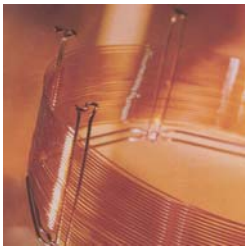
BOOK OF ABSTRACTS

4th International Symposium on

RECENT ADVANCES IN FOOD ANALYSIS

November 4–6, 2009
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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Edited by Jana Pulkrabová, Marie Suchanová and Monika Tomaniová
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4th International Symposium on

RECENT ADVANCES IN FOOD ANALYSIS

November 4–6, 2009 • Prague • Czech Republic

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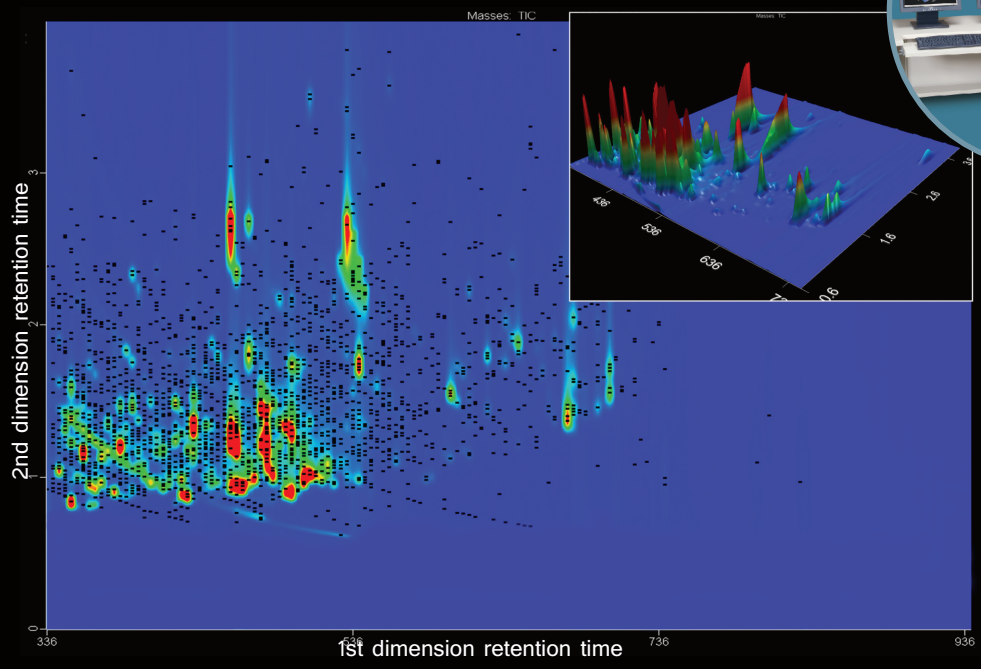
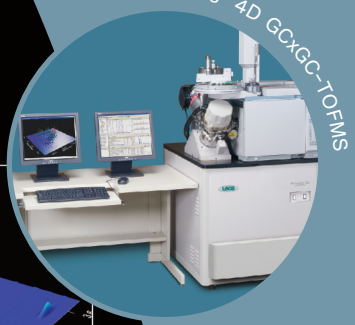
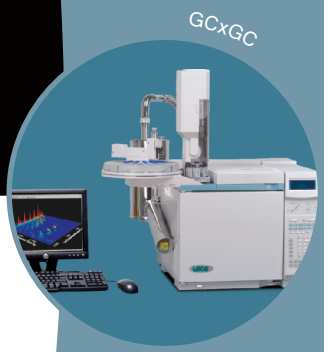


Total ion current (TIC) chromatogram as a contour plot for a urine extract sample generated using the Pegasus® 4D GCxGC-TOFMS. The black dots in the figure represent peak markers. The upper right corner of the figure shows the same chromatogram displayed in a three-dimensional view with the Z-axis showing peak intensity.





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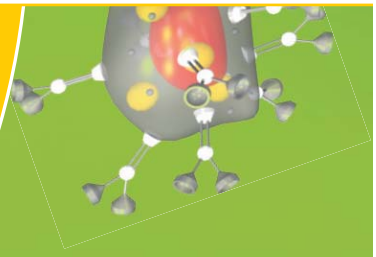

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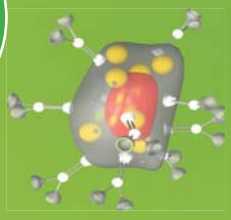

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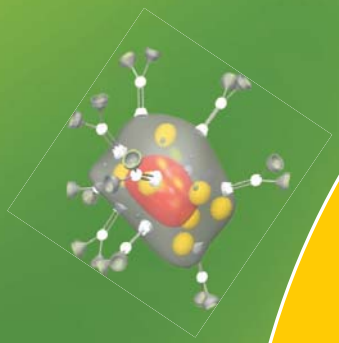

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VENDOR SEMINARS

PRE-SYMPOSIUM VENDOR SEMINAR:

MOST ADVANCED COMPREHENSIVE GC×GC SOLUTIONS UTILIZING TOF MS

NOVEMBER 3, 2009 (16:00–18:00)

Sponsored by

GC×GC TOF MS FOR FOOD FLAVORS ANALYSIS

Henryk Jelen, Poznan University of Life Sciences, Poland

Analysis of flavor compounds in food is a challenging task due to their chemical diversity, low concentrations and matrix interferences. Among hundreds of volatile compounds in food products analyst searches for key odorants. Peak capacity provided by comprehensive gas chromatography GC×GC opens new perspectives in separation of volatile compounds and its hyphenation with high speed mass spectrometry with an aid of deconvolution provides reliable identification of analytes. In the presentation a comparison between 1D GC and GC×GC will be discussed and applications of GC×GC-TOF MS in the selected food flavor applications will be provided: analysis of lipid oxidation products, volatile phenols in beer, wine flavor compounds.

QUECHERS AND GC×GC-TOFMS FOR THE ANALYSIS OF PESTICIDES IN DIETARY SUPPLEMENTS

Jack Cochran, Restek, USA

Dietary supplements are concentrated blends of a variety of botanical substances that now must be tested for pesticides. The QuEChERS sample preparation method offers a rapid way to prepare these samples for analysis, but the extracts can be so complex as to make trace-level pesticide determinations problematic. GC×GC-TOFMS has the capability to provide the separation power and sensitivity necessary for the task. This presentation will discuss the QuEChERS method, including extract cleanup by dispersive solid phase extraction, and compare GC-TOFMS and GC×GC-TOFMS, for dietary supplement extract pesticide analysis.

SEPARATION SCIENCE GC×GC TOF MS CASE STUDY: EDIBLE OIL – WHAT IS IN IT?

Sjaak de Koning, European application manager Separation science, LECO, Germany

Edible oils are analysed on a daily basis both in routine as for research. One can think here on target analysis of potentially poisoning compounds like 3-MCPD, group type analysis of trans-fats or fingerprinting analysis on flavours. Due to the often complexity of these type of analysis, is GC×GC nowadays more and more implemented as the method of choice. This contribution will give an overview of the three mentioned applications, showing the overwhelming separation power of GC×GC TOF MS in combination with the powerfull ChromaTOF software for data acquisiton and processing.

RECENT DEVELOPMENT IN GC×GC TOF MS TECHNIQUE

Tomas Kovalczuk, Separation science specialist, LECO, Czech Republic

Although, the mass-spectrometry became the method of choice in different types of analyses, there is a permanent demand for minimizing the costs of analyses. In GC×GC technique several approaches to reduce the expenses can be considered, especially taking in account the consumables required for GC×GC modulation, e.g. liquid and gaseous nitrogen. The presentation will not demonstrate only the potential of novel consumable-free modulator, but also focusing on the advanced data handling such as mathematical filtering of MS spectra (called scripting), and others.

LUNCH VENDOR SEMINAR:

LATEST ACHIEVEMENTS IN FOOD TESTING

NOVEMBER 4, 2009 (12:45–14:00)

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ADVANCING FOOD TESTING THROUGH INTEGRATED TECHNOLOGY, SAMPLE PREPARATION AND WORKFLOW SOLUTIONS

Sandra Rontree, Waters MS Technology Centre, Manchester, UK

Innovations in tandem quadrupole MS have allowed the analysis of food contaminants to be set-up and completed more rapidly. New software tools automate the creation of methods whilst providing real-time QC decision making on the data as acquisition occurs. During acquisition the unique Dual Scan MRM capability enables simultaneous matrix monitoring, helping reduce method development timescales and providing essential QC/QA during an analytical run. High performance quantification is complemented by the capability to perform long term studies of trends with the generation of electronic control charts.

Time of flight mass spectrometry (ToF MS) screening has gained popularity due to benefits such as historical data interrogation, simplified instrumental method set-up and reduced compromise in method performance when increasing the scope. However, processing and reviewing TOF screening data is often a complex workflow where positive peaks are first identified then quantified to assess the risk posed to the consumer. Frequently the transfer from qualitative to quantitative processes is performed manually, which places a significant drain on data review resource and introduces a high probability for errors.

The use of ACQUITY UPLC coupled to quadrupole time of flight (Xevo QTof MS) for the screening of more than 1250 pesticides in food will be discussed and compared to those obtained on tandem quadrupole MS. The data was processed using POSI±IVE software, enabling exact mass data to be qualitatively and quantitatively reviewed in a single pass.

INCREASING THE CAPABILITIES OF FOOD TESTING LABORATORIES USING ATMOSPHERIC PRESSURE GC (APGC) AND AMBIENT SAMPLING TECHNOLOGY (ASAP)

Peter Hancock, Waters MS Technologies Centre, Manchester, UK

In food analysis there has been a move towards the utilization of LC/MS with atmospheric pressure ionization (API) techniques. API has primarily been used to interface MS with LC, but it is also a powerful ionisation method that can be applied to GC.

A novel atmospheric pressure ion source (APGC) for tandem quadrupole and quadrupole time-of-flight LC/MS instruments will be described, allowing laboratories to switch rapidly between LC and GC applications to analyse compounds traditionally analysed by dedicated vacuum GC/MS instruments. Ionization of a GC eluent at atmospheric pressure is a softer process, giving molecular weight information for compounds which are extensively fragmented in traditional Electron Ionization (EI). Examples related to food will be shown illustrating the advantage of softer ionization

and of the improvement in separation that can be achieved with the column eluting at atmospheric pressure rather than in a vacuum.

The food testing laboratory also faces the challenge of being able to successfully and rapidly screen for the presence of analytes using a simple technique with minimal sample preparation and no chromatographic separation would be advantageous. The Atmospheric Solids Analysis Probe (ASAP) allows the direct analysis at atmospheric pressure with little or no sample preparation and no chromatographic separation. Examples will be shown where ASAP allows the direct analysis of both solid and liquid samples and is particularly useful for compounds which are non-polar and not normally amenable to analysis by API techniques.

LUNCH VENDOR SEMINAR:

ADVANCES IN THE USE OF LC/MS/MS FOR ROUTINE FOOD TESTING

NOVEMBER 4, 2009 (12:45–14:00)

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With the recent issues around imported food products and ever increasing regulation by the EU, there is an increased need for the testing of a greater number of products, at lower contaminant levels in order to ensure product quality and human health. In order to do so, LC/MS/MS is being adopted as the analytical technique of choice due to its ability to analyze for numerous compounds at lower levels with less sample preparation than traditional techniques such as LC, GC or GC/MS. The following session will provide a brief overview of the benefits of LC/MS/MS and go on to outline advances in the use of new tools, technologies and sample preparation techniques to be able to simplify the ability to screen for, identify and quantify pesticides, antibiotics, mycotoxins and other common food contaminants from a range of food matrices.

THE USE OF LC/MS/MS FOR THE ROUTINE TESTING AND THE HIGH THROUGHPUT ANALYSIS

Andre Schreiber, Food Technical Marketing Manager, Applied Biosystems

This talk will provide a brief introduction to the benefits of LC/MS/MS and go on to talk about new tools like *Scheduled*MRM™ algorithm that provide the ability to screen for more than 500 compounds in a single injection. Recent advances to QTRAP® system technology to be able to perform enhanced compound screening, identification, routine and high selectivity quantification from a single instrument will also be discussed with regard to pesticide and antibiotic testing.

NEW TOOLS TO SIMPLIFY ROUTINE FOOD CONTAMINANT TESTING

David Lavorato, Software Product Manager, Applied Biosystems

This talk will outline the issues to consider when looking to adopt LC/MS/MS for routine testing and go on to discuss Cliquid® Software, with its simple four step workflow, and pre-configured iMethod™ Tests as part of a total solution designed to reduce the barriers to adoption. The new Cliquid® MPX™ High Throughput solution for multiplexing of multiple HPLC systems to a single mass spectrometer to increase throughput and productivity will also be discussed.

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LUNCH VENDOR SEMINAR:

RAPID TESTS FOR ALLERGENS AND MYCOTOXINS

NOVEMBER 4, 2009 (12:45–14:00)

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RAPID DETECTION OF ALLERGENS – NEW APPROACH OF LFD TECHNOLOGY

Sigrid Haas-Lauterbach, Dr. Ulrike Immer (R-Biopharm AG, Germany), Karl Schmitt (Bioavid Diagnostics GmbH & Co. KG, Germany)

The key point in allergen and gluten analysis is the availability of efficient and sensitive methods for the detection of the targets from both unprocessed and heat-processed food samples including an all purpose extraction procedure. To ensure an adequate labeling of allergenic food compounds for consumer safety, and to enable enforcement of the allergen regulations in place, different analytical methods have been developed. Most of them are antibody based like ELISA (enzyme linked immunosorbent assay) which has considerable applications for food and cereal analysis.

Hidden allergens caused by cross contamination are a not discernible risk for allergic consumers. This potential risk is not covered by any labeling regulation. Therefore, industries often use precautionary labeling, which is not of help for the allergenic consumer. To minimize this kind of allergen labeling of products, appropriate allergen sanitation procedures are required. For monitoring the efficiency of cleaning procedures of the environment and shared equipment, the lateral flow devices (LFD) have the needed features like sensitivity, specificity, easy handling and rapid evaluation. Such tests are available for a number of individual allergens. They allow qualitative detection of traces of allergens in about 15 minutes of total assay time and do not require any equipment for evaluation. While the method of choice in sanitation control is swabbing, with a little more sample preparation these tests can be used also for qualitative detection of allergen residues in raw materials or even processed food.

RIDA®QUICK MYCOTOXIN LATERAL FLOW ASSAYS – A NEW APPROACH FOR MYCOTOXIN ANALYSIS

Walter Luebbe, Michael Maettner, Johannes Winkle, Martin Mehl, Ronald Niemeijer (R-Biopharm AG, Darmstadt, Germany, info@r-biopharm.de)

The RIDA®QUICK Mycotoxin product line is a novel immunochromatographic lateral flow format for the detection of aflatoxin, and deoxynivalenol from grain and cereals. The inverted competitive assay format is based on the directly proportional reaction of the target molecule with specific gold-labelled antibodies. This means as soon as mycotoxins above detection level are presented, a result line occurs. A control line assures the validity of the test run. After extraction and sedimentation the sample is applied to the test membrane. Depending on incubation time, between 2 and 16 minutes, different concentrations of applied mycotoxins can be determined semi-quantitative visually. For documentation purposes the reaction can be stopped and the sticks can be stored for several months.

Increasing demands on quality control and consumer protection requires more and more fast and reliable testing of raw materials and processed food. Contemporary assessment of raw materials guarantee a cost and time efficient distribution and production. The new RIDA®QUICK DON and RIDA®QUICK Aflatoxin, optional combined with the RIDA®QUICK SCAN, fulfils the requirements of modern mycotoxin analysis.

LUNCH VENDOR SEMINAR:

RAPID METHODS FOR FOOD CONTROL BASED ON PROVEN ESI-(UHR)-TOF TECHNOLOGY (MICROTOF, MAXIS) AND NEW GENERATION OF HIGH-SENSITIVITY ION TRAP (AMAZON)

NOVEMBER 5, 2009 (12:45–14:00)

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MULTI-TARGET SCREENING OF SEVERAL HUNDRED PESTICIDES IN A SINGLE LC/MS RUN BY EXACTED ION TRACES USING ESI-(Q)TOF OR UHR-TOF TECHNOLOGY

Rob van der Heijden (Bruker Daltonics BV, Wormer, The Netherlands; rob.vanderheijden@bruker.nl), Arnd Ingendoh, Petra Decker, Carsten Baessmann, Marcus Macht, Romano Hebeler (Bruker Daltonik GmbH, Bremen, Germany)

The usefulness of LC/MS/MS methods for the unambiguous identification and quantification of pesticides in complex matrix samples are well known. Triple quadrupole systems have proven to be useful for this task due to their high specificity in MS/MS mode and their low detection limits. However, working in MS/MS mode makes any MS system blind for all other compounds than the current MS/MS transition is designed for. Therefore, it is difficult to develop methods for simultaneous analysis of high numbers of pesticides. Thus, other ways of achieving specificity are of interest, such as the high mass accuracy and mass resolution of an ESI-(Q)TOF system or even UHR-TOF system. It can generate high specificity without limiting the number of simultaneously observed target compounds.

The ESI-TOF MS approach in general enables the screening for several hundred of possible pesticides within one run. The selectivity is based on the accurate mass, with mass traces defined within 0.002 Da over a dynamic range of about 4 orders of magnitude. By using a database of several hundred pesticides, spiked samples can be easily detected. Sensitivity in the range of low ppb range or even below can be achieved. Results for various matrices will be presented and discussed for potential need of sample preparation. An excellent linear range of 4 orders of magnitude is achieved, allowing the quantitation of the pesticides. In contrast to classical screening approaches by triple quadrupole instruments there are several benefits:

1) A high number of targets can be screened at the same time without loss of sensitivity
2) Unknown peaks can be identified based on accurate mass and true isotopic pattern
3) Data can be reprocessed later for additional compounds (archiving)
4) Profiling of the data allow for further statistical data evaluation.

Benefits and requirements of the method will be discussed in detail.

RAPID METHODS FOR FOOD CONTROL BASED ON NOVEL GENERATION OF HIGH-SENSITIVITY ION TRAP BRUKER AMAZON

L.J. Fremlin, M. Pelzing (Bruker Daltonics Division, Preston, Australia; rob.vanderheijden@bruker.nl, A. Ingendoh (Bruker Daltonk GmbH, Bremen, Germany)

Ion traps have been shown only limited use so far in quantitation studies. Main reason was an unsufficiently high RSD on low sample amounts in matrix loaded samples. However, with recent improvements in ion trap trap technologiy with regard to ion transmission, ion storage capacity and data acquisition rate, ion traps show a much improved LLQ for many applications.

E.g., adulteration of food and beverages with industrial chemicals has become an issue of late, as evidenced in September 2008 with the contamination of infant milk formula in China with melamine. There is a requirement for rapid and sensitive methods to detect and quantify such chemicals in complete matrices. LC-MSMS methods are superior to GC-MS methods in that they have an easy sample preparation and do not require sample derivatization. The high capacity ion trap mass spectrometer is a highly robust system capable of operating in full scan, MS^n and MRM modes. Here we report a recently developed method for the extraction, detection, and quantitation of melamine, cyanuric acid residues and other unwanted food stuff.

Chromatographic separations were carried out using a Dionex UltiMate 3000 using an Acclaim Mixed-Mode WAX-1 (Dionex, Sunnyvale, CA, USA) column (2.1 × 150 mm, 5 µm) maintained at 30 °C and operated in a HILIC mode. For the simultaneous detection of melamine and cyanuric acid the mobile phase gradient consisted of water / 0.1% formic acid (10%) and acetonitrile (90%) to 50%:50% over two minutes before returning to initial conditions at a flow rate of 300 µL/min, and a run time of five minutes. 10 µL injections were made. The ion-trap mass spectrometer was optimized for the detection of melamine in MRM mode.

Using the novel high capacity ion trap mass spectrometer it was possible to establish a calibration curve for melamine ranging from 1 to 1000 ppb.. Melamine was spiked into infant milk formula (1 ppm and 2.5 ppm) and extracted as per the conditions above. Despite the low detection limits, these concentrations were chosen on the basis of infant milk contamination recommendations by the FDA. Furthermore, it was possible to detect and quantify melamine contamination in a number of samples of contaminated infant milk power from China well above the limits determined by the FDA.

LUNCH VENDOR SEMINAR:

NEW APPROACHES TO SOLVING EMERGING FOOD SAFETY ISSUES

NOVEMBER 5, 2009 (12:45–14:00)

Sponsored by Thermo SCIENTIFIC

THE BENEFITS OF HIGH RESOLUTION MASS SPECTROMETRY IN SCREENING ANALYSIS OF MYCOTOXINS IN FOOD

Michal Godula, Thermo Fisher Scientific CZ, Prague, Czech Republic, michal.godula@thermofisher.com

Screening of pesticides, mycotoxins and veterinary drugs is of great importance in regulated environments such as food and animal feed analysis. Due to the broad variability of physico-chemical properties of the screened residues it is critical to employ very simple sample preparation procedure to maintain the recovery of the broad range of analytes. This however unavoidably leads to the fact that final extracts injected into the chromatographic system contain significant amounts of coextracts. For the chromatographic analysis it is therefore necessary to use the system with high selectivity but still capability to identify potential unknowns.

Traditionally these types of screening experiments have been carried out using SRM scanning with triple quadrupole instruments. This approach has certain limitations: (*I*) no post acquisition re-interrogation of data (*II*) limited number of compounds per analysis (*III*) little possibility to scan for unknown compounds at high levels. Because of these limitations, there is currently a trend towards full scan MS experiments in residue analysis. Current screening approaches employ high performance ToF instruments, with mass accuracies of < 5 ppm and resolutions max 15,000, coupled to Ultra High Performance Liquid Chromatography (U-HPLC). However, most of the techniques and instruments currently available suffer from either poor mass accuracy and its variability and more significantly from resolution not sufficient to separate analytes of interest from coeluting species. Especially the mass resolution plays an important role in the successful identification of the most of present residues in samples containing high amounts of matrix coextracts.

The presentation will focus on the main problems and issues related to the application of the screening MS techniques using accurate mass technology and will introduce the new system based on the proven Orbitrap™ technology. Mass spectrometers based on the unique performance of this type of mass analyser are routinely achieving mass resolution up to 100,000 and mass accuracy below 2 ppm. Those parameters significantly improve the efficiency and accuracy of the residue screening methods and allow successful screening of various residues at even very low concentration levels. This fact will be documented on practical examples from the field of the analysis of priority mycotoxins in the samples of food and feed.

HIGH SENSITIVITY MULTI-RESIDUE PESTICIDE ANALYSES IN FOODS USING THE TSQ QUANTUM GC-MS/MS

Richard J. Fussell and Michael T. Hetmanski; Food and Environmental Research Agency, Sand Hutton, York, YO41 1LZ, UK; E-mail: richard.fussell@fera.gsi.gov.uk

GC-MS/MS was evaluated for the multi-residue analysis of approximately 100 pesticides in various matrices. Samples were extracted and cleaned-up using the QuEChERS procedure[1]. The samples were extracted with acetonitrile in the presence of magnesium sulfate, sodium chloride, disodium hydrogen citrate and trisodium citrate. Extracts were then cleaned-up by dispersive SPE using magnesium sulfate, and PSA.

A TSQ Quantum GC-MS/MS system was used for this study. The system comprised a Trace GC Ultra gas chromatograph equipped with a TriPlus autosampler interfaced with a TSQ Quantum triple quadrupole MS/MS detector operated in EI mode.

The pesticides evaluated included captafol, captan, chlorothalonil, dichlofluanid, dicofol folpet and tolylfluanid. These compounds have caused analytical problems, especially with GC-MS analyses of sample extracts produced by the QuEChERS procedure. The use of PTV, backflush and H-SRM (Highly-Selective Reaction Monitoring) techniques were also evaluated. The data from the optimized GC-MS/MS methodology was also directly compared to data derived from analysis of the same samples by "conventional" methodology (e.g. acetone or ethyl acetate-based extraction and single quadrupole GC-MS); to assess detection and quantitation limits, reproducibility and robustness for both analytical approaches.

[1] http://www.quechers.com

QUICK AND AUTOMATED SCREENING METHOD FOR PRIORITY BETA-AGONISTS IN URINE

Thorsten Bernsmann, Chemisches Landes- und Staatliches Veterinäruntersuchungsamt Münster (CVUA), Postfach 1980, 48007 Münster, Germany

β-agonists are synthetically produced compounds that, in addition to their bronchodilatory and tocolytic effects, can promote live weight gain in the food producing animals. There have been documented cases when consumption of liver and meat from animals illegally treated with clenbuterol has resulted in serious human intoxication[1]. Due to their adverse effects, the use of clenbuterol and its analogues from the beta-agonists group has been banned by the European Union[2] and other regulatory agencies worldwide. Monitoring programs have shown that β-agonists are still illegally used by food producers, moreover, newly developed analogues with modified structures are being continuously introduced in routine practice. There is a clear need for quick and simple screening methods to routinely and accurately control levels of β-agonists in samples of animal origin (urine, plasma, tissues).

The current CVUA screening method for β-agonists in urine employs column clean-up and reconcentration before LC/MS determination. This is both expensive due to the use of molecularly imprinted polymer columns during sample preparation and laborious due to the need of fully trained laboratory staff. The LC/MS analysis is typically performed using a high performance ToF instrument, with mass accuracies of < 5ppm and resolutions of about 10,000 to 15,000, coupled to Ultra High Performance Liquid Chromatography (U-HPLC). However, most of the currently available ToF instruments suffer from resolution not sufficient to separate analytes of interest from potential coeluting species. The mass resolution plays a major role in the successful identification of most present residues in samples containing high amounts of matrix coextracts.

This presentation will describe a newly developed method based on a fully automated sample preparation procedure using the online Turboflow™ chromatography clean-up step and screening of priority β-agonists performed on a new system based on the proven Orbitrap™ technology. Mass

spectrometers based on the unique performance of this type of mass analyser are routinely achieving mass resolution up to 100,000 and mass accuracy below 2 ppm.

The unique parameters of the Orbitrap based mass spectrometer together with quick and automated sample preparation using Turboflow™ technology significantly improved the throughput and efficiency screening method for β-agonists in urine samples.

[1] Botsoglou, N.A., Fletouris D.J., *Drug Residues in Food. Pharmacology, Food Safety and Analysis,* Marcel Dekker: New York, 2001
[2] EU COUNCIL DIRECTIVE 96/22/EC of 29 April 1996, OJ L 125, 23.5.1996

LUNCH VENDOR SEMINAR:

LATEST INNOVATIONS IN FOOD ANALYSIS – AGILENT TECHNICAL SEMINAR RAFA 2009

NOVEMBER 6, 2009 (12:45–14:00)

Sponsored by  Agilent Technologies

DETERMINATION OF TARGET COMPOUNDS AND UNKNOWN CONTAMINANTS IN COMPLEX MATRICES WITH QQQ AND TOF ANALYZERS: A COMPREHENSIVE DISCUSSION ON PRACTICAL SOLUTIONS TO EVERY FOOD ANALYST

Paul Zavitsanos, Food Market Worldwide Manager, Agilent Technologies, USA

The investigation of food samples for pesticides, mycotoxins, allergens, drugs and other contaminants can be broadly classified into two categories: the measurement and confirmation of known or expected target compounds and the identification and estimation of unknown contaminants. Target compound quantitation and confirmation and the identification of unknown contaminants, in either liquid or gas phase have significantly different analytical strategies, objectives and ultimately are ideally addressed by different analyzers.

Triple quadrupole MS instruments are ideal for trace quantitation and confirmation but what happens to the sensitivity and viability of the technique as the number of compounds increases into the hundreds of compound per run? What happens as chromatographic peak widths drop below two seconds width at the base? Is there a point where the inherent scan sensitivity speed of a high resolution (15k resolution) TOF-MS analyzer with a 0.001 *m/z* extraction window is better suited? Where is the crossover point? In contaminant discovery applications, MS Scan methods are the standard approach. What would the impact of accurate mass high resolution TOF instrument with deconvolution software tuned to find unknown compounds or known and dangerous ones? Are there fundamental differences in instruments designed for LC or GC service? The results of experiments into these questions will be presented and investigated in this presentation.

THE IMPACT OF INNOVATION IN LIQUID CHROMATOGRAPHY ON FOOD ANALYSIS

Pat J. Sandra; Director of the Research Institute of Chromatography, Kortrijk, Belgium

The current trend of LC analyses is biased toward high throughput, high productivity and high resolution. In response to these increasingly demanding requirements, over recent years, innovative technologies and improvements in instrumentation have emerged which are having a significant impact on our daily work.

