and 2006 several reports have been published regarding the toxic effects that can occur in animals and in particular in leisure horses as a result of the presence of certain plant species, specifically ragwort (*Senecio jacobaea L.)* in the raw fodder (hay and/or silage). These toxic effects, which cause irriversible liver damage, are related to the presence of pyrrolizidine alkaloids (PA). As a result concern has also been raised with regard to possible human dietary exposure and the human health risk of these substances. In this respect, honey has been identified as a possible significant source of pyrrolizidine alkaloids in the human diet[1].

Tolerance levels for the presence of PAs in honey are largely lacking or are unclear. In Australia a tolerable daily intake has been established of 0.1 µg/kg bw/day while in Germany a tolerable daily intake of 1 µg/pp/pd is generally used. In The Netherlands a tolerance of 1 µg/kg on a product basis is established however this applies to herbal preparations for human consumption only. Recently however, an intake tolerance of 30 ng per person per day was proposed which is much stricter than the tolerances proposed previously. This strict tolerance (Virtually Safe Dose, VSD) is based on the genotoxic carcinogenic properties of some of the PAs.

EFSA recently published a scientific opinion[1], which states that PAs indeed may pose a threat to human health and further research is required. Recommendations made by EFSA include monitoring of PAs, which are representative for a range of plant species, including *Senecio* species, in animal feed as well as food products intended for human consumption and secondly to quantitatively assess the contribution of honey to human dietary exposure to PAs.

This paper describes an analytical method based on LC-MS/MS for the analysis of PAs in honey. The method includes several PAs *viz.* riddelline (RID), retrorsine (RET), senecionine (SCN), seneciphylline (SCP) and integerrimine (INT), which can occur in ragwort (*Senecio jacobaea L.*) as well as in other species. In addition a number of their N-oxides were included *viz.* retrorsine-N-oxide (RET-NO), senecionine-N-oxide (SCN-NO), seneciphylline-N-oxide (SCP-NO). Limits of detection between 0.5 and 2 µg/kg proved achievable, depending on the compound.

For the identification of the PAs, the requirements of Commission Decision 2002/657/EC were applied yielding unambiguous identification of the compounds detected in honey.

In this paper we also present the results of PAs monitoring in honey. The prevalence of PAs in commercially available honey intended for human consumption was studied over the last two years. In total 171 samples of honey were analysed and a considerable number of those samples contained detectable amounts of PAs (43 of the 171 (25%)). It is therefore beyond any doubt that the human consumer could be exposed to considerable levels of PAs via consumption of honey.

In addition to the analytical results, this paper also discusses the challenges and focal points for future research.

[1] Anonymous, Opinion of the scientific panel on contaminants in the food chain on a request from the EC related to pyrrolizidine alkaloids as undesirable substances in animal feed (question EFSA-Q-2003-065), 25 January 2007, The EFSA Journal (2007) 447, 1-51.

F-52

DEVELOPMENT OF A MULTIPLEX QUANTITATIVE IMMUNOASSAY BASED ON THE ELISA REVERSE M&D FOR THE QUANTIFICATION OF DEOXYNIVALENOL, ZEARALENONE, OCHRATOXIN A MYCOTOXINS, SIMULTANEOUSLY

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An innovative application of the ELISA Reverse m&d for simultaneous quantification of mycotoxins (deoxynivalenol, zearalenone, ochratoxin A) in cereals was established. The assay was designed in support to the Commission Recommendation (EC) 576/2006 concerning the threshold limits for mycotoxins in cereals and cereal products intended for animal feeding. In particular EC 576/2006 recommends to the Member States to test simultaneously the presence of deoxynivalenol, zearalenone, ochratoxin A, fumosinin B1 + B2 in cereals and cereal products in feed. Here we describe an innovative multitarget protocol based on the ELISA Reverse m&d (ER) able to quantify simultaneously the presence of deoxynivalenol, zearalenone, ochratoxin A in cereals within the limits imposed by the Raccomandation. In particular the method was successfully applied to detect 2, 4, 8, 16 ppm of deoxynivalenol, 0.5, 1, 2, 3 ppm of zearalenone and 0.05, 0.1, 0.25, 0.5 ppm of ochratoxin A. The method, the experimental design and the obtained results are here reported and discussed.

G-25
SURVEY OF ORGANOTIN MIGRATION FROM FOOD CONTACT MATERIALS

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The industrial use of organotin compounds was 20,000 tons /year (2002), of which PVC stabilizers accounted for approximately 15,000 tons/year, and the other four important usages were biocides, catalysts, agrochemicals and glass coatings [1].

For food contact materials (FCM), certain mono- and dialkyl tins have been approved as PVC stabilizers, and listed in the octa- and mercapto forms on the plastic positive list [2]. However, other organotins may be present, due to impurities in the starting compounds and because organotins are used also as catalysts in silicones e.g. used on baking paper and in (aliphatic) polyurethane, e.g. used in agglomerated wine cork stoppers [3].

In 2006/07 a survey of organotin migration from 33 FCM samples was conducted in Denmark. Samples included silicone-coated baking paper, silicone forms, PVC cling films, metal screw-cap lids with plastisized PVC gaskets, and polyurethane agglomerated cork wine stoppers, some coated with silicone.

GC-ICP/MS was used for the analysis of nine different organotin compounds and proved to be a versatile tool for organotin speciation analysis with detection limits from 0.01-0.04 µg/kg and 8-31% RSD (average 15%).

In a pre-study to find the worst case migration conditions, the results of 3% acetic acid vs. 95% ethanol were in-conclusive: In 3% acetic acid at 100 °C/4 hours/reflux the recovery of di-butyl-tin (DBT) in particular was low, but without the degradation product mono-butyl-tin (MBT) being formed. It is possible that the surfactant nature of organotin compounds could affect their solubility in a temperature and matrix dependent way. For this reason 95% ethanol/60 °C/6 hours/oven was used as the worst-case and/or screening test for the majority of the samples.