This contribution will review the features of Ultra High Pressure (UHP) and Elevated Temperature (ET) LC in extending speed, productivity and peak capacity for food analyses. Special emphasis will be given to robustness and the introduction of the principles of green chemistry in state-of-the-art liquid chromatography.

The recent developments also have an important impact on the hyphenation of LC with mass spectrometry. This will be illustrated with the determination of residues and contaminants in food products by UHPLC-QQQ and UHPLC-(Q)-TOF.

AGILENT LC/MS AND LC/MS/MS APPLICATION KITS FOR PESTICIDE RESIDUE ANALYSIS

Jerry Zweigenbaum, Senior LC/MS Applications Scientist, Agilent Technologies, USA

Agilent solutions for screening large numbers of pesticides have been developed for either LC/TOF or QTOF and LC/QQQ applications. These included a database of accurate masses for over 1600 pesticides and related compounds and a QQQ MRM database to facilitate customized screening and confirmation. The Personal Compound Database and Library (PCDL) software for pesticide analysis is populated with almost 1600 compounds, their exact masses, formulae, and structures (among other useful information) that represent most pesticides used over the last century. This presentation will describe how the database is used with LC/TOF or QTOF MS for screening targeted and non-targeted pesticides. For the most sensitive targeted pesticide screening the LC/QQQ is needed. The Agilent Application Kit for LC/QQQ with a MRM database of over 600 compounds monitored throughout the world will be discussed. Combined with Agilent's Dynamic MRM for the QQQ, the database can be used to construct customized multi-residue methods for all the pesticides in the database in food at the lowest possible concentrations. As new compounds emerge or if a lab monitors pesticides not in the MRM database, they can be added. Both databases are customizable to meet the changing monitoring needs of laboratories throughout the world.

HIGH THROUGHPUT TARGET COMPOUND ANALYSIS OF FOOD CONTAMINANTS USING THE 7000A GC TRIPLE QUADRUPOLE

Chris Sandy, Senior GC/MS Applications Chemist, Agilent Technologies, United Kingdom

The GC/MS detection, confirmation and quantitation of trace level agrochemicals in food-stuffs such as fruit, vegetables, herbs, spices and meat products can be impaired by co-extracted matrix components. Matrix components that elute during the chromatographic run can mask the presence of contaminants and interfere with their confirmation / measurement due to co-eluting ions. Furthermore, higher-boiling matrix components can remain on the capillary column between sample analyses.

These components can cause loss of chromatographic peak shape, retention time shifts of target analytes and contamination of the mass spectrometer ion source requiring more frequent maintenance of both the gas chromatograph and the mass spectrometer ion source.

This presentation will show you the robustness of the Agilent 7000A GC-QQQ system when Capillary Flow Technology is used to facilitate capillary column backflush between every sample analysis. Data will be shown from both a vegetable extract and meat fat extract analysed in MS/MS mode that demonstrates both quantitative accuracy and reproducibility for more than 60 agrochemicals spiked into the extract samples at concentration levels below the regulatory reporting level of 10 ppb.

LUNCH VENDOR SEMINAR:

MODERN MULTIDIMENSIONAL GC×GC MS TECHNOLOGIES FOR ANALYSIS RELATED TO FOOD QUALITY & SAFETY

NOVEMBER 6, 2009 (12:45–14:00)

Sponsored by SHIMADZU Solutions for Science since 1875

QUALITATIVE AND QUANTITATIVE COMPREHENSIVE GC×GC(Q)MS ON PCBS AND DIOXINS IN FATS USING NEGATIVE CHEMICAL IONISATION

Dr Hans-Ulrich Baier, Product Specialist GC&GCMS, Shimadzu Europa GmbH; hub@shimadzu.de

Thermal Modulation is the only suitable modulation technology for Comprehensive GC×GC when a MS is used as an detector. That is because Flow modulation techniques dilute the analytes by a relatively high pulse flow (Seeley et al). As quadrupol MS belong to concentration dependend detectors this results in considerably reduction in sensitivity. Here a fast quadrupol detector (small interscan delay) is used to produce at least 10 data points across each modulated fraction in full scan mode. PCBs and Dioxins can be sensitively and selectively analysed by using negative chemical ionization (NCI). By using NH_3 as the reagent gas molecular ion respons can be observed also for the smaller congeners (in contrast to dissociative resonance electron capture). Data achieved with EI and NCI mode are compared and the selectivity of the NCI mode for PCBs and Dioxins ares shown with bovine fat matrices as an example. Calibration curves were recorded and quantitative determination was performed.

FLAVOUR PROFILING WITH MDGC WITH MS IDENTIFICATION IN 1st AND 2nd DIMENSION

Hans-Ulrich Baier, Product Specialist GC&GCMS, Shimadzu Europa GmbH; hub@shimadzu.de

The characterization of Flavours in food is a widely spread analytical task in the food industry. However the analysis results very often in coelutions of some minor components which are important for the quality of the flavour with other ingredients or with some matrix signals when analyzing food products like cheese. Multidimensional technologies are very suitable to fix those coelution problems. In classical heart cut MDGC in the past however an GC-FID chromatogram was recorded to firstly obtain an area percent report and secondly to find coelution regions in order to cut and transfer those into the second dimensional column which has usually a different polarity (or is a chiral phase) compared to the first dimension. The detector can be an FID or an MS. However in the first dimension there was no identification possible. Here we demonstrate a setup where the signal coming from the first dimension is splitted into the FID and the MS detector in the second dimension. The result is 2 chromatogramms from the first dimension (FID and MS) which allows identification and a better definition of the cut positions in a subsequent run. For those cut runs the FID/MS split line is set to have a reverse flow in order to prevent coelutions from the first and second dimension. Due to the fact that a multi Deans switch is used no shifts in any of the chromatograms are observed (first or second dimension) regardless how many cuts have been programmed. Cuts will be defined by mouse click operations and the corresponding time parameters are automatically set and stored as method parameters. This is a breake through for easy MDGC operation.

LUNCH VENDOR SEMINAR:

ELISA METHODS DETECTING FOOD ALLERGENS, MYCOTOXINS AND MELAMINE

NOVEMBER 6, 2009 (12:45–14:00)

Sponsored by ROMER Romer Labs®

NEW TEST KITS FOR FAST AND RELIABLE FOOD ALLERGEN TESTING

Elisabeth Halbmayr, Romer Labs Division Holding GmbH, Technopark 1, 3430 Tulln, Austria

A food allergy is typically an immune system response to a protein present in food that the body mistakenly believes is harmful. Common food allergens are gluten-containing cereals, crustaceans, eggs, fish, peanuts, soybeans, lupines, nuts, milk, mustard, sesame, celery, sulphur dioxide, sulphites and molluscs. Food allergies affect 1–3% of the whole population and 5–8% of children. Even minor exposure to a food allergen in the nano-gram range can cause symptoms from mild skin rashes to a fatal anaphylactic shock. Cross contamination during the production process often occurs so residues of food allergens in different products may be present. Testing with the sensitive AgraQuant® Allergen ELISA Test Kits ensures safe food and contributes to consumer protection. The presentation on Food allergen ELISA Test Kits will give an overview of the common ELISA techniques used for detecting food allergens as well as important criteria when using these methods. In the presentation, information about validation process and parameters used for characterization of Test Kits will be provided.

APPLICATION OF ELISA TEST KITS FOR MYCOTOXIN ANALYSIS

Eva Wanzenböck, Romer Labs Diagnostic GmbH, Technopark 1, 3430 Tulln, Austria

Mycotoxins are naturally occurring hazards, entering the food chain in the field or during storage, with severe toxic effects on humans and animals. Since this fact is generally agreed upon worldwide, responsible governmental bodies all over the world try to protect their population by setting regulatory limits for the most dangerous mycotoxins.

An overview of the most important mycotoxins and their occurrence, with focus on validation studies of AgraQuant® Mycotoxin ELISA Test Kits and criteria for official Test Kit approvals, is given. The presentation will also give an overview of the sampling and analysing processes for mycotoxins using ELISA technology.

THROWING LIGHT ON THE MELAMINE ISSUE USING ELISA TECHNIQUES

Elisabeth Halbmayr, Romer Labs Division Holding GmbH, Technopark 1, 3430 Tulln, Austria

The widespread recall of melamine-contaminated food products–including infant formula in China, chocolates in Australia, pizza cheese in Taiwan, instant coffee in the U.S. and cheese crackers in

Hungary – has been worrying consumers all over the world and impressively illustrates the globalization of food supply. Melamine contamination was driven by monetary incentives when unscrupulous traders and producers added melamine to watered-down milk to fool the nitrogen-based standard tests, such as the Kjeldahl and Duma tests, into believing that the product contained more protein than it actually did. Regulatory bodies have set maximum allowed levels of melamine in food stuff and feed. The workshop will give an overview about the methods for detecting Melamine and focus on enzyme-linked immunosorbent assays (ELISA) methods, since this is probably the best alternative for food and feed producers who do not have OR have no access to sophisticated laboratories. A comparative study on several commodities comparing AgraQuant® Melamine ELISA test kits and HPLC methods will be presented.

About the presenters:

Dr. Eva Wanzenböck

Area Manager at Romer Labs®

PhD in Genetics, University of Natural Resources and Applied Life Sciences, Vienna

Degree in Genetics, University of Vienna

Dipl.-Ing. Elisabeth Halbmayr

Product Manager for Rapid Methods at Romer Labs®

Degree in Biotechnology and Food Science, University of Natural Resources and Applied Life Sciences, Vienna

ORAL SESSION

(L-1 – L-46)

L-1
OPENING LECTURE
EUROPEAN FOOD RESEARCH PERSPECTIVES

Dirk Pottier

on behalf of Antonio Di Gulio
European Commission–DG Research, Unit Food, Health and Well-being, Brussels, Belgium

L-2
EMERGING PROBLEMS IN GLOBAL FOOD SAFETY

Zhihua Ye[1*], Shuming Yang[2], Gang Chen[3]

[1 2 3] Institute of Quality Standards & Testing Technology for Agro-Products, CAAS, Beijing 100081, China
[*] Corresponding author–E-mail: zhihuaye@mail.caas.net.cn; Phone: +861082106501; Fax: +861082106288

Food safety is a very import issue, not only in a country, in a region, but in the whole world. Emerging problems of global food safety, from BSE to melamine incident, has resulted in various incidents in the domestic markets and in the international trade as well.

Since the first mad cow was found in 1984 in Britain, some 190,000 BSE cases have been reported in more than 30 countries. The incident of dioxin-contaminated poultry feed firstly occurred in Belgium in 1999 and, again, in North Ireland last year. Sudan red contaminated foods were detected in UK, China and several other countries, including products of some well-known brands such as KFC in 2005. In 2007, US recalled melamine contaminated pet feed in which the raw material was imported from a Chinese company. In 2008, high content of melamine was found in infant formula in China, which affected some 296,000 Chinese infants and young children with six infants died. The biggest Chinese dairy company *Sanlu* collapsed because of this incident. Huge economic loss and social impact were observed. The consequences were also felt globally as melamine-contaminated milk and relevant food products were exported to other countries. This presentation will give an overview of these emerging incidents of global food safety.

Joint efforts of national governments and international organizations have been taken to meet the challenges of global food safety crisis. International bodies such as WHO, FAO and OIE as well as CAC have worked efficiently to ensure food safety in the world. Efforts in legislation, administration and capacity-building for food safety at the national level were further enhanced than ever before. As an example, policies and actions taken by the Chinese government for resuming consumers' trust and confidence after the melamine incident are introduced and discussed in this presentation.

Food safety is increasingly and will continuously be a global challenge. It is suggested in this presentation that collective efforts be urgently needed for all countries to take food safety actions to a new level more than ever. Efficient collaborations of all relevant partners at the international and national levels are crucial for resolving the emerging problems and reducing the incidents of food safety in the future.

Keywords: Food, Safety, Crisis, Incident

L-3
QUALITY CONTROL IN ANALYSIS OF FOOD CONTAMINANTS

Thomas Wenzl[1*]

[1] European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium
[*] Corresponding author–E-mail: thomas.wenzl@ec.europa.eu; Phone: +32 14 571 320

Quality control makes part of quality assurance which covers both managerial and technical aspects. Fundamental guidance on quality assurance in among others analytical chemistry laboratories is provided by EN ISO/IEC 17025:2005 [1]. In EU official food control laboratories have to be accredited according to this standard and are regularly audited by the competent accreditation body [2]. Hence the quality of routine analyses should be under control.

However different contaminants were detected in the last few years in different types of food which caused urgent demands of analysis data. Accreditation of the respective analysis methods prior to the data generation, which would require an assessment of the data quality by an external body, was mostly not feasible with respect to timing. Hence quality assurance elements in particular quality control measures become elemental.

Quality control consists of "a set of procedures undertaken for the continuous monitoring of operation and the results of measurements in order to decide whether results are reliable enough to be released". It comprises a set of operations that are linked to each other like a chain. And as usual, the strength of the chain is only as strong as its weakest link.

This presentation focuses on a few links of the chain only and aims to demonstrate on practical examples the importance of a well established quality control system. In particular it will highlight the influence of calibration on the final result. Reasons for potential bias will be pinpointed and measures to control the bias of instrument calibration will be discussed.

[1] International Organization for Standardization (2005), General requirements for the competence of testing and calibration laboratories, EN ISO/IEC 17025:2005.
[2] Regulation (EC) No 882/2004 of the European Parliament and of the Council (2004), on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, Official Journal of the European Union L 165 of 30 April 2004.

Keywords: quality control, instrument calibration, contaminants

L-4
ADVANCED APPROACHES IN POPs ANALYSIS

Jean-François Focant[1*]

[1] CART, Organic and Biological Analytical Chemistry, Mass Spectrometry Laboratory, Chemistry Department, University of Liège, Allée de la Chimie 3, B-6c Sart-Tilman, B-4000 Liège, Belgium
* Corresponding author–E-mail: JF.Focant@ulg.ac.be; Phone: +32 495 92 95 64; Fax: +32 4 366 43 87

The 'Quest for the Holy Grail' in the 'dioxin' analysis area is dedicated to the development of reliable procedures that can offer congener-specific results on a short time scale, at a low cost, while avoiding down time issues. Such a procedure obviously has to fulfil strict QA/QC requirements such as the ones listed in Eurachem analytical guidelines and EU or other Directives, but also has to comply with ISO17025 and/or GLP procedures. Each part of such a procedure, namely extraction, clean-up, fractionation, chromatographic separation, and physico-chemical (or biological) measurement, has to be fine tuned to its optimum capabilities.

Whatever the measurement method used, either physico-chemical or biological, the sensitivity has to be at the parts-per-quadrillion (ppq, 10^{-15}) level. This represents an extreme case of ultra-trace analysis and a real challenge in terms of analytical chemistry. Large sample sizes have to be processed and extremely large amounts of matrix-related interferences have to be removed before one can even think about measurement.

Extraction and clean-up procedures can be automated and coupled to a certain extend but the global approach remains time and resource consuming. Automated solid-liquid adsorption chromatographic separations are often used to ensure high sample throughput, but also fractionation into sub-analyte groups that fit the peak capacity of the chromatographic instrument used for conger separation.

Next to the reference gas chromatography (GC) electron impact (EI) isotope dilution (ID) high resolution mass spectrometry (HRMS), other GC-MS methodologies are available. They exhibit some limitations but can also offer alternative solutions that found specific areas of application.

The presentation will highlight various aspects of some recent investigations of alternative methods for the measurement of dioxins, PCBs, OCPs, and PBDEs in biological matrices.

Keywords: dioxins, GC, MS, sample preparation, PCBs

L-5
AMBIENT MASS SPECTROMETRY EMPLOYING DIRECT ANALYSIS IN REAL TIME (DART) ION SOURCE: A NEW CHALLENGE IN FOOD ANALYSIS (?)

Jana Hajslova[1], Tomas Cajka[2], Lukas Vaclavik[3], Jan Poustka[4], Michal Godula[5]

[1 2 3 4] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[5] Thermo Fisher Scientific CZ, Slunečná 27 Praha 10, 100 00, Czech Republic
*E-mail: jana.hajslova@vscht.cz; Tel: +420 602833424

In the recent years, several new ambient desorption ionization techniques, such as desorption electro spray ionization (DESI), atmospheric-pressure solids analysis probe (ASAP), direct analysis in real time (DART) and some others, have become available thus providing further challenges in food analysis employing mass spectrometry. Their main advantages compared to conventional ionization techniques, involve the possibility of direct sample examination in the open atmosphere and minimal sample preparation requirements. On this account, fairly increased sample throughput is achieved. DART, which has been investigated in our laboratory, represents one of APCI-related techniques employing a corona discharge for the ionization. Metastable helium atoms, originated in the plasma, react with ambient water, oxygen, or other atmospheric components to produce the reactive ionizing species. DART ion source has been shown to be efficient for soft ionization of a wide range of both polar and non-polar compounds. DART produces relatively simple mass spectra characterized by $[M]^+$ or $[M+H]^+$ in positive ion mode, and $[M]^-$ or $[M-H]^-$ in negative-ion mode.

Coupling DART to time of the flight mass spectrometry (ToF MS) enables rapid sample profiling (finfgerprinting), under relevant conditions, identification /quantification of target /unknown analytes is possible. It should be noted, that screening approaches are most often realized using „high performance“ ToF MS instruments, with mass accuracies of < 5ppm and resolutions not exceeding 15,000. However, these parameters may not be sufficient for unambiguous identification of some analytes in food samples containing high amounts of (potentially interfering) matrix. To overcome these limitations, ultra high resolution mass spectrometer based on the Orbitrap™ analyzer was also employed in our experiments.

The application potential of ambient mass spectrometry in modern food analysis will be demonstrated on several examples including: (*i*) distinguishing of geographical and species origin of food commodities; (*ii*) assessment of meat freshness (in both cases based on metabolomic fingerprinting); (*iii*) control of processing contaminants and prediction of their formation; (*iv*) characterization of food supplements authenticity. Since a large volume of data is typically generated during the measurements of positive /negative mass spectra, smart chemometric tools such as linear discriminant analysis (LDA) and artificial neural networks (ANN) are used for this thein processing.

Keywords: ambient mass spectrometry, DART. Orbitrap™ analyzer

The financial support by the Ministry of Education, Youth and Sports of the Czech Republic (MSM 6046137305) and by the European Commission, under the FP6 Food Quality and Safety Priority, within the framework of the Integrated Project TRACE–006942 – entitled “Tracing Food Commodities in Europe”, is gratefully acknowledged.

L-6
NEW ANALYTICAL APPROACHES TO AUTHENTICATING FOOD: AN OVERVIEW

Paul Brereton[1*]

[1] The Food and Environment Research Agency, York, UK
[*] Corresponding author–E-mail: trace.secretariat@fera.gsi.gov.uk; Phone: +44 1904 462700; Fax: +44 1904 462133

Food fraud and traceability continues to have a high profile with several major incidents being reported in the press and media. There has been an increased emphasis from the food industry on marketing of foods with perceived food quality attributes to an ever more discerning consumer. The lack of objective methods for verifying some of these labelling claims is to the detriment of the consumer but also the food industry, as the honest producer is not protected nor the purchasers of such products within the food chain. Many of these perceived quality attributes cannot easily be verified using current analytical methods. In particular, labelling claims that relate to: provenance, organic, identity, sustainability are difficult to substantiate and require the development of new analytical approaches and processes.

Analytical methods for use in detecting food fraud rely on detecting/quantifying marker(s) of the authentic product or of the adulterant and pose considerable challenges in terms of detection, quantification and interpretation. Some of the latest analytical and chemometric approaches used to authenticate labelling claims will be described together with specific examples of the application of metabolomic profiling methods and stable isotopic techiques for confirmation of food authenticity and traceability.

Keywords: food fraud, traceability, analytical methods

This work is funded by the European Commission, under the FP6 Food Quality and Safety Priority, within the framework of the Integrated Project TRACE–006942 – entitled "Tracing Food Commodities in Europe".

L-7

AUTHENTICATION OF BEER AND WINE USING ADVANCED MASS-SPECTROMETRIC TECHNIQUES

Tomas Cajka[1*], Katerina Riddellova[2], Jana Hajslova[3]

[1 2 3] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: Tomas.Cajka@vscht.cz; Phone: +420220443142

Up to now, a lot of scientific effort has been spent to develop rapid, reliable, and cost effective analytical methods applicable for the authentication of various food commodities. Besides of spectroscopic techniques employing nuclear magnetic resonance (NMR), Raman, or infrared spectra, a wide range of methods employing gas chromatography–mass spectrometry (GC–MS), and/or high-performance liquid chromatography–mass spectrometry (HPLC–MS) have been implemented for this purpose. In addition to these approaches, matrix assisted laser desorption/ionisation mass spectrometry (MALDI), direct head-space mass spectrometry (HS-MS), and/or direct infusion MS allow reduction of analysis time thanks to elimination of chromatographic separation step.

Over the few recent years, a large number of novel ambient desorption ionisation techniques, have become available providing further improvements. Their main advantages compared to conventional ones, involve: (*i*) easy method development and optimisation, (*ii*) significantly reduced workload and, consequently, increased laboratory throughput. One of the most challenging techniques in this field is MS utilising a direct analysis in real time (DART) ion source.

For the processing of a large volume of data generated by these profiling techniques, smart chemometric tools are usually needed to fully utilise this information. Typically, the principal component analysis (PCA) is used for a preliminary inspection of the data structure, followed by various classification methods such as linear discriminant analysis (LDA), partial least squares discriminant analysis (PLS-DA), or artificial neural networks (ANNs).

In this presentation, the potential of DART–TOFMS strategy to distinguish wines and beers according to their species and/or brand origin will be demonstrated. The data generated by this emerging technique will be compared to those obtained by a “gold standard” represented by solid-phase microextraction (SPME) coupled to GC–TOFMS. Advanced chemometric strategies, mentioned above, were employed for interpretation of acquired data sets.

Keywords: DART; SPME; GC–MS; Authentication

The financial support by the European Commission through the 6th Framework Programme (contract no FP6-FOOD-2004-006942 – TRACE) and the Ministry of Education, Youth and Sports of the Czech Republic (MSM 6046137305) is gratefully acknowledged.

L-8

DETERMINATION OF HONEY ADULTERATION BY NEAR INFRARED SPECTROSCOPY BASED ON DISCRIMINANT PARTIAL LEAST SQUARES

Lanzhen Chen[1], Zhihua Ye[2*], Jing Zhao[3], Qiu Jin [4]

[1] Institute of Bee Research, CAAS, Beijing 100093, China; Institute of Quality Standards and Testing Technology for Agro-Products, CAAS, Beijing 100081, China
[2 4] Institute of Quality Standards and Testing Technology for Agro-Products, CAAS, Beijing 100081, China
[3] Institute of Bee Research, CAAS, Beijing 100093,China
[*] Corresponding author–E-mail: zhihuaye@mail.caas.net.cn; Phone: +861082106501; Fax: +861082106288

The annual production of honey is estimated at 200,000 tons in China and 50% of the products are exported to the world market. As a high nutritional and medical product, honey is very popular for consumers. However, honey has been the target of adulteration with cheaper commodities in China in recent years. Authenticity of honey is a very important issue for processors, retailers and consumers as well as regulatory authorities. There is an increasing demand for appropriate methods to ensure a fair competition among producers and to protect consumers against fraud.

The well-known adulterant is high fructose corn syrups (HFCS) in China. Since the adulterant composition is similar to that of honey, it is very difficult to detect the adulteration by conventional laboratory methods. Stable carbon isotopic ratio analysis (SCIRA) has been used to determine syrup additions of Chinese honey as an authoritative method for the evaluation of such products. The technique, while reported to have many potential advantages, is expensive, time-consuming and requires considerable analytical skills which are not suitable for routine monitoring analysis. Therefore, an attempt has been made to use near infrared spectroscopy (NIR) to detect adulteration of honey.

In this presentation, a NIR spectrometer equipped with fiber optic diffuse reflectance probe was employed for identifying pure honey and honey adulterated with various concentrations of HFCS. NIR data from 71 commercial samples were collected and 44 samples were identified as HFCS-adulterated. The contents of HFCS varied from 7% to 59% by SCIRA. NIR spectra in the range of 12,000–4,000 cm^{-1} were recorded by immersing the fiber optic probe into each of the samples contained in little bottles. The raw spectra showed no significant difference between the pure honey and those adulterated with HFCS. The samples were further randomized into calibration sets and validation sets. A classification model was constructed by using principal component analysis (PCA) and discriminant partial least squares (DPLS) regression based on PCA scores. The results demonstrated that excellent classification can be obtained after optimizing spectral pre-treatment and spectral ranges. The classification accuracy of the calibration data set as well as the validation data set for pure honey and HFCS adulterated honey samples were both greater than 90% using DPLS after first derivative, 6700–10000 cm^{-1} NIR ranges and 8 principal components(PCs). The NIR spectroscopy together with DPLS techniques is presented as a rapid, non-destructive and cost-efficient method for determining authenticity and/or adulteration of Chinese honey.

Keywords: Honey, Adulteration, Determination, NIR

L-9

DETERMINATION OF FRUCTANS AND NITRATES TO CHARACTERIZE ONIONS (ALLIUM CEPA) FROM DIFFERENT ITALIAN ORIGIN BY HPAEC-PAD AND CZE TECHNIQUES

Chiara Borromei[1*], Antonella Cavazza[2], Claudio Corradini[3], Marco Giannetto[4], Cristiana Merusi[5], Giovanni Mori[6]

[1 2 3 4 5 6] University of Parma, Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Italy
University of Parma, Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Italy
[*] Corresponding author–E-mail: chiara.borromei@nemo.unipr.it; Phone: +390521905476

The onion (Allium cepa L.) is a species of the Alinaceae family of great economic importance, and is the second most important vegetable crop in the world. The major fraction (65–80%) of the dry matter of onion bulb consists of so-called non-structural carbohydrates which mainly are glucose, fructose, sucrose and fructans. Fructans are fructose polysaccharides, which are formed by the cumulative addition of a fructosyl group to a sucrose molecule. The degree of polymerisation (DP) of these fructans can vary to a large extent amongst *Allium* species. Fructans composition of the onion is variable and depends on cultivar, ripening stage, environment and agronomic conditions. Furthermore, these carbohydrates have recently been shown to be of particular interest for human health. They have a prebiotic effect, improving the intestinal flora, especially the bifidobacteria intestinal conditions against pathogen agents. We have been interested in using currently available chromatographic procedures to screen for the range of fructans present in onion from different Italian regions. In this work the determination of glucose, fructose, sucrose and fructans was carried out by high performance anion-exchange chromatography coupled with pulsed electrochemical detection (HPAEC-PAD), aiming at developing a reliable method in the traceability of the origin of onions. Several variables are studied as geographical origin, cultivars, calibre and storage. Our work was mainly focused on onions having Protected Geographical Indication (PGI).

Moreover, considering that vegetables are the major source of nitrate and nitrite in human diet, the assessment of these ions in the investigated onions has also been considered and measured by a capillary electrophoretic method carrying out the separation on an uncoated capillary and reversing the EOF just by employing an acidic buffer. Furthermore determination of such anions was carried out on both soil and onion bulbs.

The data were processed by means of the chemiometric approach of linear discriminant analysis (LDA) that allows classifying unknown samples after checking possible differentiation of samples of known origin.

Keywords: Onions, Fructans, Nitrates, PGI, LDA

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L-10
FOODSTUFF QUALITY EVALUATION BY MAGNETIC RESONANCE IMAGING SPECTROSCOPY

Sara Cozzolino[1], Caterina Cafiero[2], Mena Ritota[3], Anna Taglienti[4], Paolo Sequi[5], Massimiliano Valentini[6*]

[1 2 3 4 5 6] Agricultural Research Council-Research Centre for Plant-Soil System, Instrumental Centre of Tor Mancina, Strada della Neve-S.P. Pascolarese Km 1, 00016 Monterotondo, Roma (Italy)
[*] Corresponding author–E-mail: massimiliano.valentini@entecra.it; Phone: +39-06-90627203; Fax: +39-06-9068309

Magnetic Resonance Imaging (MRI) is becoming a popular analytical tool in food analysis.[1, 2] It offers the almost unique opportunity of studying foodstuff in their wholeness without any preparative manipulation of the sample. ^{1}H-MRI provides highly spatially resolved images of internal sections or volumes of any food. Images can be obtained with different weighing factors (e.g. spin density, relaxation times and diffusion coefficient) chosen depending upon the structural feature to be highlighted. MRI has been successfully applied in postharvest quality analysis, elucidation of internal morphology, histology, etc., of several foods, most likely fruits and vegetables.

Here we present some application of this technique, which appears, also in consideration of the novel instruments suitable for on-line or off-line measurements, highly appealing for the food industries.