Low levels of DBT and tri-butyl-tin (TBT) were found in metal lids and corks in 11 of the 33 samples. As DBT and TBT are not on the positive list, an EU sum-migration limit of 6 µg/kg was derived based on a recent evaluation by EFSA [4] for DBT, TBT, TPhT and DOT (similar mode of immunotoxic action), using Article 3 in the work regulation[5].

All samples were compliant when testing according to the Technical directives[6], but 2 lids had DBT migration > 6 µg/kg, when exposed to a double-sided test with 95% ethanol. Migration of organotin compounds from FCM may therefore cause organotin concentrations of toxicological concern in foods.

[1] EU report: Assessment of the risks to health and the environment posed by the use of organostannic compounds, EU DG Enterprise, 2002.
[2] [Plastic Directive for FCM, EU 2002/72/EC with amendments
[3] Jiang, G, Liu, J. and Zhou, Q., Environ. Sci. Technol, 2004, 38, 4349-4352.
[4] EFSA Opinion on the health risks to consumers associated with exposure to organotins in foodstuffs, The EFSA Journal (2004) 102, 1-114
[5] EU work regulation (EC) No 1935/2004
[6] Technical Migration directives 828711/EC + amendments 93/8/EC and 97/8/EC

LM-1

BIODEGRADATION OF 5-(HYDROXYMETHYL)-2-FURFURAL (HMF) IN MODEL FOODS USING SOME LACTIC ACID BACTERIA AND YEAST

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5-(Hydroxymethyl)-2-furfural (5-HMF), a common product of the Maillard reaction, occurs in many foods in high concentrations, sometimes exceeding 1 g/kg. The toxicological relevance of this exposure has not yet been clarified. But induction of aberrant colonic crypt foci had been reported for HMF. In this study, biodegradation of 5-HMF in model food were determined. A simple and reproducible HPLC-diode array detection method for the qualitative and quantitative analysis of 5-HMF in model dairy based foods was modified and developed. For the method of the biodegradation of 5-HMF in model foods, firstly four kinds of bacteria (*Lactobacillus bulgaricus, Lactococcus cremoris, Lactobacillus casei, Leuconostoc mesenteroides*) and secondly, three kinds yeast (*Zygosaccharomyces bailii*, *Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae*) were included. Reconstitute milk, reconstitute whey and salep beverage were determined as model foods and the initial 5-HMF level of these foods adjusted by using stock solution as 124.37, 151,50 and 149.45 ppm respectively. Selected model foods were fermented with bacteria (10^7 colony-forming units [CFU]/mL at 37 ºC for 48 h) and yeast (10^5 colony-forming units [CFU]/mL at 25 ºC for 48 h). HMF levels and number of microorganisms in the model foods were determine in certain time interval (0, 6, 12, 24 and 48 h). End of the yeast fermentation decreasing of 5-HMF level of the model foods found as 22-97%. And also decreasing of 5-HMF level of the model foods determined as 11-99% in bacterial fermentations.

Keywords: 5-HMF, degradation, lactic acid bacteria, yeast

LM-2

FATTY ACID COMPOSITION AND VOLATILE COMPOUNDS OF CAVIAR FROM FARMED WHITE STURGEON (ACIPENSER TRANSMONTANUS)

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Caviars are the salt-cured and preserved eggs of many sturgeon species that have been separated from the supporting connective tissue. The most famous and valuable caviars are produced from harvested sturgeons in the area of Caspian Sea, namely Beluga (*Huso huso*), Osetra (*Acipenser gueldenstaedtii*) and Sevruga caviar (*Acipenser stellatus*). Over the past few decades, owing to the strong demand for sturgeon caviar, the over-exploitation of natural stocks has led to a dramatic decreases of the supplies. To protect these endangered species, in 1997 the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) decided to limit trade of caviar by listing all sturgeon species on the Annex II of the convention.

The lack of supply and the continuous demand for this product have increased the feasibility of caviar production from aquaculture. There are high quality and environmentally friendly alternatives available such as caviar produced from farmed white sturgeon (*Acipenser transmontanus*) in Italy, Siberian sturgeon (*Acipenser baerii*) in France and Germany, Adriatic sturgeon (*Acipenser naccarii*) in Italy and Spain. Among pure species, *Acipenser stellatus,* the beluga and the paddlefish (*Polyodon spathula*) are reared mainly in extensive or semi-intensive farming on a limited scale. The successful of producing caviar from farmed sturgeon has arisen several investigations aiming to produce the chemical characterization of the product. Biochemical assays together with sensory studies have been carried out with the purpose to determine whether compositional and sensory differences might be observed between caviar from farmed and wild sturgeon.

The study was designed to characterize caviar from farmed white sturgeons (*Acipenser transmontanus*) subjected to different dietary treatments before eggs collection. Fifty caviar samples from fifty farmed sturgeons have been analyzed for proximate composition, fatty acid and volatile compounds.

LM-3
LIQUID CHROMATOGRAPHIC DETERMINATION OF ERGOT ALKALOIDS IN CEREALS AND CEREAL PRODUCTS

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Ergot alkaloids are mainly produced by the parasitic fungus *Claviceps spp*. which is capable of producing sclerotia in the ears of cereals such as rye, wheat, barley and oat and other *Poaceae*. These brown-coloured sclerotia contain a variety of toxic indol alkaloids which are related to numerous epidemics of ergotism in the middle age.

Alkaloid content and pattern show a strong variability depending on the producing fungal strain, the host plant, the geographic origin of host and fungus and the climatic conditions. Up to know, forty alkaloids have been isolated.

Acute intoxication – 5 to 10 g can be lethal – expresses itself in nausea, headache, paraesthesia, convulsion, death by respiratory paralysis and circulatory collapse. Pregnant women suffer uterus contraction associated with bleeding, tetanus uteri followed by suffocation of the embryo, miscarriage and uterus rupture.