Changes occurring during ripening and post-harvest as a function of temperature and atmosphere composition have been highlighted for hazelnuts and kiwifruits.[3] For the latter the effect of Plant Growth Regulators on shelf-life was assessed. The use of arsenic contaminated irrigation water on radish quality was studied, as well as variation of truffle quality during storage and fungi attack was addressed. The effects induced by novel bio-stimulator based on bio-available orthosilicic acid applied to grape wine, kiwifruits, tomato and strawberry was elucidated by means of MRI.

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[3] A. Taglienti, R. Massantini, R. Botondi, F. Mencarelli, M. Valentini *Food Chem.*, 114, 1583-1589 (2009)

Keywords: MRI, non-destructiveness, shelf-life, morphology, post-harvest

L-11
THE USE OF LC-qTOFMS FOR DISCRIMINATION AND CLASSIFICATION OF RED WINES ACCORDING TO THEIR VARIETY

Lukas Vaclavik[1*], Ondrej Lacina[2], Jana Hajslova[3], Jerry Zweigenbaum[4]

[1 2 3] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic [4] Agilent Technologies, Wilmington, DE, USA
[*] Corresponding author–E-mail: lukas.vaclavik@vscht.cz; Phone: +420220445119

In this study, reversed phase liquid chromatography–electrospray–quadrupole time-of-flight mass spectrometry (RPLC–ESI–QTOFMS) was used to obtain metabolomic profiles of red wine samples (no sample preparation employed). In total, 51 wines representing three varieties (Cabernet Sauvignon, Merlot, and Pinot Noir) of various geographical origins were sourced from European and US retail market and analyzed under positive and negative ionization mode. To find compounds detected in analyzed samples, an automated compound (feature) extraction algorithm was employed for processing of background subtracted single MS data. After alignment of mass-to-charge ratios and retention times of the molecular features across all samples, stepwise reduction of the data dimensionality was carried out based on frequency of occurrence, abundance of the features, and results of analysis of variance (ANOVA). In positive ion mode, approximately 20000 initially extracted features were reduced to 26 for discrimination and classification of the samples. For this purpose, multivariate statistical methods, principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA), were employed. While the validated mathematical model produced by PLS-DA of data measured in positive ionization mode enabled correct classification of 96% of samples, recognition ability of the model constructed with the use of negative ion mode was lower (91%). Using the positive ion single MS model, 5 wine varieties not used in the model were correctly classified.

With a good working model it would very useful to identify key marker compounds that determine the class of each wine. Determination of molecular formula, and, in some cases, tentative identification of selected compounds was carried out using accurate mass measurement of full single MS spectra. More definitive identification was made by correlating the fragments obtained by MS/MS accurate mass spectra using the QTOF with collision induced dissociation (CID) of parent ions. Using a wide bandbass filter for the quadrupole, the accurate mass of the fragments and their isotopes were used for structure confirmation.

Keywords: Wine, Authenticity, LC-QTOFMS, PLS-DA

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L-12

COMPARISON OF AN ELECTRONIC NOSE, GAS CHROMATOGRAPHY AND OLFACTOMETRY MEASUREMENTS TO DISCRIMINATE BETWEEN RED WINES AGED IN OAK BARRELS AND WINES AGED USING PIECES OF WOOD

Natalia Prieto[1*], Maria Luz Rodríguez[2], José Antonio de Saja[3]

[1 2 3] University of Valladolid. Valladolid. Spain
[*] Corresponding author–E-mail: nprietoc@fmc.uva.es; Phone: +34691806818; Fax: +34983423192

In previous works, our group has developed an electronic nose, specifically dedicated to the analysis of red wines. The system consists in an array of metal oxide (MOX) sensors, coupled to a Solid Phase Microextraction (SPME) system [1,2]. This system has been able to discriminate among wines made using different varieties of grapes, or made using grapes of different geographical origins. The system can also be used to follow the ageing process of wines. In this work, the capability of the multisensor system has been used in one of the most important problems of the wine industry, the discrimination between red wines aged in oak barrels and red wines matured in steel tanks in contact with oak wood chips and staves. The volatile compounds have also been analysed by gas chromatography-mass spectrometry (GC/MS) coupled to an olfactometry system connected to a SPME [3]. Using chromatography certain compounds important in the ageing process (2-furaldehyde, guaicol, cis/trans-whiky lactone and eugenol) have been quantified with their odour activity.

Results obtained using the electronic nose have demonstrated that after ten months in bottle, it is possible to discriminate between wines aged using traditional methods from those aged using oak chips or staves. The responses of the gas-sensors correlated well with the GC/MS and olfactometry results suggesting that they can detect volatile difference that has been detected in red wine ageing with alternative systems.

Principal component analysis models have demonstrated a good-quality ability to establish correlations between three systems that have been used. Bi-plot analysis has discriminated and moreover it has associated the most important aromatic characteristics in the different ageing systems of red wines (barrels, oak chips and oak staves). The results confirmed the multivariate relationships between the volatile compounds responsible of the aged wine aroma.

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Keywords: electronic nose, olfactometry, wine

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L-13
DISCRIMINATION OF EUROPEAN BEEF ORIGIN BY STABLE ISOTOPE ANALYSIS

Micha Horacek[1*], Andreas Roßmann[2], Simon Kelly[3], Freddy Thomas[4], Katharina Heinrich[5], Federica Camin[6], Luana Bontempo[7], Claus Schlicht[8], Antje Schellenberg[9], Jurian Hoogewerff[10]

[1] Austrian Institute of Technology GmbH, Seibersdorf, Austria
[2] Isolab GmbH, Schweitenkirchen, Germany
[3 10] University of East Anglia, Norwich, UK
[4] Eurofins Scientific Analytics, Nantes, France
[5] The Food and Environment Research Agency, York, UK
[6 7] IASMA Research and Innovation Centre, San Michele, Italy
[8 9] LGL Bayern, Oberschleißheim, Germany
[*] Corresponding author–E-mail: micha.horacek@ait.ac.at; Phone: +43 50550 3575; Fax: +43 50550 3520

The control of origin of food products is an important topic for food quality and safety, consumer confidence and brand development. Food scandals connected with unhealthy meat have proven that a tool of origin verification beyond the control by paperwork is needed. We present the first large dataset of stable isotope analyses of beef samples from different European regions in order to differentiate the geographical origin of their production.

Stable isotope data for H, C, N and S were measured and reported against the appropriate international standards. We demonstrate that some of the regions can be grouped by their similar isotope patterns, due to comparable climatological and ecological conditions and cattle rearing practices:

Group 1: oceanic and moderate in climate with extensive cattle breeding regime, *Group 2*: regions with cool to moderate climates or at higher altitudes without significant influence from the sea and extensive cattle rearing regimes, *Group 3*: regions influenced by the Mediterranean Sea and with intense cattle rearing regimes, *Group 4*: regions with only limited or no influence from the sea and moderate in climate with intense use of maize in the cattle feed.

Keywords: beef; origin; authenticity; isotope; meat

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L-14
MONITORING PERFLUORINATED COMPOUNDS IN FOOD CHAIN

Stefan van Leeuwen[1], Jacob de Boer[2*]

[1 2] VU University, IVM, Amsterdam, The Netherlands
[*] Corresponding author–E-mail: jacob.de.boer@ivm.vu.nl; Phone: +31205989530; Fax: +31205989553

In recent years, perfluorinated compounds (PFCs) have received considerable attention from scientists and policy makers. PFCs have been and are still being used widely as surfactants in industrial applications and consumer products. PFCs are persistent and can enter the environment at several stages, e.g. the application to a material, the in-service life and after disposal of a product. PFOS (perfluorooctanesulfonate) and PFOA (perfluorooctanoic acid) are the most well-known PFCs. Other compounds belonging to this class have different chain lengths and/or functional groups [1]. PFOS accumulates in fish, whereas PFOA is more water-soluble. PFCs can also enter food products through contact with packaging materials. In 2008, EFSA has completed a risk assessment on PFOS and PFOA [2]. It was noted that the estimated exposure (60 ng/kg bw/d) was close to the derived TDI (150 ng/kg bw/d). Also, a lack of reliable data on PFCs in food was observed. Interlaboratory studies show improving data, but still a number of analytical issues needs to be solved [3]. The number of relevant PFC compounds is continuously growing. There is an urgent need for good quality certified reference materials. An accurate dietary exposure assessment is not possible without reliable data.

The surfactant type properties of PFCs call for different analytical approached as compared to other persistent lipophilic contaminants. Fish samples are mostly extracted using medium polar solvents (acetonitrile, methanol), combined with a clean up step using Envicarb [4]. Instrumental analysis is generally performed by HPLC-ESI-MS/MS [1]. Liquid samples like milk and drinking water are generally analysed using solid phase extraction (SPE) [2]. In recent studies methods were developed for PFCs in a wide range of foods [5,6]. These methods rely on KOH digestion of the samples, dilution with water and extraction of the PFCs from the diluted digest by SPE. This enabled the use of higher sample intakes (10 gram) in order to lower the limits of quantification (0.03–0.15 ng/g fresh weigt) [6].

Recently, the EU funded project PERFOOD was started. The aims of this project are to develop robust and reliable analytical tools including reference materials for the determination of PFCs in food items, and to use these to (*i*) qualify and quantify PFCs in the European diet; (*ii*) understand the transfer of PFCs into food products, and (*iii*) quantify the contribution of food/beverage contact materials and food and water processing to the overall PFC level in our diet.

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Keywords: Perfluorinated compounds, PFOS, PFOA, Food

L-15

FULL SCAN MS SCREENING IN FOOD SAFETY ANALYSIS: HOW TO FIND WHAT YOU'RE (NOT) LOOKING FOR

Hans Mol[1*], Arjen Lommen[2], Arjen Gerssen[3], Martijn van der Lee[4]

[1 2 3 4] RIKILT, Institute of Food Safety, Wageningen, Netherlands
[*] Corresponding author–E-mail: hans.mol@wur.nl; Phone: +31 317 480318

The potential presence of residues and contaminants is an important issue in the field of food and feed safety. The variety of matrices is enormous and the number of analytes (pesticides, veterinary drugs, natural toxins, environmental contaminants, etc) to look for is extensive (>> 1000). Chromatography combined with mass spectrometry has proven to be a very efficient approach for screening and determination of multiple residues and contaminants. Full scan MS detection is particularly promising in this respect since it involves non-targeted data acquisition with, in principle, no limitations regarding the number of analytes that can be measured in one run. Further more, it allows retrospective data analysis for detection of 'new' analytes.

GC(×GC)-MS and LC-high resolution MS (TOF, Orbitrap) are typical instruments used for wide-scope screening. By now, generic sample preparation procedures and instrumentation have reached a stage that allows implementation for sample analysis. In contrast to efforts and improvements made in instrumentation, development of software tools for efficient data evaluation has received much less attention and is the current bottleneck for routine application. Full scan data files are huge, slowing down processing and challenging electronic archiving of raw data. With the large numbers of analytes involved, manual data evaluation by an analyst is no longer an option. Instead, one has to rely on automated library-based screening of the raw data. As will be discussed in this presentation, many parameters determine the success of this approach. These include sample related parameters (complexity of the sample, analyte LODs), QC related parameters (numbers of false positives and false negatives), quality of the raw data (reliability of retention time, spectrum quality, mass accuracy), the size of the library, and last but not least the software tool(s) available for data evaluation. With respect to the latter, each instrument supplier has its own proprietary software package. Possibilities and limitations of current hardware/software combinations will be shown by examples both for GC-MS and LC-MS. Furthermore, a new generic (i.e. instrument independent) software tool, developed in-house originally for data preprocessing in the field of metabolomics [1], will be presented for application to targeted data analysis in the field of residues and contaminants. The software tool can handle data formats from a variety of instruments. It reduces the data size >50 fold by performing noise analysis, baseline corrections, peak-picking and accurate mass calculations. The individual reduced GC(×GC)-MS or LC-MS data files can be searched fast in a targeted mode using custom made or commercial libraries. The processed reduced data files can be exported back to several MS software platforms for additional evaluation if desired, or easily archived for future retrospective data mining against new or updated target libraries. The developments presented demonstrate that the necessary improvements in data handling are on-going, taking wide-scope screening a step closer toward application in routine practice.

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Keywords: mass spectrometry, data mining, residues/contaminants

L-16

MONITORING OF GROWTH HORMONE FATE IN DAIRY PRODUCTS UNDER INDUSTRIAL PROCESSES BY LC-MS/MS

Marie-Hélène Le Breton[1*], Sandrine Rochereau-Roulet[2], Gaud Pinel[3], Thierry Delatour[4], Bruno Le Bizec[5]

[1 4] Nestlé Research Center, Lausanne, Switzerland
[2 3 5] LABERCA, Nantes, France
[*] Corresponding author–E-mail: marie-helene.le-breton@rdls.nestle.com; Phone: +41 21 785 80 96

Recombinant bovine growth hormone is used in dairy farming to enhance milk yield. Even if this practice has been regulated more than ten years ago, it is still subject to controversy due to the lack of analytical methods. Indeed, the low level of residue of the hormone as well as the strong homology between endogenous and recombinant form have prevented the development of analytical tools able to discriminate treated from non-treated animals. Only recently a method has been developed for the direct detection of recombinant bovine growth hormone in plasma and serum [1–2].

The purpose of this work was the development of a mass spectrometry based method for the direct detection of recombinant bovine growth hormone in milk and dairy products. A protocol based on solid phase extraction, enzymatic digestion and analysis of the N-terminal peptide specific of the hormone has been developed. Recombinant equine growth hormone was used as internal standard. The method has been validated according to the criteria described in the EU Commission Decision 2002/657 and allowed a limit of detection of 10 ng/mL in milk and dairy products. It was then used to study the consequence of various industrial processes on the fate of the hormone in products. The effects of heating, freezing, defatting, pasteurization and spray-drying were evaluated. It showed that temperature related processes such as pasteurization and spray-drying induce a loss of the hormone up to 95%. Further experiments should be carried on in order to determine if this loss is due to a product degradation or structural modification.

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Keywords: growth hormone, mass spectrometry, milk

L-17
RESEARCH METHODS, ANALYTICAL CRITERIA AND STANDARD METHODS: HELP OR HINDRANCE?

Martin Rose[1*]

[1] Fera, York, United Kingdom
[*] Corresponding author–E-mail: martin.rose@fera.gsi.gov.uk; Phone: +44 1904 462655; Fax: +44 1904 462111

MoniQA ("**Moni**toring and **Q**uality **A**ssurance in the Food Supply Chain") is a Network of Excellence funded by the European Union. This aims to make food safer by harmonising methods for food analyses. Part of this process involves the development of new methods, analytical criteria and standard methods to be used for food control.

High throughput methods, screening methods and rapid methods are all important tools for food control. Harmonisation and standardisation can be difficult when new technologies are introduced. One way this can be achieved is by using common standards. Standard analytical methods are produced by various organisations, such as CEN, AOAC and National Standards bodies. These methods have been subject to rigorous peer review and inter-laboratory validation and are of great value to control laboratories that need to cover a vast array of work and need access to reliable methodology with known performance characteristics. But the development of standard methods can take many years and can be seen as a hindrance to implementing the most up to date new and improved procedures. Harmonisation may also be achieved by agreeing upon a criteria based approach. This gives flexibility to choose any analytical method including application of the most recent developments whilst ensuring that certain agreed quality criteria are met.

Keywords: standard-methods, analytical-criteria, harmonisation, screening-methods

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L-18
INVESTIGATION OF PESTICIDE METABOLITES IN FOOD MATRICES BY LC/MS

Felix Hernandez[1*]

[1] University Jaume I, Castellon, Spain
[*] Corresponding author–E-mail: felix.hernandez@uji.es; Phone: 34-964-387366; Fax: 34-964-387368

Pesticide residue research in food is an important topic of modern analytical chemistry. Because of the toxicological properties of some pesticides could remain in their metabolites, designing efficient strategies for pesticide metabolite investigation in food is necessary. There are thousands of potential metabolites resulting from the metabolism of hundreds of parent pesticides in plants, in animals, and from different transformation processes in the environment. Some pesticide metabolites are specifically included in the residue definition and they are commonly determined in routine analysis, as they are included in analytical methods as target analytes. However, there are still differences in the international regulations when considering a metabolite as relevant. At present, the analyst has to face to many potential unknown compounds with little information available. In addition, many reference standards are commercially unavailable (especially if the metabolite is unregulated), being their discovering in samples still more difficult.

LC-TOF MS has shown a great potential for searching pesticide metabolites in food [1], especially when these metabolites are unexpected, due to the combined characteristics of high full-spectral sensitivity and increased mass accuracy and mass resolution, which facilitate the reliable identification of the compounds detected in samples. Moreover, hybrid QTOF MS provides additional features for confirmation [2], because of the useful information given by accurate mass product ion spectra after performing MS/MS experiments.

Investigation of parent-positive samples is an interesting option when searching for pesticide metabolites in food. In this lecture I will show the use of two different strategies that facilitate this task, avoiding the tedious manual selection of “relevant” peaks in the Total Ion Chromatogram given by TOF MS. The work has been performed using UHPLC coupled to (Q)TOF MS. One strategy is based on considering a common fragmentation pathway between the parent pesticide and their potential metabolites. After an enhanced fragmentation in the collision cell when acquiring in TOF MS mode (it was feasible thanks to the use of a QTOF instrument), this approach was applied to several fruit samples collected in the market. Parent positive samples were analyzed in this way, and it allowed the discovering of (non-target) post-harvest fungicide metabolites in lemon and insecticide metabolites in grape without previous selection of the compounds to be searched.

The other strategy is based on using specific software, applying it to treated and untreated samples from field residue trials. This has led to the discovering of insecticide metabolites in treated samples. In both strategies, every metabolite detected after a first acquisition in TOF MS has been confirmed using QTOF and/or triple quadrupole (QqQ) instruments. Accurate masses given by TOF MS together with the valuable information on product ions given by QTOF MS/MS experiments were crucial for the safe identification of metabolites.

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Keywords: pesticide residues, food, metabolites, LC-TOFMS

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L-19

EFFICIENCY OPTIMIZATION FOR PESTICIDES AND MYCOTOXINS MULTI-RESIDUE ANALYSES BY INTEGRATED SAMPLE PREPARATION METHOD

André De Kok[1*], Ionara Pizzutti[2]

[1] VWA–Food and Consumer Product Safety Authority-National Reference Laboratory (NRL) for Pesticides and Mycotoxins Analysis in Food, Amsterdam, The Netherlands
[2] UFSM-CEPARC, Federal University of Santa Maria – Chemistry Department – Center of Research and Analysis of Residues and Contaminants (CEPARC), Santa Maria, RS, Brazil
[*] Corresponding author–E-mail: andre.de.kok@vwa.nl; Phone: +31205244711; Fax: +31205244700

Laboratories for the analysis of residues and contaminants in food and feed products are nowadays challenged to increase their sample throughput and scope of analysis, but also to reduce costs and maintaining the required quality of results. Therefore, more efficient and faster analytical methods have to be developed and implemented in a routine environment.

In the past years, our laboratories have developed and fully validated various comprehensive and integrated sample preparation methods for mycotoxins and pesticide residues analysis in sample types in which these contaminants may be expected to be present concurrently. These samples comprise, for example, rice, wheat, corn, soya, cocoa beans, coffee, breakfast cereals, wine and grape juice. Very homogeneous slurries were prepared by mixing the matrix with water in an optimized proportion for each individual matrix. Then, 10 g subsamples were extracted with acetonitrile + 1% acetic acid, followed by partitioning induced by MgSO4. Aliquots were taken directly for mycotoxins and LC-pesticides analysis. After a subsequent dispersive cleanup and drying step, aliquots were taken for the various GC-MS(/MS) techniques.

During method optimization, the influence of pH, use of buffer, different combinations and types of sorbents (alumina, silica, Florisil, C_{18}, PSA, Extrelut) and their amounts (100–800 mg) in dispersive SPE, were studied for 3 groups of target analytes: mycotoxins (≈35), polar (>133) and non polar (>100) pesticides. The first 2 classes of analytes were analyzed by liquid chromatography tandem mass spectrometry (UPLC-MS/MS) in positive electrospray ionization mode and the third one by GC-ITD (EI-MS and EI-MS/MS), GC-MS single-quad (NCI-SIM mode) and GC-MS/MS triple-quad (EI-MS/MS). The final optimized method for all residues studied was fully validated at 3 spiking levels (n=6) in terms of quantitative and qualitative parameters (accuracy, precision, matrix effect, linearity, ion ratios for identification).

Generally, the results for analytical curves linearity (standards in solvent and in matrix), method detection and quantification limits, recoveries (accuracy) and repeatability relative standard deviations (precision) fulfilled the EU requirements (SANCO/3131/2007) for the majority of the compounds. The use of buffering with HAc/NaAc and the dispersive C18 SPE cleanup was always needed for GC pesticides analyses. For LC-MS/MS analysis, cleanup appears not to be necessary. Recoveries between 70 and 120% were achieved for almost all analytes with RSD values < 20%. In general (except for cocoa and coffee), no significant matrix effects were present either in the mycotoxins or pesticides measurements by LC-MS/MS. However, for GC-MS analysis, matrix effects occurred despite the cleanup applied.

[1] European Commission, DG-SANCO, “Method validation and quality control procedures for pesticide residues analysis in food and feed”, Document no. SANCO/3131/2007, Brussels, 31 October 2007.

Keywords: pesticides, mycotoxins, mass spectrometry

VWA, CEPARC/UFSM

L-20

GAS PERMEATION THROUGH POLYMERIC FILMS USED IN FOOD PACKAGINGS: DEVELOPMENT OF A NEW EXPERIMENTAL METHODOLOGY

Catarina M. Marques[1], Maria Teresa S.R. Gomes[2*]

[1 2] CESAM & Department of Chemistry, University of Aveiro, Aveiro, Portugal
* Corresponding author–E-mail: mtgomes@ua.pt; Phone: +351 968661256; Fax: +351 234370084

Polymeric films are widely used to protect food either in industrially processed products and at home. They act as a barrier to prevent contact with other products, dust or contamination by manipulation. Considerable effort in preventing migration from packaging compounds into food is being made, and technological solutions are nowadays evaluated by chemical analysis. Sealing packages are also being evaluated in which concerns to oxygen permeability, although permeation of other gaseous compounds is seldom of concern. However, it is a common experience to detect some odd flavours in food, originated from other products stored in the same refrigerator. Contamination is not restricted to other food products but can arise from odours from very different sources. It can come from disinfection products, paints and varnishes but also from unsuspected and odd origins, as accidents in big supermarkets can arise from any volatile compound accidentally released by a costumer when a bottle or jar is broken in the food hall.

In this work, diffusion and permeation coefficients of n-butylamine, which is found in fish, through commercial polyvinyl chloride (PVC) films, low density polyethylene (LDPE) films and sacs and polyethylene terephthalate (PET) films were determined.

A new methodology using an acoustic wave sensor was developed. The sensor, based on a coated piezoelectric quartz crystal, allows the real time detection of the gas quantity that permeates through the membrane. The high sensitivity of the sensor allowed quantifying small quantities of permeated amine, which in the present work never exceeded a few hundreds of nanograms. The sensor is not specific to amines and other compounds as free acids, alcohols and ethers can also be detected with it. After calibration, the frequency decrease observed as gas permeates can be converted in concentration. Diffusion coefficient was calculated by the time lag methodology

The experimental apparatus was simple and inexpensive. The permeation chamber and the quartz crystal cell were made of glass, at the workshop of the University. The oscillator was mounted at the lab and quartz crystals are very cheap, as they are widely used in electronics and are available from local shops.

Besides a detailed description of the apparatus and an explanation about the sensor, results will be presented. Diffusion and permeation coefficients at several temperatures will be calculated from the sensor responses.

Keywords: permeation, diffusion, sensor, polymeric film

L-21

THE CURRENT STATE OF ANALYTICAL METHODOLOGY FOR FOOD SAFETY AND TRACEABILITY IN DEVELOPING COUNTRIES

Andrew Cannavan[1*]

[1] Agrochemicals Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Joint FAO/IAEA Programme, International Atomic Energy Agency, Vienna, Austria.
[*] Corresponding author–E-mail: A.Cannavan@iaea.org; Phone: +43 1 2600 28395

The implementation of integrated, farm-to-fork, food safety systems to meet today's stringent requirements for consumer protection and trade in food commodities requires a well developed analytical capacity for contaminants and residues. Analytical laboratories engaged in monitoring and surveillance provide essential feedback on the effectiveness of control systems. Advanced analytical techniques are often necessary to achieve the required method performance in terms of sensitivity, specificity, selectivity, precision and trueness for the analysis of residues of veterinary drugs and other agrochemicals and contaminants such as dioxins and mycotoxins. New technology for these applications is constantly being developing or improved. Techniques such as triple quadrupole LC-MSMS, which were considered state of the art only a few years ago, are already being replaced in some quarters by, for example, high resolution TOF-MS techniques, Direct Analysis in Real Time (DART) MS, multi-contaminant bioanalytical and advanced fingerprinting techniques. There appears to be trend away from targeted analysis to non-targeted techniques, made more useable by the development of powerful chemometric and analytical software packages. The implementation of effective traceability systems also requires analytical support, and analytical techniques are also advancing apace in this field, often using technology developed in parallel with that mentioned above.

Unfortunately, developing countries or those with economies in transition are frequently at least one step behind the latest developments. Many developing countries, especially those which have established trade with the developed world, have only recently implemented the last generation of analytical techniques, such as LC-MSMS, GC-MSMS and optical biosensor instrumentation. A common problem is that instrumentation has been installed to address a specific problem which impacted on trade – for example, residues of chloramphenicol or nitrofurans – and is not fully utilised for the broad range of applications for which it may be suitable. In such situations, effort is better invested in mastering and optimising proven techniques rather than trying to keep pace with technological developments. Only those emerging techniques that are robust, wide scope, cost-effective and likely to be applicable in the medium to long term should be considered for use in developing countries to complement the more proven techniques.

L-22
BIOANALYTICAL SCREENING OF MULTIPLE CHEMICAL CONTAMINANTS IN FOODS

Christopher T. Elliott[1*], Hanspeter Naegeli[2], Julie P. Meneely[3], Katrina Campbell[4], Mark Mooney[5]

[1 3 4 5] Institute of Agri-food and Land Use, School of Biological Sciences, Queen's University of Belfast, David Keir Building, Stranmillis Road, Belfast BT9 5AG
[2] UNIVERSITÄT ZÜRICH, Institut für Veterinärphar-makologie und –toxikologie, Switzerland
[*] Corresponding author–E-mail: Chris.Elliott@qub.ac.uk; Phone: +442890976549; Fax: +442890976513

Chemical contaminant monitoring in foodstuffs is a highly important and complex issue. A huge investment in time and effort is placed on these activities by regulatory and industrial laboratories. As fresh demands from consumers and regulators grow to improve the quality and safety of food the need for improved technologies has never been greater.

The range of contaminants that have to be dealt with are enormous, compounds such as drugs, toxins, heavy metals and pesticides are only a selection of these. The detrimental effects each of these have on the consumer is still a subject of great debate and opinions differ widely. Some believe that minute traces of chemicals in foods are an irrelevance to consumer protection while others believe that the problems are not fully understood and may have a major impact on health related problems.

The presentation will give an overview of the research performed as part of a large EC funded project Biocop. Particular reference will be made to the development and validation of methods which detect the biological effect of the contaminants rather than measuring the contaminants themselves. A perspective on how such innovative methods can be used to provider safer food for the European consumer will be given.

Keywords: Chemical, Contaminants, screening, Biocop

FP6 Food Safety Programme

L-23

DEVELOPMENT, VALIDATION AND APPLICATION OF HIGH THROUGH PUT BIOASSAY SCREENING METHODS: THE ADDED VALUE

Toine Bovee[1*], Jeroen Rijk[2], Ron Hoogenboom[3], Michel Nielen[4]

[1 2 3 4] RIKILT–Institute of Food Safety, Wageningen University and Research Centre, Wageningen, the Netherlands
[*] Corresponding author–E-mail: toine.bovee@wur.nl; Phone: +31-317-480391; Fax: +31-317-417717

Yeast cells were constructed that either express the human estrogen receptor α or the human androgen receptor in combination with a consensus ERE or ARE repeat in the promoter region of a green fluorescent protein (yEGFP) read-out system. These bioassays were proven to be highly specific for their cognate agonistic compounds, were fully validated according to EC decision 2002/657 for calf urine and animal feed, and an inter-laboratory ringtest was performed with the yeast estrogen bioassay for screening estrogenic activity in calf urine.

In addition we will show the value of these yeast bioassays for analysing compounds with antagonistic properties as well. Many of these compounds will find their use in therapeutic treatments and might end up in the food chain by waste incidents. However, some of them will also have a high potential for misuse in veterinary practice and the sporting world.