The Federal Institute for Risk Assessment concluded in its expert report in 2002 that data about ergot alkaloid exposure are insufficient at present. Hence, it was the aim of this project to develop, establish and validate a method that is suitable for residue analysis. In addition, food samples (cereals, flour, bread etc.) are to be analysed to gain reliable data to estimate human exposure.

An effective and time-saving method has been developed for the determination of twelve ergot alkaloids in cereals and cereal products.

Samples are extracted with a mixture of ethylacetate, methanol and aqueous ammonia and extracts are purified using a basic alumina column for solid phase extraction. After a solvent shift and a centrifugation step, liquid chromatographic determination is performed either by fluorescence or tandem-mass-spectrometric detection.

Validation data will be present for both analytical systems.

LM-4

A COMPREHENSIVE MULTI-RESIDUE SCREENING METHOD FOR THE TARGET ANALYSIS OF MORE THAN 200 PESTICIDES IN VARIOUS CROPS USING LIQUID CHROMATOGRAPHY – TIME-OF-FLIGHT MASS SPECTROMETRY

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Many countries have passed legislation to ensure that pesticides are used safely. Detection of trace levels of pesticides in food, feed and the environment is the main objective of regulatory laboratories. The introduction of mass spectrometry instrumentation with atmospheric pressure ionization has revolutionized the way analyses using liquid chromatography are approached. Analysis by liquid chromatography tandem mass spectrometry is already widely used in regulatory laboratories due to its excellent capability to perform multiresidue analyses.

Recent improvements in TOF technology, including orthogonal acceleration, ion mirrors (reflectrons), and high-speed electronics, have significantly improved TOF resolution. This improved resolution, combined with powerful and easy-to-use electrospray (ESI) and matrix-assisted laser desorption ionization (MALDI) ion sources, have made TOF MS a core technology for the analysis of both small and large molecules.

The potential of an alternative screening strategy, based on the ability of an orthogonal-acceleration time-of-flight mass spectrometer (oaTOF MS) to routinely perform accurate mass determination at 10 ppm is explored. A 5mM ammonium formate -methanol gradient elution has been used in conjunction with electrospray ionization oaTOF mass spectrometry to detect residues of pesticides and/or their metabolites in different crop matrices. The positive ion electrospray full-scan mass spectra consist of one or a few ions, that are usually adducts of the molecule with a proton, a sodium ion, or an ammonium ion.

Accurate mass / database searching with TOF is providing an attractive alternative to library searching. Searching for the exact molecular mass of $[M+H]^+$ and/or $[M+NH_4]^+$ within a retention time window, the pesticides were identified with a relative mass error of less than 5 ppm. Confirmation of the identity of the pesticides depends largely on the exact mass measurements and the retention time. The combination of full-scan spectra incorporating exact mass measurements of the protonated molecules with the evidence from resolved isotopic cluster patterns provides the ability to confirm compounds in difficult food matrices. In addition, the ability to discriminate and unambiguously identify pesticides in mixtures of isobaric compounds was also investigated. Further unambiguous confirmation could be achieved by increasing the fragmentor voltage to promote in-source collission induced dissociation (CID). In this way, characteristic accurate mass fragment ions of the pesticide could be seen in the full-scan mass spectra.

In this study, LC – oaTOF MS is shown to enable the determination of more than 200 pesticides from various compound classes in crops down to concentration levels in the range of 10 – 50 ppb. The limits of detection for a group of indicator pesticides were assessed and compared with those of our routinely applied multiresidue method based on triple-quadrupole tandem mass spectrometry. The rate of false positive / false negative findings was evaluated, in relation to the set permissible mass accuracy (3-10 ppm error) and retention time window.

LM-5

MULTIRESIDUE METHOD VALIDATION FOR PESTICIDE RESIDUES IN SOY GRAIN

PART 2: DETERMINATION OF 169 PESTICIDES BY LC-MS/MS (POSITIVE AND NEGATIVE ESI MODES), WITHOUT USING ANY CLEAN UP

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LC-MS/MS (positive and negative ESI) was used for the multiresidue pesticide analysis of soy grain samples, using two different extraction methods (based on acetone and acetonitrile). Clean up appeared not to be required for soy extracts.

Both extraction methods in combination with both LC-MS/MS modes were fully validated for 169 pesticides. From those 169 pesticides, 155 were analyzed in the ESI positive mode in a single chromatographic run time of 32 minutes, and 14 in the ESI negative mode with a run time of only 10 minutes. For recovery studies, milled and homogenized, blank soybean samples were spiked at 3 different spike levels (10, 50 and 100 µg kg^{-1}, n=6).

Even though no clean up was applied, no significant matrix effects were observed for most of the pesticides. LC-MS/MS thus showed a considerably lower matrix effect than that observed with the GC-MS methods. The linear dynamic range of the analytical curves was between 0.1 or 0.25 and 10 ng mL^{-1}. Circa 90% of the pesticides showed $r^2 \geq 0.999$, using standard solutions prepared either in matrix extract or in solvent. The ESI positive mode generated LOQs of 10 - 50 µg kg^{-1}, for 80% of the pesticides studied. In the ESI negative mode, it was not possible to detect flusulfamide at all spike levels, while 2,4–D, flucycloxuron and chlorfluazuron were not reliably quantified, due to low recoveries and/or high RSD values.

Comparing the two extraction methods tested, acetone and acetonitrile, it can be clearly observed that recoveries obtained from the last one are consistently (10 – 20%) higher than those from the first one. Especially at lower residues levels (10 – 50 µg kg^{-1}), precision is better (lower RSD%) for acetonitrile. The lower acetone extraction recoveries, with mostly still acceptable RSDs, though, could indicate to a less favorable partitioning of the pesticides between the organic phase and the aqueous phase.

Comparing GC- and LC-MS/MS, the latter technique was more efficient for pesticide analysis, taking into account sample throughput time, robustness and sensitivity. Also, the possibility to perform the determination of a large number of pesticides in just one single chromatographic run is another major advantage.

LM-6

DETERMINATION OF MACROLIDE ANTIBIOTICS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY IN FOOD PRODUCTS

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Macrolide are antibiotics, produced by actinomycetes fungi species streptomyces, and consist of macrocyclic lactone ring, containing 14, 15 or 16, atoms to which sugars are bonded. They are very important antibacterial compounds, widely used in veterinary medicine to treat respiratory diseases, or as growth. The presence of drug residues in food products could attempt the consumers health; for this reason the European Union (EU) has established maximum residue limits (MRLs) for most of these veterinary drugs.

The aim of the present work is the development of an analytical method in order to confirm the presence of residues of spiramycin, tylosin, erythromycin, josamycin and, virginiamycin in milk, eggs and edible tissues of food producing animals.

High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) for multiresidue determination of macrolides and other antibiotic drugs was developed using roxithromycin as internal standard.

Samples were minced with tris buffer at pH 9.0 and the drugs were extracted with ethyl acetate. The extract was cleaned up on a Strata X (60 mg) SPE cartridge. The chromatographic separation was achieved on a Waters Symmetry C_{18} column (150×2.1 mm x 5 µm) with a gradient system of 0.1 % Formic Acid / MeOH 90/10 to 0.1% Formic Acid / MeOH 10/90 as the mobile phase; the system was set at a flow-rate of 0.15 mL/min with a loop injector of 5 µL. Multi reaction monitoring (MRM) acquisition by positive electrospray ionisation (ESI) was performed checking the linear range from 5 to 100 ng for each drug.

Detection limit was between 10 ng/g and 30 ng/g depending on the specific drug; the recoveries was evaluated at the levels of 10 ng/g, 20 ng/g, 30 ng/g and ranged between 70.8 and 86.4%, for each drug.

The developed method results helpful for such laboratories involved in official analysis for monitoring the proper or illegal use of such antibiotics in food producing animals.

LM-7

DEVELOPMENT OF A PRESSURISED LIQUID EXTRACTION AND LIQUID CHROMATOGRAPHY WITH ELECTROSPRAY IONIZATION AND TANDEM MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF SUDAN DYES IN FOOD

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A lot of dyes are used as food additives and include both natural and synthetic substances, covering a wide range of chemical entities. The most common artificial food colours are azo dyes [1]. Synthetic Sudan dyes are non-authorised and illegally used in the food industry to enhance and maintain the appearance of food products such as in chilli-, curry-, curcuma-, and palm oil-containing foodstuffs [2].

The U.K. Food Standards Agency (FSA) alerts for the contamination with Sudan I dye of various meat preparations on the market in the U.K. and issues warnings about frozen meat products, spice mix, and chips containing contaminated chilli powder [3]. As a consequence, the EC Commission has adopted a decision requiring that all chilli-, curry-, curcuma-containing food products and palm oil coming into any EU state are certified to be free of Sudan dyes [4].

Sudan I is considered to be a genotoxic carcinogen [5] and its presence is not permitted in foodstuffs for any purpose at any level. Sudan II is the dimethyl derivative of Sudan I and it has been tested in mice by bladder implantation, resulting in a high incidence of bladder carcinomas [6] . Sudan III and Sudan IV are fat-soluble dye predominantly used for demonstrating presence of triglycerides in frozen sections. In addition, Sudan III and Sudan IV are commonly used for coloring waxes, oils and spirit varnishes. As regards to Sudan III and the dimethyl derivative Sudan IV, no LD50 /LC50 information relating to normal routes of occupational exposure is available. Sudan I, Sudan II, Sudan III and Sudan IV have been classified by IARC in the Group 3, i.e. not classifiable as to their carcinogenicity to humans.

For this reason, sensitive, selective and accurate analytical methods should be developed in order to identify and quantify such substances in complex matrices as processed foodstuffs.

An automated and sensitive method has been developed to determination of Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Orange G and Sudan Red 7B from foods. The proposal method includes pressurized fluid extraction (PFE) followed by gel performance chromatography (GPC) and chromatographic detection by LC-MS-MS.

Several solvent systems have been investigated for PFE extraction. Operating PFE conditions were optimized using a design of experiments (DOE) [7]. Acetone gave the best recoveries at 95ºC and 1250 psi, in a total time of 2 x 5 min.

The purification by gel permeation chromatography allowed obtaining “clean” extracts for the LC-MS-MS system and avoiding the use of matrix-matched solutions [2].

A reversed-phase high-performance liquid chromatography (RP-HPLC) method-mass spectrometry detection using electrospray ionization (ESI) and working in selected reaction monitoring mode (SRM) was developed for the quantification and confirmation of the analyte. The ion source settings including capillary temperature, seath gas pressure, auxiliary gas pressure and spray voltage, were optimized using a central composite design (CCD), and collision energy and tube lens were automatically optimized.

The method was applied to the determination of sudan dyes in spices, sauces and meat products in order to evaluate the compliance in the work of the European Legislation.

[1] Cornet V., Govaert Y., Moens G., Van Loco J., Degroodt J-M., 2006. *J. Agric. Food Chem*. 54, 639-644.
[2] Calbiani, F., Careri M., Elviri, L., Mangia A., Pistarà, L., Zagnoni, I., 2004. *J. Chromatogr. A*. 1042, 123-130.
[3] Food Standards Agency: http://www.food.gov.uk.
[4] Comission Decision 2005/402/EC of 23 May 2005 on emergency measures regarding chilli, chilli products, curcuma and palm oil. *Official Journal of European Communiities*, L135, 34-36.
[5] M. StiborovÃ¡, V. MartÂ´ınek, H. RydlovÃ¡, P. Hodek, E. Frei, *Cancer Res*. 62 (2002) 5678.
[6] A. Pielesz, I. Baranowska, A. Rybak, A. Wlochowicz, Ecotoxicol. *Environ. Saf*. 53 (2002) 42.
[7] Massart D.L., Vandeginste D.G.M., Buydens C.M.C., S. de Jong, P.J. Lewi, J. Smellers, V.R. Beke, Handbook of Chemometrics and Qualimetrics, Elsevier Science, Amsterdam, 1997.