The added value of bioassay screening is demonstrated by screening eighteen different dietary supplements, already analysed by a liquid chromatography tandem mass spectrometry method (LC-MS/MS) for the presence of anabolic steroids, for androgenic activity. Eleven samples containing at least one anabolic steroid according to LC-MS/MS, were also positive in the bioassay. Seven samples did not contain any of the 49 compounds screened for in LC-MS/MS, but in contrast two of them were positive in the bioassay. Bioassay-directed identification, using the bioassay as an off-line LC-detector and LC-time of flight-MS with accurate mass measurement was carried out in these two samples and revealed the presence of 4-androstene-3β,17β-diol and 5α-androstane-3β,17β-diol in the first and 1-testosterone in the second supplement, showing the added value of the bioassay in comparison with a LC-MS/MS screening method alone. We also report our findings with a 60-year old man who was surgically treated for gynaecomastia. It is shown that the breast growth in this male was most probably caused by an orally taken 'natural' herbal supplement, marketed on the internet for prostate problems. The supplement showed a strong effect in a yeast estrogen bioassay, indicating the possible presence of huge amounts of an estrogenic compound(s). Using LC/TOFMS, the responsible synthetic compound was identified. This case once more demonstrates the potential of bioassay screening and that physicians need to be aware of the use of supplements with illegal components that may be responsible for unwanted side-effects.

Keywords: supplements; screening; applications

L-24

CHALLENGES IN THE DEVELOPMENT OF LATERAL FLOW DEVICES FOR THE DETECTION OF ALLERGENIC FOOD CONTAMINANTS USING RABBIT-IGG

Judith Rudolf[1*], Parisa Ansari[2], Christoph Hasenhindl[3], Sabine Baumgartner[4]

[1 2 3 4] University of Natural Resources and Applied Life Sciences, Vienna, Austria
[*] Corresponding author–E-mail: judith.rudolf@boku.ac.at; Phone: +43-2272-66280-456; Fax: +43-2272-66280-403

Lateral Flow Devices are immunochromatographic strip tests for the rapid detection of specific analytes in complex matrices. The most famous application is the pregnancy test. Lateral Flow Devices (LFDs) exist for the detection of microbial analytes, different toxins, antibiotics, and food allergens.

The principle of LFDs is based on the combination of immunological antibody-antigen reaction, similar to ELISA, and the migration of a fluid on a membrane, similar to thin layer chromatography. For immediate read-out of the result, applied antibodies have to be labelled with coloured particles.

In this project LFDs were developed for the rapid onsite screening for allergenic contaminants during food production. Great impact has to be laid on the development of such tests in order to provide correct and reliable results. Tests were developed using the sandwich format. A capture antibody is applied onto a chromatographic membrane, labelled detection antibody and sample are mixed and the test strip is incubated for a few minutes. Presence of the target analyte in the sample leads to formation of an antibody-antigen sandwich indicated by a coloured test line on the membrane.

In the presented work rabbit-IgGs were used, which were produced against protein extracts of potentially allergenic food such as peanut, hazelnut, and egg white. Using polyclonal rabbit antibodies in immunodiagnostic tests offers the advantages of rapid availability of antiserum, high yield, and usually high specificity and sensitivity for the antigen. Colloidal gold was chosen for labelling the antibodies. The quality of the antibody-gold conjugate is essential for the performance of the test as instable conjugates may lead to false positive or false negative results. Success of the conjugation procedure is dependent on various factors, such as purification of IgG from serum, quality of colloidal gold sol, determination of the optimum coupling ratio, pH value, buffer system, incubation time, and even tube material. An overview is given about the challenges during development of Lateral Flow Devices for the detection of potentially allergenic peanut proteins.

Keywords: LFD, rabbit-IgG, colloidal gold

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L-25

A NOVEL PLATFORM FOR RAPID DETECTION OF FOOD BORNE PATHOGENS INTEGRATING BIO- AND NANOTECHNOLOGY

Jörg Schemberg[1*], Andreas Grodrian[2], Gunter Gastrock[3], Phillip Weber[4], Juliane Steingröwer[5], Elke Boschke[6], Karen Lemke[7]

[1 2 3 7] Institute for Bioprocessing and Analytical Measurement Techniques
[4 5 6] Technische Universität Dresden, Institute of Food and Biochemical Engineering
[*] Corresponding author–E-mail: joerg.schemberg@iba-heiligenstadt.de; Phone: +493606671162; Fax: +493606671200

The presence of pathogenic bacteria (e. g. *Staphylococcus aureus*, *Campylobacter* spp. or *Salmonella* spp.) in foods currently presents the most significant problem internationally. Therefore rapid methods and cost-effective detection platforms are needed to generate authoritative results for quality assurance preferably within 8 hours. The major drawback not only of modern immunological and molecular based methods is the time consuming pre-enrichment. It can be shortened by the use of a biomagnetic separation step in order to separate targets from samples. Afterwards they can be resolved in a smaller volume, stained or labeled and analysed in a microfluidic system with an integrated fluorescence detector.

In the presented research project, food samples were pre-treated and contaminants captured by superparamagnetic beads functionalized with specific ligands, e. g. short peptides selected from a phage-displayed library or aptamers – short DNA sequences screened during Systematic Evolution by EXponential Enrichment (SELEX).

After incubation and subsequent isolation of magnetic bead-target cell-complexes (MTCs) by means of a magnetic field and rejection of unbound sample components, MTCs were re-suspended and prepared for subsequent coupling with fluorescent microcapsules which were functionalized with the same ligands as mentioned above and able to couple with MTCs to form MT-microcapsule-complexes (MTMCs).

Next, to generate droplets in nL-scale with at most one MTMC per compartment, microfluidic tools like glass- and PDMS-chips or a two-fluid probe (ZFT) were integrated into one platform. The differences in the channel geometry, the handling and production regime as well as the advantages and disadvantages of these droplet-based components will be presented.

Additionally, these modules were included into an automatic spectroscopic or microscopic system respectively. Thereby detection was based on the measurement of the fluorescent signals. In this way the stability of the MTMCs during and after the generation of droplets could be tested. Furthermore signals from microscope are suitable for validation of aimed spectroscopic detection as well as for quality control during routine analyses. In this context, spectroscopic detection and quantification system is based only on a yes- or no-decision. Hence a cost-effective prototype for spectroscopic detection is available for many potential applications.

Keywords: Pathogens, biomagnetic separation, microfluidic tools

Project was founded by BMWi–AIF/ZUTECH (217 ZBR)

L-26

A FLOW CYTOMETRY-BASED IMMUNOASSAY FOR POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN FOODS

Anastasia Meimaridou[1*], Willem Haasnoot[2], Linda Noteboom[3], Mirjam van Aalderen[4], Monique E. Bienenmann-Ploum[5], Michel W.F. Nielen[6]

[1 2 3 4 5] RIKILT– Institute of Food Safety, Wageningen UR, P.O. Box 230 Wageningen, the Netherlands
[6] RIKILT– Institute of Food Safety, Wageningen UR, P.O. Box 230 Wageningen, the Netherlands*Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, the Netherlands
[*] Corresponding author–E-mail: anastasia.meimaridou@wur.nl; Phone: +31(0)317480233; Fax: +31(0)317 417717

Polycyclic aromatic hydrocarbons (PAHs) are environmental/processing contaminants with carcinogenic and/or mutagenic potency. For the general population, the major routes of exposure are from food and inhaled air. Benzo[a]pyrene (B[a]P) is one of the most potent PAHs with a maximum level of 1 ppb wet weight for cereal food and 5 ppb for smoked fish [1]. The chromatography- or radioimmunoassay-based detection methods for PAH's are accurate and quite sensitive but they are time-consuming, laborious or expensive. Thus, there is a need for validated screening tools, which are simple, inexpensive, rapid, and can detect several priority PAHs simultaneously and, ideally, that can be combined with other screening assays for food contaminants in the near future.

The objectives within our project are the development and application of an immunoassay for PAHs in food using a flow cytometry-based multiplex technology (Luminex™). This technology involves 56 different colour-coded magnetic microspheres (6.2 μm). Each microsphere can be coated with a particular biological probe, allowing the capture and detection of specific analytes. Within the Luminex™ analyzer, lasers excite the internal dyes that identify each microsphere and also reporter dye captured on the microspheres during the assay. With this combination it is possible to simultaneously measure up to 56 different biomolecular reactions in a single well, which is an advantage, compared to ELISA (Enzyme-Linked ImmunoSorbent Assay).

So far, we developed a flow cytometry-based immunoassay to detect B[a]P in buffer with an IC_{50} value of 0.3 ±0.1 ppb, which is comparable to the IC_{50} of 0.2 ppb obtained by Matschulat *et al*. [2] using the same biochemicals. The sensitivity of the assay was tested for more than 20 other PAHs. In addition to that, the assay was shown to be effective in food matrix such as smoked carp extract.

[1] Regulation (EC)No.1881/2006
[2] Matschulat et al.,2005. Development of a highly sensitive monoclonal antibody based ELISA for detection of benzo[a]pyrene in potable water. The Analyst, 130,1078-1086

Keywords: PAHs, Flow cytometry

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L-27
FOOD PROCESSING CONTAMINANTS: MODERN ANALYTICAL STRATEGIES

Richard Stadler[1*]

[1] Nestlé Product Technology Centre, CH-1350 Orbe, Switzerland
[*] Corresponding author–E-mail: richard.stadler@rdor.nestle.com; Phone: 079 3480103; Fax: +41 24 442 2021

Rapid analytical developments particularly over the past decade are clearly evident in the area of food-borne chemicals that are considered as ‘‘undesired’’ in foods. Such chemicals are part of our daily diets, and the very low detection limits of the analytical methods enable their quantification in many different foods at the low part-per-billion (ppb) level. Research in terms of method development for this group of substances has in the past years particularly focused on acrylamide, furan, and MCPD esters and related compounds.

In the case of acrylamide, several excellent reviews have been published on the different methods

employed for acrylamide determination in foodstuffs. The analytical procedure broadly speaking encompasses (*i*) extraction (*ii*) clean-up and (*iii*) the instrumental technique. In all method developments, the extraction and clean-up steps are optimized/adapted to the corresponding instrumental tool, to which the protocol invariably refers. LC-MS/MS is considered to be the more appropriate instrument for that purpose as it is adapted for hydro-soluble analytes whilst achieving the adequate performance. The use of a mass detector is a major advantage in terms of analyte confirmation and quantitation when using stable isotope-labelled internal standards. However, the relatively high cost of this instrument may explain why the use of ‘‘cheaper’’ GC-based methods are also frequently described.

Several methods for the determination of chloropropanols at trace amounts in foodstuffs such as acid-HVP, soy sauces and related products, as well as processed foods have been published in the literature. The absence of suitable chromophores has made approaches based on GC the methods of choice, and in the earlier stages of method development the native compound was determined without derivatization using an MS detector or electrolytic conductivity detection. More recent GC-MS methods have been adapted to afford stable volatile derivatives that can be readily characterized by selective MS detection. In addition, the commercial availability of stable isotope-labelled 3-MCPD has contributed significantly to the reliability of the data. Recently, the issue of MCPD esters in refined and deodorised vegetable oils have raised concern. To date, there are only a few methods reported for the analysis of MCPD esters and the isolation and measurement of all chloroesters is a lengthy process due to the many species arising from the different fatty acid combinations associated with each chloropropanol moiety. The quantification and ratio of 3-MCPD mono to -diesters are important to assess the contribution of foods to the bioavailability of 3-MCPD. Further challenges are the quantification of glycidol esters that may also be formed during the analytical work-up when using certain methods.

However, the rapid pace of research in this field will continue, and more compounds with potential health concerns in foods will be discovered albeit at very low amounts. Consequently, there is an urgent need of reliable mechanisms whereby the compounds can be prioritized based upon the margin of safety (effect/exposure relationship), as well as future guidance toward the toxicological evaluation of food within a holistic, i.e., avoid testing individual compounds but rather the complete foods.

Keywords: Process contaminants, MCPD, acrylamide, furan

L-28
ANALYSIS OF ACRYLAMIDE ADDUCTS IN FOOD

Michael Granvogl[1], Peter Koehler[2], Peter Schieberle[3*]

[1] Technical University of Munich, Garching, Germany
[2] German Research Center for Food Chemistry, Garching, Germany
[3] Technical University of Munich and German Research Center for Food Chemistry, Garching, Germany
[*] Corresponding author–E-mail: peter.schieberle@lrz.tum.de; Phone: +49 89 289 13264; Fax: +49 89 289 14183

After acrylamide was found in different kinds of processed foods in 2002, a lot of efforts have been undertaken to minimize the formation of this "food-borne" toxicant by industry and academic research centers. But, up to now, only little is known about reaction products of acrylamide in food. Recently, our group has proven the formation of the corresponding epoxide, the so-called glycidamide, which is seen as the main toxic compound in the acrylamide problem by many toxicologists. Glycidamide was found in potato chips as well as in French fries, especially if prepared in fats and oils containing triglycerides with unsaturated fatty acids.

Beside this epoxidation to glycidamide in the presence of fatty acid hydroperoxides, a reaction of acrylamide in food during processing with nucleophiles, e.g. thiols and primary amines, leading to stable thioethers or secondary amines formation, is also thinkable. However, up to the present, no quantitation methods of these acrylamide adducts have been published and no data on their occurrence in food are available.

Therefore, the aim of the present study was to develop new quantitation methods on the basis of stable isotope dilution assays (SIDA) using synthesized isotopically labeled [$^{13}C_3$]-cysteine-acrylamide and [$^{13}C_3$]-glutathione-acrylamide, respectively, as internal standards. To increase the sensitivity as well as the selectivity, a derivatization procedure using dansyl chloride was additionally established.

Application of the newly developed SIDAs on model systems (wheat flour) as well as on several food samples proved the presence of cysteine and glutathione acrylamide adducts in different processed foods for the first time. In the model systems, a clear dependance on the temperature as well as on the heating time was analyzed. The varying amounts found in food were also dependent on the processing conditions as well as on the food itself.

Keywords: acrylamide, cysteine, glutathione, SIDA, food-borne-toxicant

L-29

SPECIATION ANALYSIS OF TRACE ELEMENTS–RECENT AND FUTURE TRENDS IN FOOD ANALYSIS

Jens J. Sloth[1*], Rikke V. Hedegaard[2], Kaare Julshamn[3], Erik H. Larsen[4]

[1 2 4]DTU Food, Soborg, Denmark
[3] NIFES, Bergen, Norway
[*] Corresponding author–E-mail: jjsl@food.dtu.dk; Phone: +45 35887625

Presently, European legislation on trace elements concerning food safety is mainly based on total element concentrations expressed as maximum levels. So far only four elements are included in the regulation; cadmium, lead, mercury and tin. However, information on the total content of an element does not always provide adequate information for evaluation of e.g. bioavailability and toxicity. These parameters may vary quite significantly depending on how the element is bound, i.e. its speciation, defined as the distribution of an element amongst defined chemical species in a system. The most important practical application of elemental speciation is in the area of toxicology. With the help of more detailed toxicological knowledge on the individual chemical elemental species should lead to more specific legislation.

Arsenic is an illustrative example, where inorganic arsenic is much more toxic than organic bound and analytical methods for selective determination of inorganic arsenic is needed in order to perform a correct risk assessment of exposure.

The lecture will use this and other examples and focus on the present situation for speciation analysis in the food and feed area and discuss the latest and expected future developments within this emerging scientific area.

Keywords: trace elements, speciation, arsenic, legislation

L-30
FOOD METABOLOMICS: FACT OR FICTION?

Leon Coulier[1*], William van Dongen[2], Renger Jellema[3]

[1 2 3] TNO Quality of Life, Zeist, the Netherlands
[*] Corresponding author–E-mail: leon.coulier@tno.nl; Phone: +31 30 694 48 88

A metabolomics approach can be very useful to solve issues that are biologically complex as is the case in the agricultural and food industry. Food metabolomics includes the development of (a combination of) analytical methods which are able to describe and predict properties of food products and processes. The ultimate goal is to identifying so-called quality markers, i.e. (sets of) metabolites that correlate with e.g. quality, safety, taste or fragrance of foodstuffs. In turn, these metabolites are influenced by factors as genetic differences of the raw food ingredients (such as crop species differences), growth conditions (such as climate, irrigation strategy, or soil type) or production conditions (such as temperature, acidity, or pressure). In cases where the routine-based measurement of a food property is unpractical, monitoring based on a limited set of crucial metabolites is a good alternative. For example, metabolites can be used for monitoring food sensory properties which is much easier than using a sensory expert panel, to deduce biological processes that are yet unknown, or to shorten the pipeline in breeding strategies.

All of these factors can play an important role in the food production chain. Modern food production approaches require a good strategy to tune the food chain factors to optimization of the quality properties. Moreover the combination of analytical methods in combination with in-vitro/in-vivo effect measurements can be applied to discover health promoting compounds in foodstuffs or ingredients.

This presentation will show the current status of food metabolomics as demonstrated by a number of practical examples. Both possibilities and current restrictions of this recent trend technology are presented and discussed.

Keywords: food metabolomics; quality; advanced analysis

L-31
CHALLENGES IN FLAVOURS ANALYSIS

Henryk Jelen[1*]

[1] Poznan University of Life Sciences, Poznan, Poland
[*] Corresponding author–E-mail: henrykj@up.poznan.pl; Phone: +48-61-8487273; Fax: +48-61-8487314

Flavour plays crucial role in consumers' acceptance of food. Compounds responsible for aroma of food products are present in a mixture of often hundreds and more volatiles, varying in their chemical character, concentration and odour thresholds. They usually interact with food matrix constituents, and technological processes and storage influence their profile and amounts. Moreover, analytical instruments have to compete with human nose in terms of detection limits of key food odorants.

Analysis of food flavor compounds combines several main areas: *i)* determination of key odorants and tastants, *ii)* real-time analysis of aroma compounds during mastication or tissue disruption, iii) use of volatile compounds profiles (usually with and aid of chemometric methods) to monitor changes in products and to determine their authenticity or traceability, *iv)* investigation of bound flavor compounds, that are released during flavor formation or can be released for flavor improvement.

In the lecture tools and techniques involved in the mentioned above analytical areas will be summarized with examples. Challenges in sampling for flavor compounds will be discussed. Special emphasis will be put on sensory-oriented determination of crucial key odorants in food products using gas chromatography (GC-O) and issues related to their quantitation. Also benefits of comprehensive gas chromatography (GC×GC) and advances in mass spectrometry in resolving complex mixtures of flavor compounds will be discussed. Various methods involving volatile/flavor compounds profiling and their application in the oil rancidity and authenticity testing will be presented. Finally, potential of the bound flavor compounds and methods used for their analysis will be discussed based on wine terpenes examples.

Keywords: flavour, aroma, gas chromatography, GC-O

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L-32
AROMA ANALYSIS BY PROTON TRANSFER REACTION–MASS SPECTROMETRY (PTR-MS). AN OVERVIEW

Katja Buhr[1*], Peter Schieberle[2]

[1] German Research Centre for Food Chemistry, Garching, Germany
[2] German Research Centre for Food Chemistry and Chair for Food Chemistry, Technical University of Munich, Garching, Germany
[*] Corresponding author–E-mail: katja.buhr@lrz.tum.de; Phone: 0049-(0)89-289-13270; Fax: 0049 -(0)89-289-14183

Proton Transfer Reaction–Mass Spectrometry was first introduced by Lindinger et al. in 1995 [1] and represents a direct mass spectrometric technique which implements hydronium ions for chemical ionisation of volatile analytes. As any previous isolation, concentration and chromatographic separation steps are skipped, discrimination and identification capabilities of the analytical technique are sacrificed in favour of speed and therefore allowing for temporal resolution of monitored mass traces. In the meantime, numerous applications of the analytical technique for environmental, medical and food analysis have been published. A typical application in the area of food and flavour analysis uses a PTR-MS spectrum obtained from the headspace of a food sample as a characteristic fingerprint without further identification of the different ion traces. Statistical analysis of the obtained data prove the excellent discrimination capacities of this electronic nose-type of application for food authenticity and prediction of sensory attributes [2,3]. Own applications of the analytical technique imply investigation of aroma-matrix interaction as well as aroma release from different food systems during consumption. Therefore, breath analysis is usually performed on a representative number of panelists during consumption of a flavoured food or model system. For such kind of applications intra- and interpersonal variations in individual aroma release patterns present a challenge for adequate data treatment and interpretation while at the same time it opens the opportunity to link individual sensory perception with actual breath concentrations in individual subjects [4]. A third application of the analytical technique lies in the analysis of human breath for disease markers or breath malodours such as allyl methyl sulfide in garlic breath. In a recent study [5] an effective method for garlic breath sampling was developed for monitoring allyl methyl sulfide and its elimination profiles from human breath upon garlic consumption.

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Keywords: PTR-MS, aroma analysis, breath analysis

L-33

QUANTITATIVE ANALYSIS OF PROTEIN IN YOGURT BY ATR-FTIR SPECTROSCOPY AND CHEMOMETRICS

Amir Bagheri Garmarudi[1*], Mohammadreza Khanmohammadi[2], Keyvan Ghasemi[3], Salvador Garrigues[4], Miguel de la Guardia[5]

[1] Chemistry Department, Faculty of Science, IKIU, Qazvin, Iran–Department of Chemistry & Polymer Laboratories, Engineering Research Institute, Tehran, Iran
[2 3] Chemistry Department, Faculty of Science, IKIU, Qazvin, Iran
[4 5] Analytical Chemistry Department, Universitat de Valencia, Jeròni Muñoz building, C/ Dr. Moliner, 50, 46100 Burjassot, Valencia, Spain
[*] Corresponding author–E-mail: a.bagheri@ikiu.ac.ir; Phone: +98 21 66282279; Fax: +98 21 66282779

The main researches in field of yogurt nutritional parameters by mean of Fourier transform infrared (FTIR) spectroscopy, are related to the determination of cholesterol and sorghum fermentation control. One of the most important ingredients of yogurt is protein. Analytical methods for determination of protein in food products are generally based on Kjeldahl and Lowry methods or related modified procedures which utilize spectrophotometry. Reversed-Phase HPLC, capillary electrophoresis and diffuse reflectance infrared Fourier- transform spectrometry (DRIFTS) are some other techniques. There are many other researches dealing with determination of protein content of different food products e.g. biuret reaction, 4th derivative UV spectrophotometry and Near-Infrared Reflectance spectroscopy. In this research a method has been introduced for quantitative determination of protein content in yogurt samples by mid-FTIR spectroscopy and chemometrics. The main signals in the mid-IR region are in 1800–1500 cm^{-1}. There is a band at about 1750 cm^{-1} which is associated with the C=O stretching of proteins. On the other hand, C=O stretching band of amide I and N–H bending of amide II are both located in this spectral region. Successive Projection Algorithm (SPA) wavelength selection procedure, coupled with feed forward Back-Propagation Artificial Neural Network (BP-ANN) model was the benefited chemometric technique. The main purpose of this algorithm is to select wavelengths which their information content is minimally redundant, in order to solve the collinearity problems. The choice of wavelengths for model building using SPA is critical if the model is to have good future predictive ability. After outlier detection, an important action is to select appropriate calibration or validation data set with a minimum error in model prediction. A methodology for this procedure is hierarchical cluster analysis (HCA). In HCA, the similarity between samples is established using the concept of a “distance” (calculated using a mathematical relationship; i.e., the Euclidian norm) between samples which are related to how similar the numerical properties of the samples are (e.g. the absorbance at different wavelengths). ANN is typically organized in layers where these layers are made up by a number of interconnected nodes which contain an activation function. Input vectors are presented to the network via the input layer which communicates to one or more “hidden layers” where the actual processing is done via a system of weighted “connections”. Most ANNs contain some form of “learning rule” which modifies the weights of the connections according to input patterns that it is presented with. Relative Error of Prediction (REP) in BP-ANN and SPA-BP-ANN methods for training set was 7.25 and 3.70 respectively. Considering the complexity of the sample, the ANN model was found to be reliable, while the proposed method is rapid and simple, without any sample preparation step.

Keywords: yogurt, ATR-FTIR, ANN, quantitative

L-34

DEVELOPMENT OF HIGH THROUGHPUT APPROACHES TO OPTIMISE THE NUTRITIONAL VALUE OF CROPS AND CROP-BASED FOODS– DEVELONUTRI

Sean Conner[1*], Derek Stewart[2]

[1 2] Scottish Crop Research Institute, Dundee, DD2 5DA, Scotland
[*] Corresponding author–E-mail: sean.conner@scri.ac.uk; Phone: 01382 562731

Plant based foods are a rich source of nutrients, minerals and beneficial bioactive components. The levels of these components cover broad analytical ranges (mg/g – pg/g) and therefore present unique challenges for simultaneous analysis. Highly sensitive methods for their quantification are available, but not yet routine. These methods can help determine the absolute levels of a wide range of components as well as assessing which treatments, before and after harvesting, impact upon the post harvest nutritive value of plant-based foods.

The aim of DEVELONUTRI, an EU funded project, is the development and validation of state-of-the-art analytical techniques for rapid quantification of micronutrients. Three widely consumed crops (potato, wheat and tomato) and their production, processing and transportation chains have been the focus for this project. Within the project both traditional and GM varieties have been analysed. The project aims to cross compare standard analytical approaches with the metbolomics (LC-MS^n & GC-ToF-MS^n) and emergent technologies, such as MALDI-ToF-MS, FT-MS, LC-NMR etc, to establish what level of detail can be obtained with respect to food analysis and to determine what advantages the more advanced analytical approaches can bring to food compositional and nutrient database construction.

Ring tests using the majority of these technologies highlighted some interesting points with many sources of variation established. International ring testing of the MS-based analytical (metabolomic) approaches, using centrally prepared (± nutrient spike) representative crop samples have produced encouraging results and have shown them to be accurate, robust and quantitative approaches to high-throughput food compositional technologies. Furthermore these technologies have been applied to biological material grown under normal conditions, varying agricultural practices and on samples derived from collaborating SME processing lines.

The use of emergent technologies for instance the high accuracy systems (LC-ICR-FTMS) were shown to be extremely valuable in a food scenario due to their ability to generate molecular formulae for all compounds separated by chromatography and deconvolution. However the scan times required to ensure this molecular accuracy were significant meaning that LC-ICR-FTMS could not operate as a high throughput technology. Conversely, the comparison with the LC-FT-MS systems, in particular the Orbitrap, showed that these systems were high throughput, and that, via the generation of (deconvoluted) peak molecular and MS^n fragmentation ions, they were able to identify and characterise known and unknown compounds. The utility of these and other analytical approaches with respect to food nutritive analysis will be discussed.

Keywords: metabolomics, potato, wheat, tomato, DEVELONUTRI

L-36
APTAMERS FOR THE FOOD ANALYSIS

Marco Mascini[1*]

[1] University of Firenze
[*] Corresponding author–E-mail: marco.mascini@unifi.it; Phone: +390554573283; Fax: +390554573384

So far, several bio-analytical methods have used nucleic acid probes to detect specific sequences in RNA or DNA targets through hybridisation. More recently, specific nucleic acids, aptamers, selected from random sequence pools, have been shown to bind non-nucleic acid targets, such as small molecules or proteins. The development of *in vitro* selection and amplification techniques has allowed the identification of specific aptamers, which bind to the target molecules with high affinity. Many small organic molecules with molecular weights from 100 to 10000 Da have been shown to be good targets for selection. Moreover, aptamers can be selected against difficult target haptens, such as toxins or prions. The selected aptamers can bind to their targets with high affinity and even discriminate between closely related targets.

Aptamers can thus be considered as a valid alternative to antibodies or other bio-mimetic receptors, for the development of biosensors and other analytical methods. The production of aptamers is commonly performed by the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) process, which, starting from large libraries of oligonucleotides, allows the isolation of large amounts of functional nucleic acids by an iterative process of in vitro selection and subsequent amplification through polymerase chain reaction.

Aptamers are suitable for applications based on molecular recognition as analytical, diagnostic and therapeutic tools. In this review, the main analytical methods which have been developed using aptamers, will be discussed together with an overview on the aptamer selection process.

Keywords: aptamers, biosensors, analytical electrochemistry

L-37

MODERN LC-MS METHODS FOR THE DETERMINATION OF NATURAL TOXINS AND THEIR METABOLITES

Rudolf Krska[1*], Michael Sulyok[2], Franz Berthiller[3], Rainer Schuhmacher[4], Colin Crews[5]

[1 2 3 4] Department for Agrobiotechnology (IFA Tulln), BOKU-University of Natural Resources and Applied Life Sciences Vienna, Konrad Lorenz Strasse 20, 3430 Tulln, Austria
[5] The Food and Environment Research Agency (FERA), Sand Hutton, York, YO41 1LZ, United Kingdom
[*] Corresponding author–E-mail: rudolf.krska@boku.ac.at; Phone: +436765598011

This paper describes the development of modern LC-MS based analytical methods for the study of selected natural toxins and their metabolites in plants and foods. This includes the determination of multiple mycotoxins and the detection and identification of pyrrolizidine alkaloids in ragwort.

A previously published multi-mycotoxin method for the determination of mycotoxins in food and feed matrices based on liquid chromatography / electrospray ionization tandem mass spectrometry (HPLC/ESI-MS/MS) has been extended by 99 fungal and bacterial metabolites to cover 186 compounds overall. The method is based on a single extraction step using an acidified acetonitrile/water mixture followed by analysis of the diluted crude extract. 87 moldy food samples from private households were analysed, including bread, fruits, vegetables, cheeses, nuts and jam. In the 247 investigated sub-samples, 49 different analytes were identified, some of which were never reported before to occur in naturally contaminated food. Enniatins and ergot alkaloids occurred in all samples of (dark) bread/pastries at low µg/kg-levels. From the remaining analytes, chanoclavine, emodin, mycophenolic acid and roquefortine C were found most frequently.

In our attempts to understand the interaction between fungi and plants we have been carrying out metabolite profiling using LTQ-Orbitrap MS. The combination of the extraordinary resolution of the MS instrument (up to 100.000) and high mass accuracy (usually around 1ppm) with specific database searches (facilitates to generate the sum formulae and to assign (tentative) structure formulae to the detected metabolites.

A new time-of-flight mass spectrometric detection method (LC-TOFMS) for determining pyrrolizidine alkaloids (PA) in ragwort will also be presented. A database covering 176 PA was generated and was used to screen for this plant toxins which can also be found in honey. Experiments have demonstrated the rapid decomposition of the toxins in ragwort stored in bags.

Keywords: LC-MS/MS, LC-TOFMS, mycotoxin, pyrrolizidine alkaloids

L-38

SUPRAMOLECULAR SOLVENT MICROEXTRACTION OF MYCOTOXINS IN FOOD PRIOR TO ELISA DETERMINATION

Sergio García-Fonseca[1], Ana Ballesteros-Gómez[2], Soledad Rubio[3*]

[1 2 3] University of Córdoba, Córdoba, Spain
[*] Corresponding author–E-mail: qa1rubrs@uco.es; Phone: 34-957218644; Fax: 34-957218644

Many foods and feeds become contaminated with mycotoxins during the growing crop, storage or processing. Among mycotoxins, the carcinogenic Aflatoxins and Ochratoxin A (OTA) are by far the most commonly found food/feed contaminants in the European internal market and border control (e.g. they caused ~1 of each 4 notifications in The Rapid Alert System for Food and Feed, RASFF, during 2007). European Union regulations have set maximum levels for both Aflatoxins (0.025–15 µg/kg) and OTA (0.5–10 µg/kg).

Among rapid methods for mycotoxin analysis, enzyme-linked immunosorbent assays (ELISA), have become routinely used in food/feed industry and for checking legal compliance. Advantages associated to ELISA are speed, easy of operation, sensitivity and high sample throughput. Major drawbacks are the high volume of organic solvents required for sample extraction (usually 25–100 ml per sample) and the well-known *matrix effect,* which frequently causes overestimation. The use of immunoaffinity columns improves selectivity, however, they are expensive, not recyclable, have a limited storage time and are not applicable to matrices like spices.

This research proposes the use of supramolecular solvents for the microextraction of aflatoxins and OTA in a variety of high matrix effect raw and processed foods in order to simplify and reduce costs in sample treatment prior to ELISA quantitation. Supramolecular solvents are water-immiscible liquids made up of large surfactant aggregates. They spontaneously form in micellar or vesicular aqueous or hydro-organic solutions by the action of an external stimulus (e.g. temperature, electrolyte, pH, solvent), which induces the formation of larger aggregates, often keeping the morphology, and causes their separation from the bulk solution.

Micoextraction of OTA in a variety of Spanish wines (white, red and sweet) and spices (paprika, black pepper and nutmeg) and aflatoxin B_1 in different wheat varieties (triticum aestivum and durum, tritordeum, and triticale) was investigated with the use of supramolecular solvents made up of reverse micelles of tetradecanoic acid. A general procedure involving the extraction with 650–800 µL of supramolecular solvent and back extraction of the mycotoxins in 1 mL of phosphate buffer permitted the quantitative recovery of OTA and AFB_1 and their exact quantitation using commercial ELISA test kits. Validation was carried out using certified reference materials. This innovative approach is a valuable and widely applicable strategy for sample treatment of high-matrix effect commodities prior to ELISA quantitation of mycotoxins.

Keywords: microextraction, supramolecular solvent, mycotoxins, food

L-39
RAPID BIOTOXIN SCREENING IN SHELLFISH BY ULTRAHIGH RESOLUTION MASS SPECTROMETRY

Jeremy Melanson[1*], Pearl Blay[2], James Chang[3]

[1 2] National Research Council of Canada, Institute for Marine Biosciences, Halifax, Canada
[3] Thermo Scientific, San Jose, USA
[*] Corresponding author–E-mail: jeremy.melanson@nrc-cnrc.gc.ca; Phone: +1 902 426 6357

Over the last several years, liquid chromatography – mass spectrometry (LC-MS) has developed into an accurate and reliable technique for monitoring marine algal biotoxins. In general, LC-MS analysis of biotoxins is performed in selected reaction monitoring (SRM) mode on a triple-quadrupole MS, whereby the first quadrupole is programmed to transmit a selected mass for fragmentation and the final quadrupole transmits a single fragment ion to the detector. For monitoring multiple analytes simultaneously, the quadruples can rapidly toggle between pre-defined masses in multiple reaction monitoring (MRM) mode. This approach is well suited for quantification due to its inherent selectivity and high sensitivity. However, the relatively low resolution of quadrupoles (typically unit resolution) renders the technique prone to interference from ions of similar mass in complex samples. In addition, due to the targeted nature of MRM, only known toxins specified in the method will be detected. Therefore, new or modified biotoxins could remain undetected indefinitely, even at high abundance.

This presentation will describe the high-resolution mass spectrometry analysis of various toxin classes commonly found in shellfish, including domoic acid, dinophysistoxins, pectenotoxins, azaspiracids, and spirolides. LC-MS data was acquired over a broad mass range with resolution up to 100,000 with high mass accuracy (< 2 ppm error). This non-targeted approach provides high-resolution data over the entire chromatographic separation, allowing detection of new or unknown compounds in addition to those of interest. Furthermore, the method requires little method development, as settings are not tuned (ie. collision energies) for individual analytes. The method was optimized using a standard mixture of marine biotoxins, and then applied to various shellfish tissue extracts. Analytical performance parameters will be described and the method's ability to detect unknown or modified biotoxins will be demonstrated.

Keywords: LC-MS, marine toxins, non-targeted

L-40

DEVELOPMENT OF A STABLE ISOTOPE DILUTION ASSAY FOR THE QUANTIFICATION OF IMPORTANT BIOGENIC AMINES IN FOOD

Christine Mayr[1], Peter Schieberle[2*]

[1 2] German Research Center for Food Chemistry, Munich, Germany
[*] Corresponding author–E-mail: christine.mayr@lrz.tum.de; Phone: 0049-89-28914181

Biogenic amines are present in a wide variety of food products, including fish, meat, dairy products, wine, beer, vegetables, fruits, nuts, and chocolate. Especially in fermented food like cheese, wine, and sausages, biogenic amines occur in a high concentration. The presence of BA in food constitutes a public health concern due to their physiological and toxicological effects. Some aromatic amines (tyramine, tryptamine and 2-phenylethylamin) have a vasoconstrictor effect and can cause hypertensive crises or dietary-induced migraine. The most frequent food borne intoxication caused by biogenic amines involves histamine which is referred to as `scromboid fish poisoning´.

Biogenic amines can be formed by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. As the microbial spoilage of food may be accompanied by the increased production of decarboxylases, the presence of biogenic amines might serve as a useful indicator of food spoilage.

In the lecture a new LC/MS-MS method for the quantification of biogenic amines in food will be introduced, which is based on a derivatization of the amines with benzoyl chloride followed by an RP-LC separation and tandem-mass-spectrometric detection which reveals superior selectivity and precision. Stabile isotopes are used as internal standard to achieve high reproducibility. The limits of detection were 0.05 µg/kg. This method allows an unambiguous identification of biogenic amines by their mass spectra in addition to only the retention time and an independency from the sample matrix. The method was developed for the quantification of 2-phenylethylamine, tryptamine, histamine, tyramine, putrescine, ethanolamine, β-alanine, spermidine, spermine, cadaverine, 2-methylbutylamine, 3-methylbutylamine, 2-methylpropylamine and 3-(methylthio) propylamine and applied in several foods with different matrices.

Keywords: biogenic amines, stabile-isotopes, LC/MS-MS, benzoyl-derivatization

L-41
HIDDEN FUMONISINS: AN EMERGING ISSUE

Gianni Galaverna[1*], Chiara Dall'Asta[2], Claudia Falavigna[3], Mattia Mangia[4], Stefano Sforza[5], Arnaldo Dossena[6], Rosangela Marchelli[7]

[1 2 3 4 5 6 7] Department of Organic and Industrial Chemistry, University of Parma, Parma, Italy
[*] Corresponding author–E-mail: gianni.galaverna@unipr.it; Phone: 0039-0521-906196; Fax: 0039-0521-905472

Fumonisins are a group of structurally related mycotoxins, produced mainly by *Fusarium verticillioides* and *F. proliferatum*, which are the most important seed-borne fungi associated with corn. Several reports on the fate of fumonisins during corn processing have shown that analytical methods often underestimate the levels of fumonisins in corn and derived food products because of low recoveries. In this communication, the results obtained by five independent methods for the quantification of fumonisins B1, B2 and B3 in raw maize are reported. Five naturally contaminated maize samples were analysed in four different laboratories. Although each method was validated and common calibrators were used, a poor agreement about fumonisin contamination levels was obtained. These difficulties and these lack of consistency have been attributed to the occurrence of bound forms of these mycotoxins in foods. Indeed, this behaviour might be due to the binding of fumonisins to the food matrix or to the modification of their structure which lead to compounds not easily detectable by the normal methods of analysis. The presence of these hidden forms has been proved in several food products applying an alkaline hydrolysis protocol to release hydrolyzed fumonisins (HFBs) from the matrix. On the base of in vitro experiment with model compounds, it has been suggested that upon thermal treatment fumonisin B1 may react with starch and proteins to form covalent adducts. More recently, hidden fumonisins have been found also in low processed food (e.g. flour, bread, pasta) or raw maize suggesting the possibility of a physical interaction among the target mycotoxins and food macroconstituents such as proteins. Indeed, checking for the presence of these forms, significant amounts of bound fumonisins were detected in all the considered samples. The application of an in vitro digestion protocol to raw maize allowed for a very higher recovery in native fumonisins, suggesting that the interaction occurring among analytes and matrix macromolecules is associative rather than covalent. Depending on the analytical method as well as the maize sample, only 37%–68% of the total fumonisin concentrations were found to be extractable from the analytical samples whereas upon digestion the amount of detectable fumonisins is almost double than the amount detectable with routine analytical methods.

Keywords: hidden fumonisins, masked mycotoxins, maize

L-42

WHICH ROLE FOR VIBRATIONAL SPECTROSCOPY TECHNIQUES (NIR, MIR AND RAMAN) IN FOOD QUALITY AND SAFETY?

Vincent Baeten[1*], Pierre Dardenne[2]

[1 2] Walloon Agricultural Research Centre CRA-W. Chaussée de namur, 24. 5030 Gembloux. Belgium
[*] Corresponding author–E-mail: baeten@cra.wallonie.be; Phone: 00 32 81 62 03 50; Fax: 00 32 81 62 03 88

In the recent years, the applications of vibrational spectroscopy techniques to food quality and safety issues have drastically increased. This trend is essentially due to the development of new generation of spectrometers which are more robust, easy to use, flexible, able to work in network and suitable for on-line and on-field applications. In addition, the last developments of microscopic and imaging techniques based on the vibrational spectroscopy are full of promises for the field of food quality and safety. Today near-infrared (NIR), mid-infrared (MIR) and Raman spectrometers are more and more considered as classical instruments and used in food and feed analysis laboratories. The success of these techniques is their versatility allowing to develop and to validate methods for a large number of quality and safety issues. Moreover, they have several features (e.g. use of internal standards) that facilitate the running of these techniques in a quality system (e.g. ISO 17025).

The Walloon Agricultural Research Centre (CRA-W) is working since more than 20 years in the development of spectroscopic based solutions for the food and feed industries. Nowadays, it has a worldwide expertise in the field of management of spectral data-base and large spectrometers network. Network of instruments allow to share the same data-bases between instruments and to assure that the analytical results provided by different labs from a same company are consistent. More recently, it has been demonstrated that large data-bases can be used to tackle food and feed safety issues (e.g. melamine). On the other hand, microscopic and imaging spectroscopic techniques have been used with success in the development and validation of methods for the detection of meat and bone meal in feedingstuffs. This approach is tested and will be validated in the work of the SA*FE*ED-PAP project (safeedpap.feedsafety.org). The interest of imaging techniques is also studied in the CON*FF*IDENCE project (www.conffidence.eu) in order to detect plant contaminants in cereals. The fingerprinting nature of the vibrational spectra is used in the TRACE project (www.trace.eu.org) for traceability and authenticity issues (adulteration and geographical origin).

Keywords: spectroscopy food feed safety traceability

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L-43
RAPID METHODS FOR FOOD QUALITY AND SAFETY CONTROL

Jacob de Jong[1*], Michel Nielen[2], Stefan Weigel[3]

[1 2 3] RIKILT–Institute of Food Safety, Wageningen, The Netherlands
[*] Corresponding author–E-mail: jacob.dejong@wur.nl; Phone: +31 317 480376; Fax: + 31 317 417717

The presence of potentially hazardous chemicals in food remains a major concern among European consumers. Recent food contamination incidents, e.g. fraudulent addition of toxic chemicals to infant milk powders in China, certainly contribute to fears about the safety of food. Currently, a variety of analytical test methods are used to help ensure the safety of food and feed in Europe both for goods produced in the EU and imported from third countries. Many of these methods are tedious, time consuming, and require sophisticated and expensive instrumentation.

The CONffIDENCE project aims to further improve food safety in Europe by the development of faster and more cost-efficient methods for the detection of a wide range of chemical contaminants in different food and feed commodities. These methods will not only save precious time in ever faster production cycles, but will also permit more food/ feed samples to be monitored due to the lower cost per test. In combination with the broadened spectrum of detectable residues and contaminants the CONffIDENCE project will significantly increase food safety in Europe.

Within CON*ff*IDENCE, multi-methods will be developed for persistent organic pollutants, perfluorinated compounds, pesticides, veterinary pharmaceuticals (coccidiostats, antibiotics), heavy metals and biotoxins (alkaloids, marine toxins, mycotoxins) in products such as fish, cereal-based food and vegetables. A balanced mix of novel multiplex technologies will be utilized, including lateral flow devices, flow cytometry with functionalized beads, optical and electrochemical biosensors, cytosensors and metabolomics-like comprehensive profiling. After validation, the simplified methods will be applied in impact demonstrators that contribute to exposure assessment and validation of hazard models.

Results will be disseminated to scientists and relevant stakeholders, including the food industry, regulatory control bodies, DG-SANCO, EFSA, exporting countries, CRLs, CEN and consumers. The consortium consists of 17 partners from 10 European countries, representing 9 research institutes, 5 universities, 2 large food and feed industries and 1 SME.

The CON*ff*IDENCE project is a large collaborative project within the 7th work Programme of the European Community. It has started 1 May 2008 and it will have a duration of 4 years. CON*ff*IDENCE is coordinated by RIKILT – Institute of Food Safety, Wageningen, The Netherlands.

In the presentation, the outline of the CON*ff*IDENCE project will be given and some first results will be shown.

Contact: coordination@conffidence.eu

Website: www.conffidence.eu

Keywords: food, chemical contaminants, detection, rapid

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L-44
RECENT AND FUTURE EU LEGISLATION RELATED TO FOOD SAFETY, WITH FOCUS ON CONTAMINANTS

Frans Verstraete[1*]

[1] European Commission, Directorate General for Health and Consumers, Brussels, Belgium
[*] Corresponding author–E-mail: Frans.Verstraete@ec.europa.eu; Phone: +32 2 29 56 359; Fax: +32 2 29 91 856

General principles and objectives

The EU legislation on contaminants in food fulfils two essential objectives: the protection of public health and removal of internal barriers to trade within the EU.

Following the principles and objectives of the General Food Law [1], food safety legislation shall pursue a high level of human health protection. To achieve this objective legislation shall be based upon risk analysis. Risk assessment shall be based on the available scientific evidence and undertaken in an independent, objective and transparent manner. Risk management shall take into account the results of risk assessment, other factors legitimate to the matter under consideration and the precautionary principle where appropriate.

When international standards exist or their completion is imminent, they shall be taken into consideration in the development of any standard at EU level

Legislation on contaminants

Council Regulation (EEC) No 315/93 of 8 February 1993 laying down community procedures for contaminants in food [2] is the work for the Community action on contaminants.

This work Regulation provides that food containing a contaminant in an amount which is unacceptable from the public health viewpoint shall not be placed on the market (food can only be placed on the market when it is safe).

Furthermore it is foreseen that

- contaminant levels shall be kept as low as can reasonably be achieved by following good practices at all stages of the production chain
- in order to protect public health, maximum levels for specific contaminants shall be established where necessary;
- the consultation of a scientific body (EFSA) for all provisions which may have an effect upon public health is mandatory.

Based on this work Regulation, maximum levels for the following specific contaminants have been established by Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs [3].

Challenges for EU legislation on contaminants in food

To reduce the presence of contamination in the food supply, "prevention is better than cure". Therefore there it is important to encourage preventive actions. Prevention requires knowledge and acquiring knowledge requires research.

The establishment of maximum levels is not contrary to prevention as maximum levels, established at a reasonably achievable level, stimulate food business operators to apply preventive actions all along the food chain in order to avoid the contamination of the food chain. Furthermore regulatory standards provide a benchmark against the effectiveness of the successful implementation of prevention programmes and provide a tool for control authorities to control the correct application of prevention measures by each actor in the chain.

Legislation on contaminants needs continuously be updated to ensure a continuous high level of human protection and to address the challenges with which the risk managers are faced such as

- emerging contaminants: brominated flame retardants, (per)fluorinated compounds *Alternaria* toxins …
- contamination incidents with "new" contaminants: melamine, mineral oil, …
- new risk assessments: non dioxin like PCBs, arsenic, …
- updated risk assessments: cadmium, PAHs, mercury, ochratoxin A, lead, …
- developments in risk assessment approaches:
    - risk-benefit assessment : nitrates in vegetables
    - Margin of Exposure (MOE): genotoxic carcinogens such as aflatoxins, PAH, …
- changing production conditions /climate change: Fusarium-toxins, …
- international developments within the Codex Alimentarius: aflatoxins, …

The presentation will focus on these challenges the risk managers/regulators are faced with, how this leads to changes to EU-legislation, in full respect of the principles and objectives laid down in the General Food Law and the work Regulation and on the very important role of science and research in these developments.

[1] Regulation (EC) 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (OJ l 31, 1.2.2002, p. 1)
[2] OJ L37, 13.2.1993, p. 1
[3] OJ L 364, 20.12.2006, p. 5

Keywords: legislation, contaminants

L-45
NANOPARTICLES IN FOOD: EMERGING ANALYTICAL TASK

Stefan Weigel[1*], Ruud Peters[2]

[1 2] RIKILT–Institute of Food Safety, Wageningen, Netherlands
[*] Corresponding author–E-mail: stefan.weigel@wur.nl; Phone: 0031 317 485518

A number of recent reports and reviews have identified the current and short-term projected applications of nanoparticles for food and beverages. These include nano-sized or nanoencapsulated ingredients and additives for food, beverages, and health-food applications as well as the use of engineered nanoparticles for the improvement of food contact materials with view to mechanical properties, gas permeability or antimicrobial activity. Although potential beneficial effects of nanotechnologies are generally well described, their potential (eco)toxicological effects and impacts have so far received little attention. A prerequisite for toxicological, toxicokinetic, migration and exposure assessment is the development of analytical tools for the detection and characterisation of nanoparticles in complex matrices such as food. Given the huge diversity of engineered nanoparticles for use in the food and feed sector in terms of chemical composition, size, size distribution, surface activity/modifications etc. and potential interaction with food matrix components (e.g. proteins) this is a challenging task requiring tailored solutions.

Following a brief introduction to current applications of engineered nanoparticles (ENPs) in food and food contact materials and analytical approaches suitable to address food safety related issues of nanotechnology first methods are presented for the detection and characterisation of inorganic ENPs in food and food related products such as food packaging materials as well as personal care products. Generally, physical preparation and separation methods are used and combined with instrumental techniques such as hydrodynamic chromatography (HDC) and ICP-MS. Types of ENPs included are silver, silica, titanium dioxide and zinc oxide. The presentation will show some of the results of these methods and highlight some alternatives that may be used for the detection and characterisation of inorganic ENPs (for instance field flow fractionation versus hydrodynamic chromatography).

Key words: nanoparticles, silver, separation, characterisation, hydrodynamic chromatography, ICP-MS

L-46
TRANSCRIPTOMIC FINGERPRINTING TECHNOLOGY IN FOOD SAFETY

Hanspeter Naegeli[1*]

[1] University of Zürich, Zürich, Switzerland
[*] Corresponding author–E-mail: naegelih@vetpharm.unizh.ch; Phone: +41 44 635 8763; Fax: +41 44 635 8910

The goal of tranomic fingerprinting is to improve food safety by using cultured human cells as versatile biological detectors ("cytosensors") of toxic contaminants. This new strategy is prompted by the finding that living cells respond to toxic chemicals by changing the pattern of genes that are converted into messenger RNA trans. Each individual RNA tran carries the information for the synthesis of a particular protein product. The term "tranome" refers to the entire spectrum of such messenger RNA intermediates in a given biological system. Accordingly, "tranomics" stands for large-scale analytical methods that can be used to monitor complex RNA profiles consisting of thousands of trans. The general scheme of contaminant detection by tranomic fingerprinting is as follows. Cultured human cells are exposed to extracts prepared from food samples (meat, milk, cereals, etc.). Following an incubation time of 3–24 hours, messenger RNA trans are isolated, labelled and detected on high-throughput DNA microchips. Different contaminants generate characteristic changes in the tranional pattern, thus giving rise to diagnostic RNA fingerprints that can be used to recognise and quantify hazardous constituents. In the of the BioCop project, we have adapted a miniaturised DNA microarray platform to determine tranional fingerprints induced by estrogenic endocrine disrupters as well as type A trichothecenes. With exception of a portable reader, this tranomic platform requires no specialised equipment and, hence, can be easily disseminated. A key advantage is that this novel test exploits health-relevant parameters in a toxicologically significant target system. With the widespread use of rapid screening tests, which are not related to any toxicological endpoint, effect-driven in vitro bioassays will become of increasing importance to support risk assessment and monitor the success of risk management actions.

Keywords: Mycotoxin, Trichothecene, Phytoestrogen, Endocrine Disruptor

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SEMINAR

"Issues and Approaches to Address Chemical Contaminants in Food: US Perspective"

(LS-1 – LS-5)

LS-1
QUECHERS AS A METHOD AND AN APPROACH FOR SAMPLE PREPARATION IN CHEMICAL RESIDUE ANALYSIS

Steven J. Lehotay[1*]

[1] USDA-ARS; Wyndmoor, PA; USA
[*] Corresponding author–E-mail: steven.lehotay@ars.usda.gov; Phone: 1-215-233-6433; Fax: 1-215-233-6642

The original "quick, easy, cheap, effective, rugged, and safe" (QuEChERS) method for pesticide residue analysis of fruits and vegetables was introduced at the 4th European Pesticide Residue Workshop in Rome in 2002 and is detailed in subsequent publications. QuEChERS incorporates streamlined extraction and cleanup techniques (sample miniaturization, salt-out partitioning extraction by shaking in a single tube, and dispersive solid-phase extraction cleanup) to provide a highly flexible sample preparation template that can be used in multiple modified versions and applications. With respect to pesticide residue analysis, there are two independent and interlaboratory-validated methods under the auspices of AOAC International (AOAC Official Method 2007.01) and European Committee for Standardization (CEN Standard Method EN 15662). Along with the original version that did not employ buffering, these official buffered QuEChERS methods have been validated for hundreds of pesticides and commodities and implemented in countless residue monitoring labs worldwide. At least 11 companies are marketing QuEChERS sample preparation products for use in pesticide residue analysis and for adaptation in other applications. This includes the analysis of acrylamide, veterinary drug residues, clinical assays, forensics, and environmental methods. More than 100 peer-reviewed papers have been published on QuEChERS and dispersive-SPE, and the original QuEChERS paper in 2003 has been cited more than 200 times. In this presentation, a history of the QuEChERS method(s) and its evolution into an approach for many applications will be described. The QuEChERS "baby" of Anastassiades and Lehotay born in the USDA-ARS lab outside Philadelphia has become a "teenager" that does not know its full potential or its limits yet, and the speaker intends to provide guidance of how QuEChERS concepts can help streamline sample preparation, and when it will likely fail to do so.

Keywords: sample preparation; chemical residues; analysis

LS-2
FOOD SAFETY RESEARCH AT USDA: HORMONES IN WATER AND POPS IN FOOD

Janice Huwe[1*], Nancy Shappell[2]

[1 2] USDA-ARS-RRVARC-BRL, 1605 Albrecht Blvd., Fargo, ND, USA
[*] Corresponding author–E-mail: Nancy.Shappell@ars.usda.gov; Phone: 701-239-1233; Fax: 701-239-1430

Large concentrated animal farms have been the subject of intense public scrutiny, in part due to concern about environmental release of endocrine disruptors, including natural hormones. Surface waters in proximity to farms were evaluated for hormones and estrogenic activity (E-Screen) by the USDA. A constructed wetland built to lower N and P in swine waste prior to land application was found to efficiently lower estrogen/ic concentrations/activity in swine wastewater from a farrowing facility. Application of ~ 54,000 tons of dairy cattle waste on ~ 2,000 acres using best management practices did not elevate estrogen/ic content of area surface waters (< the proposed lowest observable effect concentration, pLOEC, for estradiol). Context for these values was provided by studying area wetlands, rivers, and municipal wastewater treatment plant (WWTP) effluent, all of which had activity < the pLOEC, with the exception of WWTP effluent. E-Screen's application in the evaluation of food will be discussed.

In efforts to monitor various persistent organic pollutants (POPs) in domestic meat and poultry, statistically-based surveys for dioxins and dioxin-like compounds were conducted by the USDA in 2002 and 2008. In both surveys, 17 toxic polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and three coplanar polychlorinated biphenyls (PCBs) were measured in beef (steer/heifer), market hog, young turkey, and young chicken samples. Sixteen polybrominated diphenyl ethers (PBDEs) were concurrently measured in 40% of the samples from the 2008 survey. Other 2008 samples and all of the 2002 samples were pooled by slaughter class and region to give 26 composites of the 510 total samples from each survey for measurement of PBDEs. The results of the 2008 survey showed total toxic equivalencies (TEQs) ranging from not detected to 4.5 pg/g lipid, and the sum of nine tri- to hepta-PBDEs ranging from not detected to 23.6 ng/g lipid. A comparison of the 2002 and 2008 surveys showed TEQs declining or remaining steady in all slaughter classes. Levels of individual tri- to hepta-BDEs declined by 40–90% and their sum by over 70% from 2002 to 2008 indicating that phasing out U.S. production of PBDEs probably resulted in a drop in these POPs entering the food chain.

Keywords: Estrogen, Endocrine disruptors, Dioxins, PBDE

LS-3
MASS SPECTROMETRIC (MS) APPROACHES TO DETECTING AND QUANTIFYING CONTAMINANT PROTEINS IN FOODS

Steven Musser[1*], Melinda McFarland[2], John Callahan[3]

[1 2 3] U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, U.S.A.
[*] Corresponding author–E-mail: Steven.Musser@FDA.HHS.Gov; Phone: 301-436-2404; Fax: 301-436-2332

Determining the presence of unknown chemical contaminants in regulated food and cosmetic products is a complex, time consuming process. While there have been significant advances in the analysis of small molecules and nucleic acids, our ability to detect and quantify contaminant proteins has proven to be significantly more difficult. Improving our capabilities in this area would significantly advance our analysis of biotechnology products, food allergens and microbial pathogens. MS approaches to solve these problems have several advantages, a high degree of sensitivity, unmatched specificity, and more importantly, the ability to measure multiple-proteins in a single analysis. Additionally, they do not require the development of antibodies, and therefore can be applied immediately in emergency circumstances.