LM-8

FAST MULTI-ANALYTE METHOD FOR THE SIMULTANEOUS DETERMINATION OF 221 PESTICIDES AND 26 MYCOTOXINS IN RICE SAMPLES - UPLC-MS/MS (ESI+) ANALYSIS OF 26 MYCOTOXINS

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Nowadays, there is a need for the analysis of various classes of residues and contaminants (such as pesticides and mycotoxins) in a wide variety of food samples (such as grains, spices, fruits, wines, coffee, nuts, etc.), in order to ensure the food safety for the consumers. Traditionally, various classes of analytes are covered by separate analytical methods. A gain in efficiency for laboratories could potentially be achieved when sample preparation for different analyte classes, and also the chromatographic analyses will be integrated. The introduction of LC-MS/MS with atmospheric pressure ionization, e.g. electrospray ionisation (ESI), has offered the residue chemists a robust detection system, with high sensitivity, accuracy, selectivity, compatibility with a wide range of analyte polarities and with less stringent sample preparation requirements.

In this study, we developed and optimised an integrated sample preparation method (homogenisation and extraction via a single-step “slurry technique”) for the simultaneous analysis of 221 pesticides and 26 mycotoxins in rice samples. The final analysis of the rice extracts was performed, without any further cleanup, via 2 fast runs for LC-compatible pesticides and mycotoxins, respectively, by ultra-performance liquid chromatography combined with ESI triple quadrupole tandem mass spectrometry (UPLC–MS/MS) using the multiple reaction monitoring (MRM) mode. For both mycotoxins and pesticides, UPLC separation conditions and MS/MS parameters of the analytes were optimised.

The extraction was based on the modified QuEChERS method. Approximately 10 g of previously homogenized rice sample was weighed into a 50 mL centrifuge tube, to which 10 mL of acetonitrile with 1% acetic acid was added. The tubes were subsequently capped and shaken manually for extraction. Then, Mg_2SO_4 was added and the tubes were shaken again for the partitioning step. After centrifugation, the extract was ready for direct injection.

A complete validation study was performed for the determination of 26 mycotoxins, replacing the original LC-MS/MS method[1] by the present UPLC-MS/MS method[2].

The calibration curves of most of the mycotoxins showed good linear correlation and good coefficients of determination ($r^2 > 0.99$) over the concentration range of 0.1– 10 ng mL^{-1}.

For the recovery studies, six portions of non-contaminated, blank rice slurry (10 g each) were spiked at 3 different levels with a mixture of mycotoxin standards and subsequently analysed. Satisfactory recoveries (70–120%) and good RSDs (<20%) were obtained for the majority (80%) of mycotoxins. The LOQ_m range for the 26 mycotoxins was 1 – 100 µg kg^{-1}.

The major advantages of the fully validated method are speed and high sensitivity, which are applied beneficially to the identification and quantification of mycotoxins in rice samples. The low limits of quantification allow the successful enforcement of the maximum limits established or proposed in the European Union. The method has proven its applicability in a survey of real practice rice samples containing positive residues of mycotoxins.

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[1] J. M. Scholten, P. M. Rensen, M.C. Spanjer, Food Addit. Contamin, 24 (2007) in press.
[2] A. De Kok, M.C. Spanjer, J. M. Scholten, P.M. Rensen and G. Kearney, Waters Application Note Nr. 720001996EN (2007).

LM-9

DEVELOPMENT OF AUTOMATIC SEDIMENTATION TESTER AND ITS APPLICATION IN WHEAT QUALIFICATION PROCEDURE

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Sedimentation value or Zeleny-index are very simple, but important parameters for qualification and grading of wheat. These parameters are widely used all over the world, the applied methods are standardized (ICC Standard No. 116/1, AACC Methods 56-60 – 56-70). In spite of the frequent application of these methods, any automated apparatus has not been available, yet. On the other hand, on special areas the available sample volume is very limited, therefore the development of small-scale equipments and methods are requied.

Recently, an automated sedimentation tester (SediCom Tester^{R}) was developed at our Department in collaboration with Hungarian industrial partner, LabIntern Ltd. The measuring system was developed also for standard- and micro-scale methods, where the sample requirements are 3.2g and 0,4 g of flour, respectively. The architecture and the operation of the equipment are compliant to the requirements of standard procedures. The sedimentation process is followed by a computerized digital imaging system and is evaluated with software. Almost all type of sedimentation procedure will be applicable on this equipment. The validation procedures for standard- and micro-scale methods were also carried out. The measuring system was introduced recently in Montpellier at AACC meeting, this year.

The newly developed system was applied in different research projects. The Zeleny-value and many other chemical and technological parameters were determined for 160 different Hungarian wheat varieties were grown in standardized, but different conditions. The stability of quality of wheat varieties and the effects of different growing condition on wheat quality were investigated. Additionally, the relationships among different parameters were also studied. The detailed results of this study will be shown on poster. Generally, it can be stated, that the new modular measuring system, the SediCom Tester, is a useful tool not only for routine analysis but also for research work.

This phase of the research work was supported by the Hungarian Economic Competitiveness Operative Program (GAK, Project No.: ALAP1-00126/2004) and by the "Gábor Baross Innovation Program" (INNOCSEKK, Project No.: INNO-4-2005-006.)