Although MS based approaches to identifying and measuring proteins in food matrices hold significant promise, there are a number of analytical problems that need to be overcome. Principal among them are sample preparation and accurate quantification. Current sample preparation methods are time consuming, involve multiple steps and could contribute to significant loss of the target protein. Typical MS based methods for quantification of proteins only address a small number of MS instrument response factors and fail to address sample loss that may occur through the sample preparation process and incomplete enzymatic digestion. The address these important issues, we have been investigating a number of new approaches to control for the enzymatic digestion step of the sample preparation process.

One of these approaches is the elimination of the enzymatic step through the application of top-down MS techniques. In these experiments, intact proteins are first separated through a chromatographic step, as then analyzed directly through electron-transfer dissociation (ETD) or collision induced dissociation (CID) on an Orbitrap mass spectrometer. Results from these experiments clearly demonstrate the ability of the approach to identify intact proteins in mixtures without an enzymatic digestion step. This approach, along with more traditional approaches to quantification of proteins can provide unambiguous identification and measurement of proteins in foods.

Keywords: Mass Spectrometry, Proteins, Quantitation

LS-4

OPTICAL SENSING TECHNOLOGY DEVELOPMENT BY USDA-ARS FOR FOOD SAFETY AND SECURITY USE

Kurt Lawrence[1*], Bosoon Park[2], William Windham[3], Moon Kim[4], Kevin Chao[5]

[1 2 3] USDA-ARS, Athens, Georgia, USA
[4 5] USDA-ARS, Beltsville, Maryland, USA
[*] Corresponding author–E-mail: kurt.lawrenc@ars.usda.gov; Phone: 1 706 546-3527; Fax: 1 706 546-3607

The U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) has been conducting food safety research with monochromatic, multispectral and hyperspectral imaging systems. In an ARS laboratory in Maryland, hyperspectral imaging systems are being used to detect systemically-diseased poultry carcasses in the harsh processing environment with better than 96% accuracy. Several in-plant trials have been completed and a commercial system is under development. Similarly at an ARS facility in Georgia, multispectral and hyperspectral imaging systems are also being developed to detect fecal contaminants on poultry carcasses. Two- and three-wavelength systems have been developed with accuracies of 97% and 99%, respectively. The three-wavelength system has reduced false positives and a high-speed prototype is being evaluated. Additionally, joint research between the two labs is integrating the two detection technologies under a common platform. Both laboratories are also developing portable, hand-held systems, based on different detection algorithms, for use in the processing environment. One system utilizes a fluorescence response while the other uses a wavelength ratio for detection of various contaminants. For fruit and vegetable safety, a hyperspectral reflectance and fluorescence imaging system is showing promise as a means to detect fecal contamination on apples destined for the unpasteurized cider market. A prototype has been attached to an apple sorter and can operate at about 3 fruit per second with near 100% accuracies. The system is also capable of performing many of the other quality sorting tasks such that one imaging system can perform both quality and safety sorting/grading.

A monochromatic imaging system is being used with a modified pressure chamber to detect very small hairline cracks in table eggs. The system utilizes two images, one captured at atmospheric pressure and another captured while the eggs are under a slight negative pressure, to determine shell cracks from other eggshell features. The system is over 99% accurate in detecting the hairline cracks with less than 0.4% false positives.

Other research is utilizing silicon nanorods to enhance pathogen detection for food products. Salmonella-specific antibodies have been attached to one end of a nanorod while the shaft of the nanorod was coated with hundreds of fluorescent markers. Identification of the pathogen was enhanced by a factor of a thousand. Additional research is being done with nanofabrication in conjunction with surface enhanced raman spectroscopy for pathogen detection.

Keywords: imaging, hyperspectral, multispectral, safety

LS-5

TECHNOLOGIES FOR REPLACEMENT OF RODENT BIOASSAYS IN SENSITIVE DETECTION OF TOXINS IN FOODS

J.M. Carter[1*], D. Brandon[2], L. Cheng[3], L. Clotilde[4], X. He[5], R. Rasooly[6], M. Scotcher[7], L. Stanker[8]

[1 2 3 4 5 6 7 8] Foodborne Contaminants Research Unit, Agricultural Research Service, US Department of Agriculture
[*] Corresponding author–E-mail: j.mark.carter@ars.usda.gov; Phone: 510.559.6428; Fax: 510.559.6429

Rapid sensitive assays for biothreat toxins that can be used to detect intentionally contaminated foods are now typically performed via bioassay in live mice. While bioassay provides essential data on bioavailability, animal models are technically, fiscally, and ethically challenging. Through careful application of state-of-the-art techniques for immunization and screening, we created new monoclonal antibody reagents (MAb) specific for detection of botulinum neurotoxin (BoNT). These MAbs bind BoNT so tightly that, in a sandwich ELISA, they are more sensitive than the rodent bioassay. These reagents are also useful for sample preparation and production of portable tests for field use. Through a CRADA we used these MAbs to develop a simple "dipstick" assay that can detect BoNT in food at levels well below the human oral LD_{50}. We also used the new MAbs to develop sample preparation methods based on immunomagnetic beads. In liquefied food extracts these beads rapidly and irreversibly bind all toxin present in a large sample. Sequestering the beads with a magnet effectively concentrates the toxin into a small volume suitable for laboratory testing. While the toxin is still bound to the beads, we can detect its highly specific peptidase activity using a fluorescence (FRET) based substrate, for detection of sublethal amounts of BoNT in foods.

ALERGENS

(LA-1 – LA-6)

LA-1
ALLERGENS – THE ANALYTICAL CHALLENGE TO MEET LEGISLATIVE REQUIREMENTS AND CONSUMER DEMANDS

Bert Popping[1*]

[1] Eurofins Scientific Group, Pocklington, UK
[*] Corresponding author–E-mail: bertpopping@eurofins.com; Phone: +44 7768 166673; Fax: +44 870 168 8047

Food allergen labelling is regulated under 2007/68/EC and gluten labelling under 2009/41/EC. The list comprises a total of 14 groups, where all IgE allergens are protein based. ELISA (Enzyme Linked Immuno Sorbent Assay) is the most common method principle to test for allergens, followed by PCR (Polymerase Chain Reaction).

However, it can be observed that in a number of cases, the commercial ELISA kits available on the market produce significantly different results. This has a particular relevance for gluten detection where not only a presence/absence information is needed but quantification is required to meet labelling requirements.

The European Commission recognised that gaps exists in several analytical areas relevant to human health and nutrition and funded the 6th work program MoniQA, which, *inter alia*, deals with the problematic of allergens. The allergen working group of MoniQA, led by Bert Popping (Eurofins) and co-led by Clare Mills (IFR) deals with all aspects encompassing consumer, authority, industry and laboratory issues. Here, a subgroup was formed to look specifically at novel method approaches, in particular mass spectrometry, leading to consistent and accurate results. This group has made good progress on the analytical side by using newly produced reference material for the detection and quantification of milk and egg in processed foods. The progress of this working group will be presented in the session.

Keywords: Mass spectrometry, Allergens, milk, egg

MoniQA (EC FP6 Contract FOOD-CT-2006-036337)

LA-2
DEVELOPMENT OF REFERENCE MATERIALS FOR THE VALIDATION OF FOOD ALLERGENS DETECTION METHODS

Valery Dumont[1*], Philippe Delahaut[2]

[1 2] CER Groupe, Rue du Point du Jour, 8, 6900 Marloie, Belgium
[*] Corresponding author–E-mail: v.dumont@cergroupe.be; Phone: 32 (0)84 31 00 90; Fax: 32 (0)84 31 61 08

Food allergies constitute an increasing problem for several years. Currently, these allergies can not be cured. A complete elimination of the allergen in the diet is the only way to avoid an allergic reaction. Thus, the allergic consumers must know the exact composition of the food they eat. The European Directive 2007/68/EC sets out labelling rules in order to inform the allergic consumers of the presence of allergens in pre-packaged foodstuffs. However, these consumers are also concerned by the problem of cross-contaminations during the food processing, for which there is no legislation. The management of food allergens requires reliable analysis tools for the detection of allergens in food. Very few validation data are available for the comparison of allergen detection methods. This is certainly due to the lack of harmonized validation protocols and of recognized reference materials. During the MoniQA project the Working Group Food Allergens intends to produce incurred reference material with egg and milk. Cookies have been selected as the foodstuff to be incurred. The first tests have been carried out with the non-fat milk powder RM 1549 and with the spray-dried whole egg for allergen detection RM 8445 from NIST (National Institute of Standards and Technology). Cookies were incurred with the two compounds at two concentrations before cooking: 100 ppm or 1000 ppm. The dough (not baked cookies) and cookies were analyzed with casein, beta-lactoglobulin and egg kits from CER Groupe, Elisa Systems, R-Biopharm, Tepnel and Morinaga. The results show that only Morinaga's assays are able to detect each allergen at 10 ppm of milk and egg powders. As expected, each kit has its own calibrators and its own quantification procedure. In the work of Working Group Food Allergen for MoniQA, cookies will be produced in great quantity and will be used for a ring trial based on the harmonized validation protocol.

Keywords: Reference Material, Allergen, Cookies

Institut des Techniques et des Commerces Agro-Alimentaires

LA-3
RISK MANAGEMENT FOR ALLERGENS IN FOOD: INTERNATIONAL REGULATORY ENVIRONMENT FOR FOOD LABELLING

Samuel Benrejeb Godefroy[1*]

Food Directorate, Heath Products and Food Branch, Health Canada
[*] Corresponding author–E-mail: Samuel_Godefroy@hc-sc.gc.ca; Phone: (613) 957-1821

In Canada, food allergy affects 6 to 8% of infants and young children and 3.5 to 4% of adults and the prevalence may be increasing. Food allergies and Celiac Disease (CD) contribute to further straining public health systems worldwide, through increased numbers in emergency room visits associated with food allergy incidents, some of which can be preventable. Other impacts such as effects on quality life of people with food allergies, their family and their social circle are still to be defined. The only viable option for food allergy and CD sufferers to prevent allergy incidents is to avoid the food to which they are known to be sensitive. Food allergic consumers must rely on information provided to them by food processors and importers to protect their health. For prepackaged foods, it is critical that such information be included on the food label. Labeling has therefore been identified as a *public health tool* by several food regulatory jurisdictions, enabling the consumer to manage avoidance, but also allowing informed choice from safe food sources. This paper will present an overview of a number of food labeling requirements developed internationally to promote the protection of food allergic consumers. Where applicable, some initiatives pertaining to improved manufacturing or labeling practices developed by stakeholders other than government agencies (e.g. consumer groups or industry) will also be cited.

LA-4
DEVELOPMENT OF A TRIPLE-QUADRUPOLE MASS SPECTROMETRIC METHOD FOR THE DETECTION OF A-S1, A-S2- AND B-CASEIN

Julia Heick[1*], Markus Fischer[2], Bert Pöpping[3]

[1] Eurofins Analytik, Hamburg, Germany
[2] Universität Hamburg, Hamburg, Germany
[3] Eurofins, Hamburg. Germany
[*] Corresponding author–E-mail: juliaheick@eurofins.de; Phone: 00494049294617

Development of a triple-quadrupole mass spectrometric method for the detection of α-S1, α-S2- and β-casein Cow's milk allergy is an abnormal immunological response to casein that may result in clinical manifestations ranging from disorders in the digestive tract to anaphylaxis. Especially children under the age of three are affected. There is no clinical treatment available; patients must avoid the allergen and therefore rely on accurate food labelling. In the EU directive 2007/68/EC regulates the declaration of all intentionally added ingredients that may cause an allergic reaction. However, the detection of accidentally added allergens remains a problem. Casein analysis is routinely done with antibody based methods (ELISA). These have the advantage of being quick and easy to handle, but cross-reactions with other food proteins may occur. Since mass spectrometry is a very specific method, it offers the opportunity to overcome this problem. Also antibody based methods cannot detect different food allergens in one method, whereas mass spectrometry has the potential to do so. The presentation focuses on the development of a mass spectrometric method for the detection of α-S1-, α-S2- and β-casein. After extraction and alkylation the proteins are digested with trypsin, separated by HPLC and analyzed with a quadrupole-linear ion trap mass spectrometer. Reproducible occurring peptides were selected for the development of transitions for single reaction mode (SRM) mass spectrometry. These transitions were evaluated and the optimized method is shown to be capable of detecting casein in different samples.

Keywords: allergen, mass spectrometry, casein

LA-5
LC-ESI M.S. FOR DETECTION OF PROTEIN COMPOSITION AND MODIFICATION IN DAIRY PRODUCTS

Phil Johnson[1*], Clare Mills[2]

[1 2] Institute of Food Research, Norwich, UK
[*] Corresponding author–E-mail: johnsonp@bbsrc.ac.uk; Phone: +441603450200

Allergenicity of proteins in egg and milk products is affected by processing techniques such as hydrolysis (*1*) and heating (*2*). Such techniques are important in the production of 'hypo-allergenic' foodstuffs. The changes in allergenic potential are presumably due to hydrolytic destruction of IgE epitopes, changes in protein folding, and covalent protein modification by non-protein constituents.

Here we describe the rapid characterisation of proteins from egg and milk powders using ESI-m.s. techniques. In particular, we describe the sizing of protein aggregates, protein identification using intact mass, analysis of hydrolysis, and characterisation of protein modifications. In addition, we address the potential of electrospray techniques to characterise the degree to which allergenic proteins are folded and the impact this may have on allergenicity of products. Such m.s. based protein characterisation is also potentially of use in areas such as treatment history, functionality, hydrosylate sizing and characterisation of bioactive peptides.

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[2] Ehn B-M., Ekstrand B., Bengtsson U. and Ahlstedt S. (2004) Modification of IgE binding during heat processing of the cow's milk allergen β-lactoglobulin. *J Agric Food Chem.* 52 398– 1403.

Keywords: allergy, milk, egg, mass spectometry

LA-6
IMMUNOCAPTURE MASS SPECTROMETRY FOR THE ANALYSIS OF THE ALLERGEN LYSOZYME IN CHEESE

Nadine Schneider[1], Monika Pischetsrieder[2*]

[1 2] Department of Chemistry and Pharmacy, Food Chemistry, University of Erlangen-Nuremberg, Erlangen, Germany
[*] Corresponding author–E-mail: monika.pischetsrieder@lmchemie.uni-erlangen.de; Phone: 004991318524102;

Currently, ELISA and PCR assays are mainly used for the detection of allergens in foods. However, these assays need confirmatory methods, to rule out false-positive and false-negative results. Liquid chromatography combined with detection by UV/Vis, fluorescence or mass spectrometry can be used as confirmatory method for immunoassays, but often lacks sensitivity in complex matrices.

In our study, we combined purification of the samples by immunocapture with detection of the analyte by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). By the use of an immunity isolation step, the analyte can be specifically isolated from a complex mixture. Afterwards, the analyte is detected by its particular mass with MALDI-TOF-MS.

The method was applied for the selective analysis of lysozyme in cheese samples. Lysozyme is used in cheese manufacturing as a preservative to prevent the late blowing of ripened cheese caused by *Clostridium tyrobutyricum*. Since lysozyme is derived from hen eggs, it belongs to the "big eight" allergens that have to be labelled both in Europe and the USA. Cheese extracts containing lysozyme were incubated with anti-lysozyme antibody-coated magnetic particles. Lysozyme bound with high specificity to the antibodies on the magnetic beads. Other proteins and interferents were washed out. After the immunocapture purification, lysozyme was detected sensitively and specifically by MALDI-TOF-MS. The limit of detection was about 5 ppm lysozyme in cheese. Peak identification could be further verified by a peptide mass fingerprint generated by tryptic digest of the eluted protein.

The new assay was used as confirmatory method for an ELISA which had been developed for the quantification of lysozyme in cheese. The presented method can be easily adapted for the detection of other allergens and protein analytes in complex matrices and therefore has the potential for a broad application in food analysis.

Keywords: Iimmunocapture mass spectrometry, cheese, lysozyme

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POSTER SESSIONS

Topics

GENERAL FOOD ANALYSIS

(A-1 – A-54)

A-1

RIDASCREEN® SALMONELLA–A NEW INNOVATIVE ELISA BASED TEST KIT FOR RAPID DETECTION OF SALMONELLA SPP. WITHIN 23 HOURS

Ronald Niemeijer[1], Reinhard Witzenberger[2*], Janina Mahnke[3], Mohsen Jrade[4], Patrick Taylor[5], Paul Dunnigan[6]

[1 2 3 4 5 6] R-Biopharm AG, Darmstadt, Germany
[*] Corresponding author–E-mail: r.witzenberger@r-biopharm.de; Phone: +49 6151 81 02 466; Fax: +49 6151 81 02 40

The bacterial genus *Salmonella* is quite commonly known as a significant cause of food-borne illness. It is one of the most important pathogens responsible for food poisoning worldwide. In the current times of advancing globalisation and international travel, potentially dangerous *Salmonella* strains previously known only to occur in specific regions of the world may no longer remain isolated. Understanding this situation it is clear that effective and reliable methods of analysis are critical for all food producers & laboratories. Consumers must be in a position to unquestionably trust in their food safety and minimise all avoidable health risks.

R-Biopharm AG (Darmstadt, Germany) has developed a new ELISA based test kit called 'RIDASCREEN® Salmonella' for the rapid and easy detection of *Salmonella* species in food, feed and environmental samples. The new test is an innovative technique with a one-step-enrichment and antibody linked capture. This new procedure allows the cultivation and detection within the same micro plate to obtain results for the analysis on *Salmonella* spp. within 23 h.

RIDASCREEN® Salmonella detects all common known *Salmonella* serotypes including species that are non-motile such as *S*. gallinarum and *S*. pollorum. The test can be evaluated visually, with a micro plate reader or may be used fully automated to offer a level of flexibility that suits every laboratory's individual requirements.

RIDASCREEN® Salmonella has been validated by the European Validation Organisation (AFNOR) according to the EN/ISO 16140 standard for the use as alternative screening method for the detection of *Salmonella* spp.

Keywords: Salmonella, ELISA, Validation,Pathogens

A-2
SHELF LIFE DURABILITY OF OAT-BASED PRODUCTS

Claudine Cognat[1*], Derek Stewart[2]

[1 2] Scottish Crop Research Institute, Dundee, Scotland, UK
* Corresponding author–E-mail: claudine.cognat@scri.ac.uk; Phone: 01382 562731

Bakery products include a large group of foodstuffs, widely consumed on a daily basis or as occasional food. These types of products often contain fats and oils for technological, organoleptic or nutritive purposes but these components can also be the cause of product deterioration, unacceptability and ultimately rancidity. Indeed, oxidation of lipids is a well reported common but undesirable chemical reaction that can impact upon flavour, aroma and nutritional quality and in some cases the texture of a product. The compounds derived from lipid oxidation are known to be responsible for rancid flavours and aromas. These are volatile compounds including hydrocarbons, aldehydes, enals, dienals, ketones and organic acids, and are generally determined by headspace analysis.
In dried cracker-like products, such as oatcakes, shelf life can often be considerable (>26 weeks) but in the case of some oat based products this can be considerably shorter with consumer acceptability declining with storage time. As part of a project in conjunction with a major producer of oat products, in this case oatcakes, to extend shelf-life and reduce the saturated fat content levels of oatcakes we endeavoured to define the basis mechanisms of this product shelf life problem with a view to its correction and elimination.
In order to understand the behaviour of the oat based products, a preliminary study on fresh and rancid biscuits was carried out by headspace analysis in order to obtain their profiles at these two different storage stages. Two techniques were used: Solid Phase Micro Extraction/Gas chromatography/Mass Spectrometry (SPME-GC-MS) and Automated Thermal desorption/Gas Chromatography/Mass Spectrometry (ATD-GC-MS). Two oat based products were analysed: Rough oatcakes and Cheese oatbakes.
The overall volatiles composition of these products comprised mainly aldehydes, ketones, alkanes, alkenes, alcohols and ethers with some of these apparent indicators of shelf life duration. Interestingly, the addition of a natural herbal food antioxidant to the product mix resulted in a significant reduction in the onset of rancidity particularly in the cheese oatcakes. An accelerated shelf life system was validated by ATD-GC-MS and this should allow product evaluation in a more economic fashion. The details of all studies will be discussed.

Keywords: bakery, headpsace analysis, shelf life

A-3
DEPENDENCES BETWEEN COMPOSITION, STRUCTURE AND COLLOIDAL COMPETENCES OF POLYSORBATES

Calin Jianu[1*], Monica Butnariu[2], Ileana Cocan[3], Alexandru Rinovetz[4], Gabriel Bujancă[5], Ionel Jianu[6]

[1 3 4 5 6] Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Processing Technology, 300645-Timişoara, Calea Aradului 119, Romania
[2] Banat's University of Agricultural Sciences and Veterinary Medicine, Chemistry Department, 300645-Timişoara, Calea Aradului 119, Romania
[*] Corresponding author–E-mail: calin.jianu@gmail.com; Phone: +40-0722-632199

Polysorbates (E 432–436) like surfaceactive structures with variable HLB value [dependent on medium oligomerisation degree of polyoxyethylene chains (PEO)] are food additives with multifunctional competences (emulsifying, aeration, moistening, mycelium dispersing – solubilizing, monitoring the crystalline form of fats, foaming, stabilization agents, etc.) appreciated and alimentary processing.

To predict technological competences must more strictly defined PEO chain (more homogeneous) (the dispersing of homologues chain restricted) and free of usual industrial by-products in variable amounts in the technical products commercializated [water (1–2%); such as sorbitols or anhydride (1–2%); polyethyleneglycols (1–5%) 1,4 – dioxan (under 1%)].

By-products in variable amounts in composition of commercial polysorbates affecting the colloidal competences in analysis bulletins. Increasing the oligomerisation medium degree in commercial polysorbates (n = x + y + z + w) affects directly proportional quantities of high polyethyleneglycols and inverse proportional the amount of sorbitol such as or anhydrid.

The paper presents (the first) experimental results of separation by selective liquid / liquid extraction, molecular distillation, elution on, open column chromatography to specify the structure and purity comparatively (the second) with polysorbates structurated with polyoxyethylenic „homogeneous" chains (PEO) (n = 3–20) by adapted Williamson method.

When x + y + z + w = 20 (Polysorbate 80) respectively x = y = z = w = 5 shall be evident differences of competence colloidal.

Keywords: polysorbates, polyoxyethylene chains, sorbitols

A-4
TRACE ELEMENT ANALYSIS OF DIETARY SUPPLEMENTS AND NUTRIENTS BY TXRF

Armin Gross[1*], Hagen Stosnach[2], Lutz Schomburg[3], James Neal-Kababick[4]

[1 2] Bruker AXS Microanalysis GmbH, Berlin, Germany
[3] Institute for Experimental Endocrinology, Berlin, Germany
[4] Flora Research Laboratories, Grants Pass, OR, USA
[*] Corresponding author–E-mail: armin.gross@bruker-axs.de; Phone: +49 30 670 990 819; Fax: +49 30 670 990 30

In the last decades, total reflection X-ray fluorescence (TXRF) analysis was proven to be an easy and versatile method for the determination of trace elements [1]. TXRF can be applied to different sample types, like solids in form of micro fragments, powders, suspensions, thin films or liquids. The required sample amount is in the low µg or µl range, respectively. In TXRF the samples are prepared as thin film or layer, thus matrix effects are negligible. Quantification is possible by means of the known concentration of an internal standard element.

However, because of the large size and high operative costs, the application of this method was restricted to large research and development laboratories. But recently with the introduction of low-power benchtop instruments, this analytical method starts to get established even in small laboratories for research or routine applications.

This has opened new doors in the rapidly growing field of Phytoforensic chemistry. This presentation will present a brief outline of the instrumental design and theory of TXRF followed by case studies demonstrating how the technique is utilized in every day laboratory analysis. In particular, heavy metal contamination, particle contamination and lot comparison profiling of dietary supplement products will be presented.

In addition, the growing interest in selenium as a health relevant nutriant will be discussed. The individual Se status, dietary Se intake and Selenoprotein expression are interrelated and become increasingly recognized as an important pathophysiological parameter for disease risk and prognosis [2]. We will describe recently developed procedures for fast Se monitoring of food and the potential relevance for the food industry.

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[2] Schomburg, L., Köhrle, J. (2008), Mol Nutr Food Res.

Keywords: dietary supplements, contamination, metal, selenium

A-5

STUDY OF CARBOHYDRATE REACTIVITY ON THE DEVELOPMENT OF THE MAILLARD REACTION IN SOLID MODEL SYSTEMS

Oswaldo Hernandez[1], Vesela I. Chalova[2], Steve C. Ricke[3], F. Javier Moreno[4], Maria Luz Sanz[5*]

[1 5] Instituto de Química Orgánica General (CSIC) Madrid, Spain
[2 3] Center for Food Safety and Food Science Department, University of Arkansas, Fayetteville, AR, USA
[4] Instituto de Fermentaciones Industriales (CSIC), Madrid, Spain
[*] Corresponding author–E-mail: mlsanz@iqog.csic.es; Phone: (+34) 91 562 2900; Fax: (+34) 91 564 4853

Glycation or non-enzymatic glycosylation of free amino groups of amino acids, peptides and proteins with reducing carbohydrates (Maillard reaction, MR), is an important and universal reaction with several implications in health and food science. Many researchers have been focused on the kinetic study of MR; however, there is still much work to be done to achieve a complete understanding of the numerous factors which have an influence on the evolution rate of MR. The effect of carbohydrate structure is one of the main factors which affects to MR progress, as well as to the diversity of MR products formed that can play an important role in a wide number of functional (biological or technological) properties. Although most of these studies have been carried out in liquid model systems (Laroquet al., 2008), the comparison of carbohydrate effect in solid model systems, which can represent a most real approximation to food system with low water content and where less interferences appeared, can be of interest.

Therefore, the aim of this work was to evaluate the influence of carbohydrate structures on the development of MR in solid model systems. For this purpose *N*α-acetyl-lysine and different carbohydrates (ribose, glucose, galactose, fructose, lactose, maltose, maltotriose and maltotetraose) at equimolar concentrations were incubated at dry-state under controlled conditions (a_w=0.44; 40ºC) for different times. Five different indicators were used to evaluate the Maillard reaction evolution: furosine (as indicator of Amadori compounds; initial products); fluorescence (as indicator of advanced glycation end products, AGEs), browning intensity at 420 nm (as indicator of melanoidines) and loss of carbohydrates and amino acids. Furthermore, the formation of the corresponding Amadori and Heyns compounds was confirmed in all model systems by ESI-MS analyses.

As expected, ribose was the most reactive carbohydrate. This model system showed the highest increase of browning and the highest decrease of the carbohydrate and the amino acid during the heating time. Concerning hexoses, galactose was more reactive than glucose, whereas fructose showed the lowest reactivity, even compared with the rest of carbohydrates. It is worth noting that the difference observed between glucose and galactose was not found for lactose and maltose probably due to that both disaccharides are covalently linked to the free primary amino group through the glucose unit.

In summary, the most reactive studied carbohydrates were the aldo-monosaccharides followed by disaccharides, trisaccharides, tetrasaccharide and finally fructose.

The evaluation of the evolution of MR for the different carbohydrates could allow the obtainment of new ingredients with different functionality, depending on their enrichment in Amadori compounds, AGEs or melanoidines.

Keywords: maillard reaction, dry-state, carbohydrates

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A-6
DETERMINATION OF INDOLE-3-ACETIC ACID IN MALVASIA FROM ISTRIA GRAPE AND WINE

Luna Maslov[1*], Ana Jeromel[2], Stanka Herjavec[3], Marko Karoglan[4], Bernard Kozina[5]

[1 2 3 4 5] Faculty of Agriculture University of Zagreb, Croatia
[*] Corresponding author–E-mail: lmaslov@agr.hr; Phone: ++385915181021

The phenomenon of untypical aging off-flavor, which is associated with higher concentrations of 2-aminoacetophenone (2-AAP) in white wines, can stem from some technological treatments applied in grape and wine production. In order to find out if a wine is liable to untypical aging off-flavor, it is necessary to determine the concentrations of indole-3-acetic acid and L-tryptophan, which are the precursors of 2-aminoacetophenone. The purpose of this investigation was to determine the influence of soil type and different yeast strains on the levels of L-tryptophan and indole-3-acetic acid in the grapes and wines of Malvasia from Istria. Grapes were harvested from two different soil type locations in Istria wine growing region and separately vinified according to the technology customary for white wines. Young wine samples were taken for analysis immediately after completed alcoholic fermentation. The investigation included the development and validation of the method for determining the levels of indole-3-acetic acid and L-tryptophan in must and wine by means of the liquid chromatography with fluorescence detection and a prior purification of samples by means of Solid Phase Extraction (SPE) on polystyrene- divinylbenzen columns. Luna C_{18} (250 x 4,6 mm, 5 µm) was used column for component separation on HPLC. The efficiency and selectivity of two types of SPE columns (PS-DVB and MAX) were compared. Chemical analyses of compared musts and wines showed differences in L-tryptophan and indole-3-acetic acid concentrations that depend more upon the soil type than yeast strain used in this investigation.