LM-10
APPLICATION OF THE LAB-ON-A-CHIP TECHNOLOGY IN WHEAT PROTEIN ANALYSIS

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The protein fractions have a significant influence on the functional and technological quality of any wheat base product. The characterization of these fractions and to investigate the relationship between protein composition and functionality become more and more important in research work and product development. Today, the electrophoretic and chromatographic methods represent the two major protein separation techniques. The different applied analytical technologies and methods such us PAGE, size exclusion and reverse phase HPLC, and partly the capillary electrophoresis are widely used. However, these processes are time consuming, experienced staff and manual labour-intensive work is required, which hinders the application of these techniques in the everyday analytical practice.

Lab-on-a-chip (LOC) technology is a newly developed microfabricated device which integrating several laboratory processes on a single chip. It can fulfil most of the criteria of automatic and rapid analysis for protein subunit characterisation. In our work we studied the applicability of LOC for the characterization of wheat protein profiles. Agilent 2100 Bioanalyzer with Agilent Protein 230 and 80 Kit and Biorad Experion with Pro260 Kit were applied in our experiments. A newly developed micromill was applied to work out as a new sample pre-treatment procedure. The protein extraction process was optimized and the analytical procedure was validated: accuracy, repeatability and reproducibility were determined. Using the validated LOC-method, a wheat variety database was constructed, based on the subunit profiles and composition of 90 Hungarian wheat species. The database was applied for developing an automatic variety identification system. The other application of the developed method was to study the variability of subunit composition in different environmental condition. A similarity index was introduced for characterization of differences in the protein subunit profiles. The results will be shown on a poster.

Our studies showed that the LOC technology can be a useful tool for wheat protein characterization. This analytical technique has several advantages over conventional methods, such us faster procedure, reduced manual labour work, automated data handling, and good repeatability. In the near future it could replace the traditional SDS-PAGE, and can be a complement process to other analytical methods.

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LM-11

THERMAL INDUCED OXIDATIVE PROCESS IN SICILIAN OLIVE OIL INVESTIGATED BY OPTICAL AND EPR SPECTROSCOPY

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It is well known that free radicals are responsible for alterations of food organoleptic properties (colour, flavour, nutritional value), leading to a loss of quality and of customer compliance. In particular, free radical-initiated oxidation is considered the main cause of rancidity in fats and oils. In this context extra virgin olive oil (EVOO) is recognized as the healthiest food for its high content of antioxidants, which forestall and slow down radical formation. Moreover, recent investigations show that olive oil has specific biologic effects able to prevent some pathologies, such as cancer and heart diseases. It is worth to note that EVOO quality is directly related to several factors as olive variety, cultivar conditions, extraction and storage procedure that can heavily influence antioxidants synergy. As a consequence, recently many research activities have been focused on extra virgin olive oil.

Here we present experimental results obtained by UV-Vis absorption and photoluminescence (PL) spectroscopy on Sicilian EVOO samples oxidized at three different temperatures (30, 60 and 90°C) for a period of 30 days. We have been able to follow how the oxidation time remarkable affects the content of antioxidants: chlorophyll, carotenoid, α-tocopherol and polyphenols. In the first stage of the oxidation process, these molecules represent primary substrate of radical action whereas unsaturated fats (especially linoleic and linolenic acid) are involved in the subsequent steps; at the final stage, such fats form carbonylic compounds responsible of unpleasant flavours, i.e. due by oldness or by not correct storage of oil.

In addition, to estimate the amount of formed free radicals during oxidation and with the aim of investigating their nature, an EPR study has been performed. Free radicals are very reactive and, for this reason, the spin trap technique has been used. Such method consists in adding suitable diamagnetic molecules (N-t-butyl-α-phenylnitrone) to the samples with the aim of capturing any radicals potentially created during heat treatment and forming a much “longer-lived” radical species.

This work is part of a technological transfer project (P.O.R. Sicilia 2000/2006 – Misura 3.15-Sottoazione C) for quality controls on Sicilian extra-virgin olive oil.

LM-12

OXIDATIVE CHANGES IN SICILIAN EXTRA VIRGIN OLIVE OIL UNDER HEATING TREATMENT DETECTED BY FTIR SPECTROSCOPY, VISCOSIMETRY AND TIME-RESOLVED LUMINESCENCE

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Extra virgin olive oil (EVOO) is recognized as one of the best food in preventing some pathologies, such as cancer and cardiovascular diseases and in reducing their incidence in western population. As a consequence, the interest of many researchers is focused on how to avoid deteriorative changes of olive oil during storage due to oxidative processes. It is well known that these processes alter the vegetable oil stability producing undesirable flavors, deteriorating the quality and shelf life of extra virgin oil and customer compliance.

This work illustrates results obtained by FTIR, viscosity and time-resolved luminescence studies performed on Sicilian EVOO samples stored at three different temperatures (30, 60 and 90°C) up to 30 days.

Main changes in the FTIR spectra are observed in the 3100-3600 cm^{-1} region. In particular, the band at about 3468 cm^{-1} becomes more intense and shifts towards higher wavenumber. The intensity increase may be assigned to the vibrational activity of the hydroperoxides generated in the first phase of the oxidation process. The shift towards higher wavenumber is related to the appearance and increase of a shoulder at about 3530 cm^{-1}, assigned to the presence of significant amount of alcohols or secondary oxidation products, such as aldehydes and chetons. Other changes due to advanced oxidation process are not present in the spectra suggesting that, at this time, the oxidation process is still at the initial stage.

Viscosity measurements show that, after thermal treatment, all examined samples exhibited a increase of eta value compared with data obtained at room temperature: the larger value being recorded at 90°C for 30 days of incubation. In agreement with FTIR data, these results can be explained assuming that autoxidation causes the formation of hydroxyl and carbonyl groups with propensity to the development of hydrogen bonds.

Finally, time resolved luminescence spectroscopy measurements, made with a tunable pulse laser exciting oil samples at 410 and 650 nm and recording the luminescence on a cooled CCD camera with ns resolution, shows that the fluorescence lifetime of natural chromophores embedded in the oil matrix, like chlorophyll, depends on the oxidation time.

This work is part of a technological transfer project (P.O.R. Sicilia 2000/2006 – Misura 3.15-Sottoazione C) for quality controls on Sicilian extra-virgin olive oil.

LM-13

DETERMINATION OF PESTICIDES FROM DIFFERENT CHEMICAL CLASSES IN OLIVES AND INFLUENCE OF WATER ON THEIR PROCESSING FACTORS IN VIRGIN OLIVE OIL

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In this work, the occurrence and levels of multi-class pesticides in olives and olive oil were determined in order to calculate their processing factors during olive processing into oil. Processing factors of selected pesticides were determined in olive oil extractions in olive mills and in various laboratory-scale olive oil extractions from fortified olives with azinphos methyl, chlorpyrifos, λ-cyhalothrin, deltamethrin, diazinon, dimethoate, endosulfan and fenthion at different concentrations in order to determine the influence of water on their processing factors in olive oil. The analysis of pesticides was performed using a multi-residue method developed in our laboratory for the determination of different insecticides and herbicides in olive oil by SPE techniques coupled to GC detection (ECD and NPD) that optimized and validated for olive fruits sample preparation.

Processing factors varied among different pesticides studied in the range of 0.29-5.83. Water addition in the oil extraction procedure (as in three-phase centrifugation system) was found to decrease the processing factors of dimethoate and α-endosulfan, diazinon and chlorpyrifos whereas those of fenthion, azinphos methyl, β-endosulfan, λ-cyhalothrin and deltamethrin residues were not found to be affected by that water addition. Water content of olives processed was found to affect proportionally pesticide processing factors. Fenthion sulfoxide and endosulfan sulfate were the major metabolites of fenthion and endosulfan respectively that detected in laboratory produced olive oils, but only the concentration of fenthion sulfoxide was found to increase with the increase of water addition in olive oil extraction process.

LM-14

DECREASING THE COST OF GPC CLEANUP OF EXTRACTS FOR TRACE PESTICIDE ANALYSIS

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Gel Permeation Chromatography Cleanup is a size-exclusion liquid chromatography method used to remove lipids, sulfur and other co-extractives from environmental and food matrices prior to analytical analysis. It is a desirable technique because it is non-destructive and separates based on molecular size.

GPC cleanup, while a beneficial cleanup technique, has been criticized for its solvent and time investments. GPC Cleanup using the traditional glass column requires one hour per sample and about 300 mL of mobile phase solvent. Additionally, the traditional mobile phase is methylene chloride (DCM), a chlorinated solvent that requires expensive disposal.

To significantly decrease the cost of GPC Cleanup, the run time must be shortened. Simply increasing the mobile phase flow rate may speed the processing time, but will not decrease the amount of solvent used. It will also create pressure problems with the column. Another way to increase sample throughput while decreasing solvent consumption is to decrease the bed volume of the column. The lower bed volume will decrease the run time, thus decreasing the amount of solvent used to process each sample. There are, however, drawbacks to decreasing column length in some situations.

LM-15

KEEPING THE NATIONS BEEF SUPPLY SAFE FROM CHRONIC LEVELS OF CHLORINATED PESTICIDES AND FLAME RETARDANTS

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The USDA FSIS National Residue Program mandates the testing of domestic meat to prevent violative levels of persistent pollutants like chlorinated pesticides from entering the food supply. Recent findings have prompted an interest in flame retardant levels in meat. Flame retardant compounds, like hexabromobiphenyl, are commonly found in flame retardants and enter the animal by ingestion of retardant-treated items. Little is known of the toxicity of fire retardant compounds in humans, but research in rodents suggests they are associated with cancer, endocrine disruption and brain impairment. Like chlorinated pesticides, fire retardant compounds are highly lipophilic and tend to accumulate in fatty tissue of animals in the food chain.

The standard method for determining chlorinated pesticide residue levels in meat employs GPC Cleanup with GC/ECD detection. In this study the flame retardant compound hexabromobiphenyl was simultaneously determined with a standard list of 20 chlorinated pesticides. Advances in GPC Cleanup column technology allows for a decrease in run time, keeping the entire procedure, extraction through analysis, close to 1 hour per sample.

LM-16

UNATTENDED IN-LINE AND SEQUENTIAL SAMPLE CONCENTRATION FOR A RANGE OF APPLICATIONS

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Many techniques are used for the concentration of samples and the level of labor, user supervision and recoveries varies with each. This unattended concentration technique allows the operator to concentrate samples from an inline process such as GPC Cleanup and Solid Phase Extraction (SPE) or concentrate pre-collected samples ranging in volumes from a few to several hundred milliliters. The samples are sequentially evaporated under microprocessor control with sensor feedback, concentrated to a final endpoint with solvent exchange, and transferred to a storage vial or GC vial ready for analysis.

LM-17

EUROPEAN PROFICIENCY TESTING FOR THE SCREENING AND CONFIRMATION OF TETRACYCLINE AND SULFATHIAZOLE RESIDUES IN HONEY

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The participation to proficiency testing schemes allows the laboratories to assess their competence, to prove the reliability of their results and to improve their analytical performances. The use of antibiotics in beekeeping is forbidden in the EU. In the field of analysis of veterinary drugs residue, honey is very special matrix beside meat, milk. The aim of this proficiency study was to test the performance of the LNRs involved in monitoring of antibiotics in honey and to know the screening and confirmation methods used for two antibiotics: tetracycline and sulfathiazole. The second step was to propose some criteria to the EC for the determination of working limits for these antibiotics in honey. The organization of the proficiency test and its statistical analysis was performed under the scope of quality assurance system according to the internationally recognized guidelines. The first remark to point out is that there was globally a wide dispersion of the results for the two antibiotics to quantify. From our experience, a second proficiency test would show improvement of the quality of the results obtained from the laboratories.