Keywords: indole-3-acetic acid, tryptophan,yeast, Malvasia

A-7
ASSESSMENT OF EGG PRODUCTS FRESHNESS BY ARTIFICIAL OLFACTORY SYSTEMS

Michele Suman[1*], Gabriele Riani[2], Enrico Dalcanale[3]

[1] Barilla SpA Research Labs, Parma, Italy
[2 3] University of Parma, Parma, Italy
* Corresponding author–E-mail: m.suman@barilla.it; Phone: 00390521262332; Fax: 00390521263452

Food industry is interested in high quality egg products in a liquefied form (obtained from eggs shelled within 4 days and subjected to homogenization and pasteurization processes) because of their usefulness as ingredients of foams, emulsions, pastry and bakery products.

Eggs contain a series of organic acids that cannot be altered through thermal restoration actions and whose presence is directly correlated to the microbial quality and enzymatic properties; currently, the legal European Union limits are: lactic acid = 1000 mg kg^{-1} dry egg; succinic acid = 25 mg kg^{-1} dry egg.

Non-destructive methods to determine egg freshness, including optical and spectroscopic measurements on shell or yolk colours, have been proposed in the past. An alternative strategy to sensing the global profile of organic volatiles emitted by eggs can potentially be achieved by using artificial olfactory systems (AOS), also called “electronic noses”.

In this work we would like to show the potential of AOS, based on metal oxide semiconductors sensor technology, to tackle the problems regarding the different freshness state of egg product lots subjected to acceptance procedures in the food industry quality control laboratories.

AOS demonstrate a high discrimination ability related to the chemical (change of the organic acid contents) and microbiological (growth of the present microbial populations) evolution of the samples during their degradation process.

Furthermore, AOS quickly supplies practical responses in around 40 min (including three repetitions for each sample) against the hours necessary to complete traditional chemical and microbiological analysis, with the contemporaneous advantages of the absence of sample pre-treatments, a reduced economic impact and the possibility to express the results in a simple, objective and easily interpretable way.

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Keywords: Eggproducts, Freshness, Artificial Olfactory Systems

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A-8

RFID SMARTCARD CHEMIRESISTORS FOR WIRELESS DETECTION OF ORGANIC VAPOURS

Zorana Grabaric[1], Miroslav Marecic[2*], David Matthew Steinberg[3], Ivana Murkovic Steinberg[4], Ivan Zura[5], Maja Kozina[6]

[1 2] Faculty of Food Technology & Biotechnology, University of Zagreb, Zagreb, Croatia
[3] Erasmus Technology LLP, Duxford, Cambridge, U.K.
[4 5 6] Faculty of Chemical Engineering & Technology, University of Zagreb, Zagreb, Croatia
[*] Corresponding author–E-mail: mmarecic@pbf.hr; Phone: + 385 1 4605 022

Passive chemiresistor sensors with integral wireless power and data links are in development, and their potential applicability for the detection of organic vapors is demonstrated. The chemiresistors comprise a planar gold electrode array onto which chemically sensitive thin films are cast using micro-fabrication techniques. The thin film sensors are interfaced to a proprietary radio-frequency smartcard designed for use as a distributed chemical or biological detector, and which is compatible with the ISO15693 radio-frequency identification (RFID) wireless protocol. The measurement of thin film sensor resistance is performed by a two-contact and four-contact methods, and is controlled with on-card electronics. Thin polymer films based on the organic semiconductors 3,4-ethylenedioxythiophene-poly(styrenesulfonate) (PEDOT-PSS) have been used as chemically sensitive thin films to demonstrate function of this platform for detection of gaseous analytes. Potential applications of smartcard chemiresistors include mobile and wearable sensors for chemical exposure and contamination monitoring and smart personal diagnostics. The standardization of short-range wireless radio protocols such as Bluetooth, RuBee, ZigBee, WiFi and RFID is creating new markets for distributed sensors and sensor networks, and the fusion of chemical and biosensor technologies with short-range, low-cost, wireless technologies will create new opportunities for chemical and biological sensor systems in healthcare, environmental monitoring, process and quality control, and chemical and biological threat detection. We are developing high-value applications beyond typical cool-chain temperature tracking by designing novel sensors onto custom RFID labels compatible with the ISO 15693 smartcard protocol. The RFID protocol is particularly suitable for short-range contactless sensor applications, since it supports both passive (batteryless) and semi-passive (powered) devices and has an anti-collision protocol that supports reading of up to 255 sensors in a single scan. This work is concerned with developing and integrating conductometric chemiresistor sensors onto a short-range wireless platform with which to address a subset of these emerging applications. RFID-based sensor chips are now commercially available for temperature logging, and investigational chemical devices have been reported for both gases and vapors. We have developed a custom RFID processor and antenna onto a credit-card sized device which includes a conductometric sensor interface. The smartcard interfaces with a low-cost disposable part on which the chemiresistor is fabricated. First results demonstrate a reversible response of the sensors on exposure to ethanol vapours. The smartcard chemiresistors described here could in future be suitable for use in distributed wireless data applications and chemical sensor networks where organic vapours are to be detected.

Keywords: conductometric sensor, RFID, passive, low-cost

This work is a part of the project "Distributed wireless sensors for smart chemical and biological detection systems: chemo- and biosensor interface and applications development" (125-0000000-3221) and "Development of Chemometric and Sensor Methods for Determination of Different Analytes" (058-0580000-3071). The financial support of the Croatian Ministry of Science, Education and Sport is appreciated.

A-9
MEASUREMENT OF TOTAL STARCH

Ida Lazewska[1*], Anna Draga[2], Barry McCleary[3]

[1 2 3] Megazyme International Ireland Limited, Bray, County Wicklow, Ireland
[*] Corresponding author–E-mail: ida@megazyme.com; Phone: +(353 1) 2861220; Fax: +(353 1) 2861264

Several methods have been developed for the measurement of starch. In recent decades, enzyme based methods have been favoured. These generally involve solubilisation of starch in boiling water, hot DMSO or KOH, followed by liquifaction of the starch and subsequent hydrolysis of dextrins to D-glucose; with D-glucose measurement with the glucose oxidase/peroxidase system, or with hexokinase plus glucose 6-phosphate dehydrogenase. AACC Method 76.13 (AOAC Method 996.11), developed by Megazyme, involves hydrolysis of starch with thermostable alpha-amylase at pH 7, followed by hydrolysis of dextrins to D-glucose with amyloglucosidase (AMG) at pH 4.5. High amylose starch is pre-dissolved in hot DMSO.

With the development of improved enzymes for industrial starch hydrolysis, it is now possible to obtain thermostable alpha-amylases that are stable and active at pH 5. This has allowed us to modify the starch assay procedure to allow both liquifaction and dextrinisation steps to be performed at the same pH (pH 5.0), which simplifies the assay and allows the use of inexpensive acetate buffer. The use of cold KOH and hot DMSO for the solubilisation of high amylose starches have been compared, and the incorporation of glucose mutarotase to accelerate the glucose determination step has been evaluated.

Keywords: total starch

A-10

APPLICATIONS OF HYDROPHILIC INTERACTION CHROMATOGRAPHY (HILIC) TO ANALYSIS OF CHEMICAL MARKERS IN MEAT AND MEAT PRODUCTS

Leticia Mora[1], Aleida Hernandez-Cazares[2], M-Concepcion Aristoy[3], Fidel Toldra[4*], Milagro Reig[5]

[1 2 3 4] Instituto de Agroquímica y Tecnología de Alimentos (CSIC), PO Box 73, 46100 Burjassot (Valencia), Spain
[5] Institute of Food Engineering for Development, Polytechnical University of Valencia, Camino de Vera s/n, 46022, Valencia, Spain
* Corresponding author–E-mail: ftoldra@iata.csic.es; Phone: +34 963900022; Fax: +34 963636301

HPLC methods based on hydrophilic interaction chromatography (HILIC) have been applied to the analysis of biochemical compounds used as markers as well as residues in meat and meat products. Chemical compounds are nucleotides and nucleosides, creatine and creatinine and dipeptides carnosine and anserine. A zwitterionic polymeric column, optimized in terms of linearity, repeatability, reproducibility and recovery, has been used for the analysis. The HILIC method allowed the separation and quantification of these compounds in complex matrices such as meat and meat products. Such separation was monitored using a diode array detector at a wavelength of 214 nm for creatine, carnosine and anserine, whereas a wavelength of 236 nm was used for creatinine detection. In the case of nucleotides, a wavelength of 254 nm was used. Detection limit values were 0.10, 0.15, 0.05, 0.16, 0.16 and 1.72 µg/mL for ATP, ADP, AMP, IMP, Inosine and hypoxanthine respectively, and 4.45, 5.94, 3.37 and 0.04 µg/mL for carnosine, anserine, creatine and creatinine, respectively. These methods were also applied to the analysis of such compounds in meat products like dry-cured hams, showing good performance. These results were very good when compared with other rather conventional methods based on ion-pair reversed-phase chromatography (IP-RP-HPLC) since they avoided complex clean-up and/or sample derivatization procedures and is compatible with further mass spectrometry analysis. Thus, these HILIC methods have proven to be useful for the analysis of such compounds in meat and meat products.

Keywords: nucleotides, creatine, dipeptides, meat, HILIC

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A-11
SEPARATION, IDENTIFICATION AND DETERMINATION OF SOME COMPONENTS IN SOME VEGETABLE OIL SAMPLES

Nabil Fakhre[1*], Hemen Khalid[2]

[1 2] Dept. of Chemistry, College of Science Education, Erbil, Iraq
[*] Corresponding author–E-mail: havras@yahoo.com; Phone: 009647504661563; Fax: 00964662260598

Different components of some vegetable oil samples had been separated and derivative spectroscopy techniques were used for identification and determination of these components with acceptable precision and accuracy in coparsion with standard methods.

A-12

USE OF A MEMBRANELESS EXTRACTION MODULE FOR THE VOLTAMMETRIC DETERMINATION OF SULPHITES IN WINE

Luís Gonçalves[1*], Miriam Anunciação[2], João Pacheco[3], José Rodrigues[4], Aquiles Barros[5]

[1 2 3 4 5] Requimte, Faculdade de Ciências da Universidade do Porto, Porto, Portugal
[*] Corresponding author–E-mail: luis.goncalves@fc.up.pt; Phone: +351 918 393 243; Fax: +351 220 402 659

In this work, a new methodology for the voltammetric analysis of sulphites in wine is proposed. It makes use of a membraneless extraction module (MLEM), recently developed by the author's research group, for the analysis of volatile and semi-volatile compounds. Sulphites are widely used as preservatives in the food industry for their several roles including the prevention of undesirable microbial growth and oxidation processes. However at high levels they produce an unpleasant aroma/taste and also become a health hazard. Therefore the relevance of sulphite analysis is widely established, particularly in wine where it is almost indispensable. Among a whole variety of methodologies proposed for the determination of sulphites in wine, the most used are based on the classical Monier-Williams procedure and the also ancient Ripper method. Nevertheless, voltammetric-based methodologies appear to be the most accurate ones. Square-wave voltammetry (SWV) is very advantageous in the determination of sulphites since there is a direct detection, i.e. it is the SO_2 molecule that is instrumentally measured in the electrode. The major drawback when using voltammetry is the previous analyte extraction step required since wine is such a complex matrix. The extraction process is based on a MLEM that works with the same principles of gas diffusion and pervaporation however it does not require a membrane, and therefore does not have the associated problems with its use. The sample is placed inside the module and the volatile compounds evaporate to the headspace. Inside the module there is a suspended small reactor, where a small volume of an acceptor solution is placed. After a period of time, the acceptor solution is collected and analyzed by SWV. The proposed method was validated by comparison to the reference methodology (the Ripper method, an iodometry), moreover, it shown to be particularly advantageous with red wines, since the iodometry's endpoint color is purple. The developed method also shown repeatability (RSD lower than 5%) and linearity (between 20 to 200 $mg \cdot L^{-1}$) as well as suitable limit of detection (6 $mg \cdot L^{-1}$) and limit of quantification (19 $mg \cdot L^{-1}$). Moreover, if needed, these limits can be easily lowered by increasing the time of extraction, which in this case was less than 5 minutes.

Keywords: Sulphites; Wine; Membraneless extraction module

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A-13
DETECTION OF NITRITE USING A PRUSSIAN BLUE MODIFIED SCREEN-PRINTED CARBON ELECTRODE

Chia-Yu Lin[1], Kuo-Chuan Ho[2*]

[1 2] National Taiwan University, Taipei, Taiwan
[*] Corresponding author–E-mail: kcho@ntu.edu.tw; Phone: +886-2-2366-0739; Fax: +886-2-2362-3040

Nitrite is present ubiquitously in soils, waters, foods and physiological systems and has been reported as a human health-hazard species. The excess uptake of nitrite would cause gastric cancer [1]. Therefore, it is necessary to develop a reliable and sensitive sensor to detect nitrite in food, drinking water and environmental samples.

In this study, a Prussian blue (PB) modified screen-printed carbon electrodes (SPCEs) were fabricated and their electrocatalytic properties to nitrite was investigated. After electrodepositing a thin layer of PB from $FeCl_3$/ $K_3Fe(CN)_6$ solution, the peak potential for nitrite oxidation on PB was 0.648 V (vs. Ag/AgCl), which is about 80 mV lower than that of the bare electrode. The lower peak potential for nitrite oxidation could suppress some interferences from other oxidizable compounds. Besides, as cetyl trimethyl ammonium bromide (CTAB) was added as a surfactant during the preparation of PB film, the sensitivity was enhanced from 0.101 to 0.218 $mAmM^{-1}cm^{-2}$, which can be attributed to the electrostatic interaction between nitrite and PB/CTAB film. Furthermore, by increasing the $FeCl_3$/ $K_3Fe(CN)_6$ concentration from 0.50 to 0.75 mM, the sensitivity was increased from 0.218 to 0.241 $mAmM^{-1}cm^{-2}$. However, further increase in the $FeCl_3/K_3Fe(CN)_6$ concentration decreased the sensor sensitivity, which could be attributed to the increased diffusion barrier for nitrite. Further study will be focused on the determination of nitrite extracted from cured meat.

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Keywords: Nitrite; Prussian blue, Electrochemical sensor

A-14

AMINO ACID ANALYSIS OF SPINACH AND APPLE USING THE QUECHERS SAMPLE PREPARATION TECHNIQUE AND AN AUTOMATED OPA/FMOC DERIVITIZATION LC METHOD

John W. Hendeson Jr.[1*], Joan Stevens[2], Ulrik Wittek[3], Thierry Faye[4]

[1 2] Agilent Technologies, Wilmington
[3] Agilent Technolgies, Waldbronn, DE
[4] Agilent Technologies, Massy, FR
[*] Corresponding author–E-mail: john_w_henderson@agilent.com; Phone: 302-633-8502

A well established online automated OPA /FMOC derivitization method for amino acids will be used to analyze QuEChERS (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe) extracts of apple and spinach produce. The chlorophyll rich spinach will be prepared using a different QuEChERS dispersive kit than the chlorophyll poor apple. Amino acid analysis of the food extracts will be compared. Scalability, batch-to-batch reproducibility, linearity, and longevity data of the amino acid method will be presented. Several column options will be shown, ranging from rapid nine minute analyses of 23 amino acids including re-equilibration using short (50 mm) Rapid Resolution High Throughput columns (1.8 µm), to 40 minute analyses using 250 mm traditional 5 µm columns.

Keywords: QuEChERS, amino acids, spinach, HPLC

A-15

DEVELOPMENT OF METHODS FOR MONITORING COD DESALTING PROCESS BASED ON FLOW INJECTION ANALYSIS AND FOURIER-MID INFRARED SPECTROSCOPY

Andrea C. Galvis-Sánchez[1*], Ildikó Tóth[2], Susana Ganito[3], Ivonne Delgadillo[4], António O.S.S. Rangel[5]

[1 2 5] CBQF/Escola Superior de Biotecnologia-UCP, Porto, Portugal
[3] Pascoal e Filhos S.A, Aveiro, Portugal
[4] Departamento de Química, Universidade de Aveiro, Aveiro, Portugal
[*] Corresponding author E-mail: agsanchez@mail.esb.ucp.pt; Phone: +351 934939852

Two analytical methods were developed for monitoring the variation of sodium chloride concentration during the cod desalting process. The developed flow injection analysis (FIA) system was based on the reaction between chloride and silver nitrate, and the formed precipitate was assessed spectrophotometrically as a turbidity change. The different chemical and physical FIA parameters were studied and the use of two different colloids, polyvinyl alcohol (PVA) and gelatin were compared. A Fourier-mid infrared spectroscopy (FT-MID) Golden-Gate attenuated total reflection (ATR) method was also applied.

Although the limit of detection (LD) values for the FIA-PVA and gelatine were comparable (0.039 and 0.035 g L^{-1}), the sensitivity of the FIA-PVA was higher. The reproducibility of the developed system with the two colloids was assessed from five calibration curves performed during 2 months period; sensitivity and standard error values of 0.0020 ± 0.0001 L mg^{-1} for the FIA-PVA system and 0.00064 ± 0.00002 L mg^{-1} for the FIA-gelatine system were obtained. The repeatability (RSD) values for the FIA-PVA system ranged between 2.6 to 3.8% (n=10) and for the FIA-gelatine the RSD values were between 4.0 and 8.3% (n=10). Along a 48 h desalting process, samples of cod extracts and brine were taken and analysed by the FIA-PVA system as well as by the reference procedure. The relative deviation (RD) found between the FIA methodology for the cod extracts *vs*. the reference methodology showed values between -4.8 and 27%, while for the brine samples these RD values were between -9.5 and -4.8%. A determination throughput of 40 h^{-1} was obtained.

In relation to the FT-MID, the PCA analysis of the spectrum of chloride standard solutions ranging from 1 to 20% (w/v) showed that linearity is observed with the increase of the NaCl concentration. The PLS1 showed that the experimental points fit well to a straight line (R^2 = 0.9996, LV = 2, RMSECV = 1.8%). The limit of detection was 5 g L^{-1}. Comparing the results obtained by the FT-MIR *vs*. the reference methodology, the RD values of the NaCl determination for the cod extracts were between 0 to -27.3% and, for the brine samples, RD values were between -10.0 to -3.2%. The determination frequency using this methodology was 20 h^{-1}.

In this work, the successful application of two analytical procedures for the determination of NaCl in cod and brine samples during a simulated desalting process is demonstrated and some of their features are presented.

Keywords: NaCl, food-quality, FIA, FT-MID-ATR, cod-fish-desalting

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A-16

EFFECT OF INCREASING NITRATE APPLICATIONS TO AN ORGANIC-NITROGEN-BASED NUTRIENT SOLUTION ON THE N ISOTOPE COMPOSITION OF PEPPER PLANTS

Pilar Flores[1*], Phil Murray[2], Pilar Hellín[3], Mª del Mar Davó[4], Alicia López[5], José Fenoll[6]

[1 3 4 5 6] Dpto.de Calidad y Garantía Alimentaria, IMIDA, Estación Sericícola, 30150 La Alberca, Murcia, SPAIN
[2] Cross-Institute Programme for Sustainable Soil Function, North Wyke Research, Okehampton, Devon, EX20 2SB, UK
[*] Corresponding author–E-mail: mpilar.flores@carm.es; Phone: 34 968 366804; Fax: 34 968366792

Natural abundance of stable isotopes has been widely used in food and environmental sourcing research. In particular, several studies have used the $\delta^{15}N$ values as a potential tool to detect fraudulent applications of nitrogen synthetic fertilizers to organic crops. However, combination of inorganic and organic sources could complicate interpretation of the results when using the $\delta^{15}N$ values as an indicator of agricultural regime. In addition, plant $\delta^{15}N$ values usually differ from those of the original source due to isotope discrimination during physiological processes of N assimilation within the plant. The aim of the present research is to evaluate the use of N isotope composition of pepper plants for discriminating between conventional and organic crops by studying the effect of increasing synthetic N applications to an organic-nitrogen-based nutrient solution, on the contribution of both N sources to the plant N pool and on plant isotope fractionation. Treatments consisted of irrigation with nutrient solutions with eleven different organic/inorganic N ratios: 100/0, 99.5/0.5, 99/1, 98/2, 96/4, 95/5, 92/8, 88/12, 81/19, 66/34 and 0/100. Organic N source was a liquid manure-derived fertilizer and inorganic N was applied as KNO_3. Application of NO_3 to the plants significantly affected plant $\delta^{15}N$ values from the 99/1 ratio with regard to the control treatment (100/0). The contribution of each N source to the plant N pool was estimated from the $\delta^{15}N$ values of plant exclusively grown with organic or inorganic fertilizers (100/0 and 0/100 ratios). Correlation between the NO_3 percentage in the nutrient solution and the contribution of the inorganic source (NO_3) to the N pool was fit by a single exponential rise to maximum curve: $f(x) = 97.88\ [1 - exp(-0.0876x)]$ ($R^2 = 0.92$, $P < 0.001$). The high increase observed at the first phase curve (from 100/0 to 92/8 ratio) could be attributed to plant preferential uptake of NO_3, in spite of this source representing a low percentage of the total N in the nutrient solution. Fractionation between shoot and root was only well correlated with NO_3 concentration in the root medium from 92/8 ratio, according to a linear equation ($f(x) = -2.44+1.81x$) ($R^2 = 0.97$, $P < 0.001$). The results suggest that low application of nitrate salts to plants could be detected even though when it is combined with high levels of organic N sources. In addition, the observed increasing isotope fractionation by increasing NO_3 supply point to the convenience of analysing the entire plant when using ^{15}N as indicator of N source.

Keywords: δ15N, nitrogen, pepper

A-17

DETERMINATION OF COPPER CHLOROPHYLLINS (E141[II]) IN FOOD BY ULTRAFAST LIQUID CHROMATOGRAPHY (UFLC) WITH PHOTODIODE ARRAY DETECTION

Fangyan Li[1*], Pei Geok Lee[2], Sheot Harn Chan[3]

[1 3] Food Safety Laboratory, Health Sciences Authority, 11 Outram Road, 169078, Singapore
[2] Department of Chemistry, The National University of Singapore, 3 Science Drive 3, 117543, Singapore
[*] Corresponding author–E-mail: li_fang_yan@hsa.gov.sg; Phone: +6562138922

Food colour additives E141 are copper complex of oil-soluble chlorophylls (E141[*i*]) and water-soluble chlorophyllins (E141 [*ii*]), which are prepared from the natural chlorophyll extract of edible plant materials such as lucerne. They are mainly used in food commodities such as sugar confectionary, desserts, soups and soft drinks. The EU acceptable daily intake (ADI) of E141 is 15mg/kg body weight. However, there is limited data on the levels of these additives in food products, thus making the intake estimation difficult.

In this study, a method using ultra fast liquid chromatography (UFLC) with photodiode array (PDA) detection has been developed for the determination of copper chlorophyllins in sweets and beverages. By using UFLC, the analysis time is significantly shortened compared with conventional high performance liquid chromatography (HPLC) method. Due to the complexity of copper chlorophyllins, a trisodium copper chlorophyllin salt was used as a surrogate standard and the copper chlorophyllins content in samples was expressed as total sodium copper chlorophyllin equivalents. Single laboratory validation of the method was performed in terms of linearity, precision and accuracy.

Keywords: food additives,copper chlorophyllins, UFLC

A-18

DETERMINATION OF ISOASCORBIC ACID IN FISH TISSUE BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY– ULTRAVIOLET AND TANDEM MASS SPECTROMETRIC DETECTION

Spyros Drivelos[1*], Nikolaos Thomaidis[2], Marilena Dasenaki[3]

[1 2 3] University of Athens, Athens, Greece
[*] Corresponding author–E-mail: spyrosdrivelos@gmail.com; Phone: 6936421918

D-isoascorbic acid (IAA) has only 5% of the vitamin activity of L-ascorbic acid (AA) and does not exist naturally in foodstuff. Due to the great usage of AA and IAA, there is always a need to develop a reliable, fast, simple, sensitive and low cost analytical method for their determination, convenient for all the fields of applications, which could separate AA and IAA and all their co-products, with instantaneous identification and quantification. In the current study, a hydrophilic interaction liquid chromatographic (HILIC) method was developed, which can separate AA from IAA. The stability of the IAA solutions was studied and oxalic acid 10mM was chosen as stabilizing agent in the absence of light and cooling conditions. For the development of the method, a UV-Vis detector was used and for the separation the following analytical columns were used: Cosmosil HILIC (150 × 2.0 mm, 5 μm), XBridge HILIC (150 × 2.1 mm, 3.5 μm), TSKgel Amide-80 (150 × 2.0 mm, 3 μm), APS-2 Hypersil (50 × 2.1mm, 3 μm), ZIC-HILIC (150 × 2.1 mm, 3.5 μm) and ZIC-pHILIC (150 × 2.1 mm, 5 μm). The optimized conditions were the percentage of the acetonitrile, the flow rate, the column temperature and the buffer concentration. The chromatographic parameters studied were: the capacity factor, peak area, symmetry and peak width, height of the theoretical plates and the resolution of the column. This analytical method was validated using APS-2 Hypersil column, in fish tissue (redfish) and presented adequate linearity, precision and accuracy and detection and quantification limits lower than the maximum permitted limit. In addition, a HILIC – negative ion electrospray (ESI-), tandem mass spectrometry (MS/MS) with selective reaction monitoring (SRM) was developed. ZIC-pHILIC, APS-2 Hypersil and Cosmosil HILIC were examined, acetonitrile, methanol and their mixtures of them as organic modifiers and ammonium acetate and bicarbonate ammonium as buffer solutions.

Keywords: isoascorbic, fish, HILIC, MS

A-19
EFFECT OF PARTICLE SIZE AND PH ON SOYBEAN PROTEIN SOLUBILITY

Adrian Caprita[1*], Rodica Caprita[2], Gheorghe Ilia[3]

[1 2] Banat's University of Agricultural Sciences and Veterinary Medicine, Timisoara, Romania
[3] Institute of Chemistry Timisoara of the Romanian Academy, Romania
* Corresponding author–E-mail: adi.caprita@gmail.com; Phone: 0040256277298; Fax: 0040256200296

Introduction&Objectives: The simplest criterion used for the characterization of proteins is their solubility in various media. The solubility of soybean proteins in water is strongly affected by the pH. Close to 80% of the protein in raw seeds or unheated meal can be extracted at neutral or alkaline pH. As in all legumes, the bulk of soybean proteins are globulins, characterized by their solubility in salt solutions.

The objective of this study was to evaluate the effect of particle size and pH on the protein solubility of soybean meal in 0.0357 M KOH solution.

Material&Methods: SBM samples were ground and several particle sizes were obtained using a series of standard sieves: 65 µm, 125 µm, 200 µm, 315 µm, and 630 µm. The KOH protein solubility test is based on the solubility of soybean proteins in a dilute solution of KOH. Protein was determined by the biuret method. The solubility of the protein, expressed as a percentage was calculated by dividing the protein content of the KOH-extracted solution by the protein content of the original soybean sample.

Results&Discussion: We investigated solubility for soybean meal proteins in the pH range 1–12. Up to pH 3 soy protein is very extractable; soy proteins are least soluble at their isoelectic point (4.2–4.6); above pH 6, soy proteins become extractable. A slurry of native soybean meal in water has a pH of 6.5 and 75% of the proteins are soluble. At pH 12 when the solvent is water, soy proteins are 87% soluble. The solubility of a protein depends on its free energy in solution relative to its free energy when interacting with other molecules and generally increases as the pH moves away from the isoelectric point.

The obtained results revealed a negative correlation (r = -0.9218) between the protein solubility and the particle size. Protein solubility in 0.0357 M KOH decreased as particle size increased**.** While the soluble fraction increased with reduced particle size, the crude protein value decreased. This suggests that grinding may cause fractionation of the nutrient components of SBM, and indicates the importance of determining both the crude protein and KOH-soluble protein for particles of the same size. Particles smaller than those, which pass through a 75 mesh (0.20 mm) sieve failed to produce a significant increase in protein solubility.