LM-18

MONIQA – A NEW EU-PROJECT TOWARDS THE HARMONIZATION OF ANALYTICAL METHODS FOR MONITORING FOOD QUALITY AND SAFETY IN THE FOOD SUPPLY CHAIN

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A globalised economy has rapidly increased international trade of a large variety of foods and food products. Consumer satisfaction and health are of utmost importance. Ensuring high quality and safety of food requires powerful and reliable tools and methods for food analysis and control. The MONIQA Network of Excellence (NoE) integrates key organizations across the food supply chain around the world to find acceptable solutions for all stakeholders including the consumer, food manufacturers, food research institutes and regulatory bodies.

The network members will investigate mechanisms to coordinate and merge research activities, personnel and infrastructure to achieve synergetic affects. The resulting harmonized analytical strategies and methods, databases and training modules will extend beyond the network to associated partners and involved stakeholders. Food production industries and SMEs will benefit through harmonized analytical methods and technologies, as will the end consumers.

MoniQA will play an important role in European and worldwide food quality and safety research by creating a virtual laboratory for joint research, training, dissemination and mobility programmes. It will allow sharing of data and knowledge for harmonizing the standards and performance quality of analytical methods for monitoring food quality and safety.

Integrating activities will facilitate shared access to world's best research facilities, technological platforms, databases, analytical tools and knowledge. Joint research is directed towards the most pressing issues to fulfill food quality and safety policies, as well as citizens' concerns. The network will develop common strategies for harmonizing and validating detection methods and technologies to set new standards in quality and safety - within food production and extending throughout the whole food supply chain.

International food trade helps promote economic development, but likewise creates risks. Complex food supply chains now require harmonized standards in analytical methods and technologies to ensure quality and safety in the global food production and supply chain.

MoniQA (www.moniqa.org) is an EU funded Network of Excellence (NoE), which works towards harmonization of analytical methods for monitoring food quality and safety in the food supply chain. The MoniQA NoE (Contract N0. FOOD-CT-2006-36337) is coordinated by ICC – International Association for Cereal Science and Technology), represented by Roland Ernest Poms. MoniQA is funded by the European Commission with 12.3 Mio EURO between 2007 and 2012 within the Sixth Framework Programme Topic T5.4.5.1: Quality and safety control strategies for food (NOE).

LM-19

A COMPARISON OF DNA EXTRACTION METHODS FOR FOOD ANALYSIS

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In this paper, two DNA extraction and purification procedures for food analysis, Wizard Magnetic DNA Purification for Food (Promega) and DNeasy Tissue Kit (QIAGEN), have been compared concerning extraction efficiency, DNA purity and DNA suitability for amplification. The comparison of two extraction methods, in this study, has highlighted the efficiency of the Wizard Magnetic DNA Purification approach for vegetable matrices while the revised DNeasy Tissue Kit for complex and processed matrices.

LM-20

ADVANCES IN THE ANALYSIS OF THE OCHRATOXIN A (OTA) PRESENT IN COCOA DERIVATIVES

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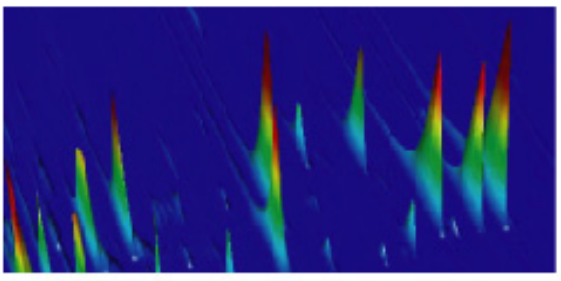
Ochratoxin A (OTA) is a secondary metabolite produced by filamentous fungi of the genera *Aspergillus* and *Penicillium* present in a wide variety of foodstuffs (cereals, coffee, wine, cocoa products, species, etc.). This mycotoxin has been described as nephrotoxic, carcinogenic, teratogenic, immunotoxic and hepatotoxic in animals, as well as being thought to be the probable causal agent in the development of nephropaties and urothelial tumors in humans. Indeed, the European Union has established regulatory levels in order to control the presence of OTA in several foodstuffs (Regulation (CE) nº 123/2005) and it is considering its extension to others like cocoa beans and their derivatives. To set regulatory levels, analytical method development and screening studies are mandatory.

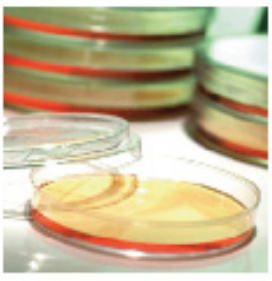
First screening study was carried out by MAFF in 1980 [1]. In this case, 16 of the 19 samples of roasted cocoa and 18 of the 56 samples of cocoa beans considered were contaminated with OTA. Since them, several research programs have been carried out. Working with more sensitive techniques, they have stated the OTA contaminating cocoa derivatives (cocoa beans, roasted cocoa, cocoa mass, cocoa powder, chocolate and chocolate drinks).

Concerning OTA analysis, in 1983, Hurst and Martin [2]. developed an analytical method based on liquid-liquid extraction with sodium bicarbonate and chloroform and on HPLC-FLD detection. Recovery was 92.8–101 % in 10–50 µg/kg range, limit of detection (LOD) was 10 µg/kg. Up to now, new analytical methods have been published achieving lower LOD and LOQ by more efficient purification and detection techniques.

Present work describes the different analytical methods and screening studies to provide an overwiew of the state-of-the-art of OTA in cocoa derivatives.

[1] Ministry of Agriculture, Fisheries and Food (MAFF). Surveillance of mycotoxins in the United Kingdom. The fourth report of the Steering Group on Food Surveillance, The Working Party on Mycotoxins. Food Surveillance Paper N. 4, 1980 (HMSO: London).
[2] Hurst WJ and Martin RA. High-performance liquid chromatographic determination of ochratoxin A in artificially contaminated cocoa beans. Journal of Chromatography 1983; 265: 353-356.



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