Keywords: protein solubility, pH, particle size

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A-20
BIOPHYSICAL METHODS USED FOR EVALUATING SOYBEAN PROTEIN SOLUBILITY

Rodica Caprita[1], Adrian Caprita[2*], Calin Julean[3]

[1 2 3] Banat's University of Agricultural Sciences and Veterinary Medicine, Timisoara, Romania
[*] Corresponding author–E-mail: adi.caprita@gmail.com; Phone: 0040256277298; Fax: 0040256200296

Introduction&Objective: Heat treatment of soybeans can reduce the activity of trypsin inhibitors and thereby improve the digestibility of protein. Excessive heat or heating time reduces the availability of amino acids due to the Maillard reaction and tends to destroy certain amino acids and reduce the nutritional value of soy protein. Protein quality of soybean meal is linked to both the reduction of antinutritional factors, and the optimization of protein digestibility. Direct analysis of both specifications is difficult in routine operations. It is therefore replaced with indirect tests such as urease index (UI), Protein Dispersibility Index (PDI) and KOH protein solubility.

The objective of our study was to compare chemical and biophysical methods for evaluating the soybean meal quality, and to establish the most suitable for evaluating over processing.

Material&Methods: Commercial SBM (43.7% crude protein) was ground to pass the 200 µm sieve. SMB was heated in a forced air oven at 120ºC for varying periods of time: 5, 10, 15, 20, 25 and 30 minutes.

The KOH protein solubility test is based on the solubility of soybean proteins in a dilute solution of potassium hydroxide. The supernatant was analyzed for the protein concentration (biuret method), the refractive index (Abbe refractometer), and the dynamic viscosity (Brookfield viscometer Model DVIII Cone CP-40).

The PDI assay is based on the solubility of soybean protein in water.

The UI assay is based on the pH increase from ammonia released from urea by residual urease enzyme in SBM.

Results&Discussion: UI is useful to determine if the SBM has been heated enough to reduce the antinutritional factors, but it is not very useful for determining if SBM has been over-processed.

KOH solubility is a good index for determining over processing, but it is not a sensitive index for monitoring under processing of SBM.

PDI is the best method of evaluating these soybean ingredients for both under heating and over heating.

The refractive index and the dynamic viscosity of dilute KOH solution extracts are highly correlated with the KOH protein solubility: r = 0.9382 for refractive index and r = 0.8943 for dynamic viscosity. Viscosity is a biophysical parameter with considerable influence in food digestibility.

Conclusion: Determination of biophysical parameters instead of chemical indices has two great advantages: the methods are very rapid and nonpolluting since they don't use chemical substances.

Keywords: protein solubility, refractive index, viscosity

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A-21
VARIATION IN THE CROP YIELD AND CHEMICAL COMPOSITION OF WILD CLOUDBERRY

Mari Jaakkola[1], Kalle Hoppula[2], Ville Korpelainen[3], Vesa Virtanen[4*]

[1 4] Biotechnology Laboratory, Kajaani University Consortium, University of Oulu, Salmelantie 43, FI-88600 Sotkamo, Finland
[2 3] MTT Agrifood Research Finland, Sotkamo Research Station, Kipinäntie 16, 88600 Sotkamo, Finland
[*] Corresponding author–E-mail: vesa.virtanen@oulu.fi; Phone: +358 8 63321311; Fax: +358 8 63321313

Cloudberry*, R. chamaemorus* L.*,* is a dioecious herb, which inhabits mainly in peaty moors, open Sphagnum bogs, forested mires, and in wet spruce forests in the Boreal zone. Individuals growing in different habitats express phenotypic variation, such as differences in mean seed, leave, and rhizome sizes. Cloudberry is nutritionally valuable berry: it is a good source of vitamins (C, A, and E), phenolic compounds (ellagitannins), and fibers. Furthermore, cloudberry has a beneficial triacylglyserol composition: over 90% of the fatty acids are linoleic, alpha-linoleic, and oleic acids.

In this study a crop yield and chemical composition of ripe-fruits of cloudberry, *Rubus chamaemorus* L. grown wild in ten different habitats in the same latitude in eastern Finland were collected and analysed in two consecutive years (2006 and 2007). Analyses of chemical composition consisted both primary (proteins and nutritional fibers, total lipids and triacylglyserol composition) and secondary metabolites (anthocyanins, β-carotene, α-tocopherol, ascorbic acid, citric and malic acids, and total phenolic compounds). Chemical composition was compared to the characteristics of the habitats and to the local climate of the year. Two habitats represented open areas whereas eight habitats were shaded. Sun shine duration was longer and rainfall lower in summer 2006 than in 2007. Crop yield varied significantly between habitats, and yields were generally lower in 2007 than in 2006. Concentrations of primary metabolites, except composition of fatty acids, were comparatively constant between habitats and consecutive years, whereas more variation was found among secondary metabolites. Higher proportion of polyunsaturated fatty acids and lower proportion of monounsaturated fatty acids were found in 2007 than in 2006. Concentrations of malic and citric acids, anthocyanins, and β-carotene varied between habitats. Highest citric acid contents were found from the berries grown in open area, and there was negative correlation in the concentrations of malic and citric acids between habitats. Significant annual variation was found in the in the content of anthocyanins and vitamin E. Habitat type and climate clearly affected to the crop yield and chemical composition of cloudberry grown in the same geographical area.

Keywords: cloudberry, chemical composition, habitat, climate

A-22

INCREASED SELECTIVITY WITHOUT LOSS OF SENSITIVITY: USE OF LC-MS^3 FOR SENSITIVE ADDITIONAL STRUCTURE INFORMATION AND LC-MRM^3 FOR HIGHLY SELECTIVE QUANTIFICATION

Axel Besa[1*], **Jan Lembcke**[2]

[1 2] Applied Biosystems, Darmstadt, Germany
[*] Corresponding author–E-mail: axel.besa@lifetech.com; Phone: +49 6151 9670 5252

Introduction: Structure determination as part of metabolism and unknown ID necessitate sensitive and selective product ion spectra. These easily can be obtained by Triple Quadrupole instruments as well as IonTraps. Due to technical setup the former suffers from bad duty cycle while IonTraps typically show weak fragment low masses (70:30 low mass cut off). The latter can be bypassed by MS^n fragmentation at the expense of loss in sensitivity and increase in cycle time.

Beside the use of MS^n experiments for gathering additional structure information MS^3 provides the capability to increase selectivity. Commonly Multiple-Reaction-Monitoring (MRM) is dedicated sensitive and selective mode for quantification on triple quad instruments but sometimes there is the need for increased selectivity to meet desired levels of sensitivity.

Method: The combination of selective precursor mass selection and sensitive fragment detection, which is necessary to get whole fragmentation pattern, can be realized by QTRAP® LC-MS/MS systems. This type of instrument combines TripleQuadrupole with linear IonTrap technology. Thus, a highly sensitive product ion scan can be realized by trapping TripleQuadrupole-like generated fragments in Q3 (linear IonTrap). In addition the linear IonTrap can now be used for secondary fragmentation (MS^3).

Unique technical enhancements of AB SCIEX QTRAP® 5500 LC-MS/MS system now enable the possibility of highly selective isolation of secondary precursor in combination with selective detection of secondary fragment. This results in most selective and MRM-like sensitive LC-MS^3 experiment for quantification (LC-MRM^3) and will solve matrix-induced interferences in MRM-mode.

Preliminary Data: In this presentation we will explain inimitably technical benefits, which enable highly sensitive Enhanced Product Ion scans as well as unique sensitivity in MS^3 experiments to increase number of detectable fragments for structural elucidation.

In terms of quantification we will present a unique strategy to increase selectivity of MRM mode in cases of complex matrices, e.g. waste-water-influent, urine of horses/cows, hair, where multiple matrix interferences were monitored and could not be solved by laborious sample preparation, derivatization, complex chromatographic development or even a combination of them. Hence, to counter those matrix induced interferences an increased selective experiment at highest sensitivity would be highly appreciated. This can be realized by LC-MRM^3 and will be shown by various applications in various matrices on AB SCIEX QTRAP® 5500 LC-MS/MS system, including fast LC conditions.

Novel Aspect: Latest generation of QTRAP® system technology: unique sensitivity in MS3 mode results in highest selectivity without loosing significant sensitivity for quantification.

A-23

OPTIMIZATION OF ACCELERATED SOLVENT EXTRACTION (ASE) OF TOTAL LIPIDS FROM FISH MUSCLE FOR GC/FID FATTY ACID AND HPLC CHOLESTEROL DETERMINATION

Saša Janković[1*], Aurelija Spirić[2], Dejana Trbović[3], Jasna Djinović[4], Danijela Vranić[5], Radivoj Petronijević[6]

[1 2 3 4 5 6] Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia
[*] Corresponding author–E-mail: sasa@inmesbgd.com; Phone: +381112650655; Fax: +3812651825

Accelerated solvent extraction (ASE), as a novel extraction technique for extraction of total lipids from fish muscle tissues, was applied. The principle of ASE is based on the use of common solvents at elevated temperature and pressure to extract fat from solid samples filled in stainless steel extraction cell containing appropriate adsorbents. The advantage of ASE technique in relation to conventional fat extraction methods is less solvent consumption, shorter extraction time, avoidance of toxic chlorinated solvents and the same, or better extraction efficiency.

The aim of this study was to optimize ASE procedure for total lipid recovery from homogenized fish muscle tissues and to compare its efficiency to the efficiency of conventional lipid extraction method, such as acid hydrolysis, followed by Soxlet extraction. The accuracy of ASE for quantitative extraction of total lipids was evaluated in relation to different solvent mixtures, various temperatures and pressures. For this purpose extraction of fat from fish muscle was carried out by using a Dionex ASE 200 instrument, in a 33 ml stainless steel extraction cells filled with diatomaceous earth, at temperatures ranging from 100°C to 125°C and nitrogen pressure ranging from 8.3 MPa to 10.3 MPa, with different solvent mixtures (n-hexane/isopropanol, n-hexane, n-hexane/acetone), by using two static cycles. Evaporation of reagent was performed by using Dionex SE 500 N_2 evaporator, at 50°C, until dryness. After statistic evaluation of fat content (n=6) obtained by using optimized ASE procedure and conventional fat extraction methods, it was evident that the best fat recoveries were achieved with ASE technique by using solvent mixture hexane/isopropanol (60:40V/V), at 100°C and 10,3 MPa, during two static cycles, lasting in total 35 min. This ASE conditions were applied to all other farmed fish samples of rainbow trout and common carp for extraction of total lipids for fatty acids and cholesterol content determination. Fat content determined in rainbow trout and common carp, by using ASE method, was 2.77±0.61% and 4.66±0.43% respectively. This fat content showed a good corelation with the fat content of rainbow trout and common carp obtained by applying conventional fat extraction method (acid hydrolysis, followed by Soxlet extraction), 2.01±0.63% and 4.57±0.63% respectively.

Fatty acids have been determined, as methyl esters, by using Shimadzu 2010 capillary gas chromatograph equipped with flame ionization detector (GC/FID) and cianopropyl-aryl HP-88 capillary column. Results obtained for fatty acid content indicate that palmitic acid is the major component of the saturated fatty acids in rainbow trout, as well as in carp. The total content of n-3 fatty acids in trout is higher than in carp, with 22:6 n-3 as the most abundant one, as well as the Σn-3/Σn-6 ratio. These data are influenced by environmental conditions and nutrition. Cholesterol content was determined by using Waters 2695 separation modul with photodiodearray detector, on Phenomenex Luna C_{18} column, at 210 nm. Cholesterol content determined in trout is lower than cholesterol content determined in carp.

Results from the present contribution show a high correlation between and ASE 200 total lipid extraction method and applied conventional fat extraction method (acid hydrolysis, followed by Soxlet extraction). However, reduced solvents, less toxicity, shorter analysis time and high sample throughoutput present a great advantage of ASE method. This enables almost complete automatization of fatty acid and cholesterol content determination in a large number of samples.

Keywords: lipids, ASE, fatty acids, fish

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A-24

BALANCE BETWEEN NUTRIENTS AND ANTI-NUTRIENTS IN EIGHT IRANIAN PLANT FOODS AS PLANT-BASED DIETS

Ali Aberoumand[1*]

[1] Behbahan University, Iran
[*] Corresponding author–E-mail: aaberoomand@yahoo.com; Phone: 00989166716540; Fax: 00986712229969

Purpose of this paper: To examine the nutritional properties of eight edible plant foods: Name them. The foods were analyzed with standard analysis methods in order to detect several nutrient and anti-nutrient compounds present in each. These included: water, starch, free sugars, such as glucose, fructose and sucrose, and,. phytic acid and trypsin inhibitors

Design/methodology/approach: A range of chemical and HPLC analysses were employed.

Key Findings: The eight edible plants formed three groups according to their nutritional properties, each being suitable for a different technological processes. *Cordia myxa* had the highest concentration of sucrose (29.09 g/100 g) probably due to a better storage process.

Practical implications: Three plants (*Momordica dioicia*, *Eulophia ochreata* and *Portulaca oleracia)* are suitable for high temperature food processes, because they have very low free sugars concentrations; thereby reducing the possibility of Maillard reaction and subsequent acrylamide formation.

Keywords: Anti-nutrients; Edible plants; Nutrients

A-25

EXTRACTION AND PURIFICATION OF INOSITOLS AND IMINOSUGARS FROM PLANT EXTRACTS

Sonia Rodríguez-Sánchez[1], Oswaldo Hernández[2], Maria Luz Sanz[3*], Ana Isabel Ruiz-Matute[4]

[1 2 3 4] Instituto de Química Orgánica General. Consejo Superior de Investigaciones Científicas, Madrid, Spain
[*] Corresponding author–E-mail: mlsanz@iqog.csic.es; Phone: +34 912 587 451; Fax: +34 915644853

In recent times there is a high interest in the obtainment of carbohydrates with biological functions for pharmacological and/or food purposes.

Inositols are cyclic alcohols which have beneficial biological properties. Myo-inositol is present in most of vegetable sources, but there is a special interest in minor cyclitols such as chiro-inositol which seems to be related to the insuline action (Larner J. et al., Int. J. Exp. Diabetes Res., 2002, 3, 47), pinitol (metil-chiro-inositol) or its glycosides (fagopyritols), etc.

Iminosugars or azasugars, which are present in microorganisms and plants, are polyhydroxylated derivatives of piperidine, pyrrolidine, indolicidine and nortropane. They have diverse ecological and pharmacological activities, being the inhibitory effect against glycosidase the most studied and reported (Oku T., Br. J. Nutr, 2006, 95, 933).

Both inositols and iminosugars can be extracted from natural sources, being a previous purification from other low molecular weight carbohydrates, such as pentoses, hexoses or sugar acids, an essential requirement. Therefore, the aim of this work was to optimise a rapid and effective method for the extraction and purification of inositols and iminosugars from other low molecular weight carbohydrate interferences of Morus sp. (Morus alba and Morus nigra) in which both inositols and iminosugars had been previously described.

Samples of leaves, branches and fruits of mulberry were used for this work. Analyses were carried out by GC-MS using a capillary column coated with methylsilicone as stationary phase. Carbohydrates were previously converted to their trimethylsilyl oximes (Sanz, M.L. et al., J. Chromatogr. A, 2004, 1059, 143).

Extraction procedure was optimised using acidulated (with 0.1% HCl) and non-acidulated methanol, ethanol, hot and cold water. Acidulated cold water was the most appropriate solvent for extracting the highest amounts of inositols and iminosugars with the lowest contents of other low molecular weight carbohydrates.

Extracts were purified using a yeast treatment, incubation time being optimised for each extract. Removal of carbohydrate interferences of mulberry was achieved by this treatment and an enriched fraction of iminosugars and inositols was obtained. However, the appearance of trehalose interferes in the yield. Further studies will be done to improve this procedure.

Keywords: inositols, iminosugars, yeast, purification, bioactivity

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A-26

APPLICATION OF HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY FOR THE ISOLATION OF POLYPHENOLS FROM AMONTILLADO SHERRY WINE

Fabian Weber[1], Mónica Schwarz[2], Enrique Duran[3], Dominico Guillen[4], Carmelo G. Barroso[5], Peter Winterhalter[6*]

[1 6] Institut für Lebensmittelchemie, TU Braunschweig, Germany
[2 3 4 5] Centro Andaluz de Investigaciones Vitivinicolas, Universidad de Cadiz, Spain
[*] Corresponding author–E-mail: p.winterhalter@tu-bs.de; Phone: 00495313917226

Amontillado wines are produced in southern Spain following a complex ageing process which varies in length, lasting at least 3 years. Amontillado wine production is carried out in wooden barrels by means of a special procedure known as the *criaderas* and *soleras* system, the same procedure that is used for the other types of Sherry wines (*Finos* and *Olorosos*). During ageing, the wine acquires its typical characteristics through the changes taking place in its composition. The wine that has been used in this study is an aged Amontillado from a *Solera*. The objective of this study is to characterise the main polyphenolic compounds to be found in this aged wine.

The wine was poured onto a glass column filled with Amberlite XAD-7 and the column was washed with water to remove sugars, organic acids, proteins and salts. Phenolic compounds were retained by the resin and eluted using a mixture of methanol-acetic acid (19:1; *v/v*). Methanol was evaporated *in vacuo* and the aqueous solution lyophilized. The lyophilizate was separated by high-speed countercurrent chromatography. The binary solvent systems MTBE/1-Butanol/Acetonitrile/Water (1.1:3:1.1:5; *v/v/v/v*, acidified with 0.1% TFA) and MTBE/n-Butanol/Acetonitrile/Water (2:2:1:5; *v/v/v/v*) were successfully used for the separation. Furthermore the XAD7 extract of Amontillado was extracted with ethylacetate and water to obtain a less complex and more apolar fraction of the XAD-7 extract. This ethylacetate extract was fractionated with Hexane/Ethylacetate/Methanol/Water (1:5:1:5; *v/v/v/v*) as HSCCC solvent system. The fractions were analysed by HPLC-PDA and HPLC-ESI-MS-MS. Structure elucidation by NMR-spectroscopy is in progress. The antioxidant activity of the fractions was also studied.

Keywords: amontillado, HSCCC, ageing, polyphenols

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A-27

ANALYSIS OF SUGAR CONTENT FROM SCHOOL MEALS IN CHUNCHEON, KOREA

Hee Yun Kim[1], Se Jong Park[2*], Jin Sook Lee[3], Young Hun Kim[4], In Sun Choi[5], Min Ja Cho[6], Min Su Shin[7], Jae Sang Song[8], So Young Chung[9], Seon Hee Choi[10]

[1 2 3 4 5 6 7 8 9 10] Seoul Regional Food and Drug Administration, Seoul, Republic of Korea
[*] Corresponding author–E-mail: sjpark517@korea.kr; Phone: 82-2-2640-1467; Fax: 82-2-2640-1364

Nutritional unbalance in teenagers is getting worse during last two decades in Korea. 12.0% of children and teenagers are overweight or obese according to the third Korea National Health and Nutrition Examination Survey (KNHANES Ⅲ). Among several reasons, eating the high sugar-containing foods can be a cause of nutritional unbalance and result in the deficiency of other nutrients by fulfilled calorie intake. So our government is about to set up the nationwide food safety system with strict control of nutrients like sugar, fatty acids and sodium as well as advanced nutrient education system. In addition, almost a hundred percent of school food service rate forced the government to consider more effective ways to upgrade the nutritional status of school meals.

The purpose of this study was to analyze the content and consumption of total sugar in school meals. We investigated meal intakes of school lunch in Chunceon, Korea. Analysis of sugar content was performed in main dishes, side dishes and desserts which were supplied from elementary and middle school foodservices. The sugar content was extracted with 50% ethanol after defatting from various types of food. Results of recovery tests were above 88%. We simultaneously analyzed sugars such as fructose, glucose, sucrose, maltose and lactose and sugar alcohols such as xylitol, sorbitol and mannitol with HPLC-RID (Refractive Index Detector). Detection limits of sugars and sugar alcohols ranged from 0.001% to 0.005%. Correlation coefficient (r^2) of calibration curve was 0.9999 in sugars and sugar alcohols. Based on the data of sugar content, sugar intake per meal of students was calculated.

Keywords: Sugar, Intake, School meal, HPLC

A-28

VALIDATION OF DUMAS COMBUSTION METHOD (LECO TRUSPEC CHNS) FOR TOTAL NITROGEN CONTENT DETERMINATION IN CEREALS AND OILSEEDS

Bojana Beljkaš[1*], Jovana Matić[2], Ivan Milovanović[3], Aleksandra Mišan[4]

[1 2 3 4] Institute for Food Technology, University of Novi Sad, Novi Sad, Serbia
* Corresponding author–E-mail: bojana.beljkas@fins.uns.ac.rs; Phone: +381 21 485 3837; Fax: +381 21 450 728

During routine laboratory analysis, there is a demand for a fast, reliable and precise determination of nitrogen (N), and hence crude protein (CP) content in oilseeds, cereal grains and their products, to determine their nutritional quality. For that purpose, we have validated the Dumas combustion method (CM), using Leco's CHNS Truspec analyzer. Validation was done on a wheat flour sample. Following parameters were determined: limit of detection (LOD=0.006% N), limit of quantification (LOQ=0.019% N), precision under repeatability conditions (Sr=0.01% N, RSDr=0.41% N), precision under reproducibility conditions (SR=0.02% N, RSDr=0.74% N), reproducibility under interlaboratory conditions and trueness (using certified reference material). For 15 cereals samples compared, the Kjeldahl procedure gave N values slightly lower than the Dumas procedure: NKjeldahl= 0,9905×NDumas- 0,0376 (R^2=0,9996). Complex cereal products in which is not easy to prepare representative sample using small sample size needed for the Dumas combustion method, are also often encountered. Thirteen samples of cereal breakfast and cereal bars have shown smaller Sr and RSDr values when analyzed using Kjeldahl method, owing to larger sample size, and thus, easier preparation of representative samples. Based on the validation data, it can be concluded that the Dumas combustion method could be used instead of the Kjeldahl method for routine laboratory analyses of cereal foods including complex cereal products. However, care must be taken to ensure sample homogenization due to small sample size taken for the analysis.

Keywords: validation, Dumas method, cereal, oilseed

A-29

CONCENTRATIONS OF TRACE ELEMENTS IN TOMATO FRUITS AND THEIR CULTIVATION SUBSTRATES

Irantzu Martinez-Arkarazo[1], Josu Trebolazabala[2], Azibar Rodriguez-Iruretagoiena[3], Silvia Fdez-Ortiz de Vallejuelo[4], Juan Manuel Madariaga[5], Eneko Atxa[6], Alberto de Diego[7*]

[1 2 3 4 5 7]Department of Analytical Chemistry, University of the Basque Country, P. O. Box 644; 48080 Bilbao, Basque Country
[6] Azurmendi jatetxea, Legina auzoa, 48195 Larrabetzu, Basque Country
[*] Corresponding author–E-mail: alberto.dediego@ehu.es; Phone: 0034946015550

Tomato is one of the most consumed vegetable all over the world. Its content in trace elements should be closely related to the chemical and mineralogical composition of the cultivation substrate. The latter can be largely altered by addition of fertilizers and other chemicals frequently used in agriculture. Healthy consumption of vegetables requires strict control of their quality, including concentrations of potentially dangerous chemicals of anthropogenic origin such as trace elements like As, Cd, Cu, Pb or Zn. Surprisingly, studies on the trace element composition of tomatoes are not abundant.

We present here analytical methods for the simultaneous and fast measurement of the concentration of several elements in tomato fruits and soils by inductively coupled plasma-mass spectrometry (ICP-MS) after acid digestion of the sample accelerated by the use of focused ultrasound energy (USF). The methods have been optimised and their reliability checked using reference certified materials.

In addition, tomato fruits from different locations of the Basque Country were sampled together with aliquots of the surrounding cultivation soil. The three different phases obtained after centrifugation of the tomato fruits (aqueous, fat and pulp) were collected and independently analysed. The soil samples were also analysed to determine the acid extractable fraction of each element, a concentration which is well above the bioavailable fraction. The results obtained were used to investigate the distribution of trace elements in the fruit and in an attempt to look for correlations between concentrations found in the different parts of the fruits and those measured in the soils.

Keywords: tomatoes, trace elements, ICP-MS

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A-30

INFLUENCE OF PERFLUORINATED CARBOXYLIC ACIDS ON SEPARATION OF POLAR BETACYANINS IN ION-PAIR HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY

Sławomir Wybraniec [1*], Paweł Stalica [2], Gerold Jerz [3], Bettina Klose [4], Nadine Gebers [5], Peter Winterhalter [6], Aneta Spórna [7], Maciej Szaleniec[8], Yosef Mizrahi [9]

[1 2 7] Faculty of Analytical Chemistry, Institute C-1, Cracow University of Technology, Cracow, Poland
[3 4 5 6] Institute of Food Chemistry, Technische Universität Braunschweig, Braunschweig, Germany
[8] Joint Laboratory of Biotechnology and Enzyme Catalysis, Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Cracow, Poland
[9] Department of Life Sciences, Ben Gurion University of the Negev, Beer-Sheva, Israel
[*] Corresponding author–E-mail: swybran@chemia.pk.edu.pl; Phone: +48-12-628-3074; Fax: +48-12-628-2036

In the last decades, high-speed countercurrent chromatography (HSCCC) has been shown in numerous applications to be a versatile preparative scale separation technique especially used in the field of natural product isolation. Recently, betalains (betacyanin and betaxanthin plant pigments) from deeply coloured poke-berries (*Phytolacca americana*) were separated for the first time by HSCCC [1].

Using ion-pair forming additives had become in our labs a very important direction for large-scale isolation of highly polar plant pigments which normally do have a very limited thermal and chemical stability [1]. Furthermore, excellent scale-up possibilities by HCCCC provide a possibility of recovering of larger amounts of pure betalains for thorough '*in-vitro*' and '*in-vivo*' physiological evaluations and other research purposes.

One of the most important physicochemical properties of betalain plant pigments are their significant polarity and ionization (zwitter-ionic behavior) in aqueous solutions. The increased polar character of betalains results in insolubilities in any of the popular organic polar or semi-polar solvents except of water and low-molecular alcohols [1]. It is known that degradation is advancing in alcoholic solutions. Hence, both factors such as high hydrophilicity and low chemical stability of betalains result in strong limitation of finding appropriate solvent systems.

In this study, polar betalains from edible ripe cactus fruits of *Hylocereus polyrizhus* (Cactaceae) were fractionated by means of preparative ion-pair HSCCC with the application of new solvent systems containing different concentrations of trifluoroacetic acid (TFA), and heptafluorobutyric acid (HFBA), respectively. The addition of 0.7% of HFBA was extremely effective to separate betanin from phyllocactin and hylocerenin. The slight increase of HFBA concentration to 1.0% highly influenced the chromatographic conditions for the pigment separation and significantly decreased the chromatographic resolution between the pigments. In the case of HFBA, we postulate that there seem to be narrow concentration optima where a HSCCC separation under ion-pair conditions is getting to its highest performance – very depending on the compounds which need to be separated. The use of ion-pair reagents during HSCCC to separate the sensitive and highly degradable betalain pigments enabled the larger scale recovery while reducing the risk of excessive degradation.

The increasing demand for research on pure betalains is clearly justifying the direction of performing ion-pair high-speed countercurrent chromatography. So far, no alternatives for perfluorinated additives for IP-chromatography were investigated and will require more research efforts.

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Keywords: betanin, phyllocactin, hylocerenin, countercurrent chromatography

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A-31
ASSESSMENT OF SODIUM INTAKE FROM SCHOOL LUNCHES IN SOUTH KOREA

S.K Lee[1*], E.J Chang[2], K.N Bahn[3], C.S Kang[4], M Kim[5]

[1 2 3 4 5] Imported Food Anlysis Division, Food & Drug Administration, Incheon, South Korea
[*] Corresponding author–E-mail: sunk93@kfda.go.kr; Phone: +82-32-450-3368; Fax: +82-32-442-4662

Chronic diseases such as obesity, diabetes and high blood pressure have been major public concerns. Sodium is an essential element to maintain the homeostasis and physical metabolism. But over accumulation of sodium in the body can cause the problems such as hypertension, stroke, stomach cancer, kidney disease and heart disease. The purpose of this study is to estimate dietary sodium exposure and determine contents of sodium from school meals in Incheon. In this study, we collected 706 samples from 4 elementary schools(5th grade, 62 males, 67 females) and 4 middle schools (2nd grade, 92 males, 94 females) for 15 days in Incheon. The samples were analyzed using AAS (Atomic Absorption Spectrometery) after microwave digestion. The average recovery of sodium in the different matrices was about 104%. Limit of detection (LOD) and Limit of qualification (LOQ) were 2.9 μg/kg and 8.9 μg/kg, respectively. The average meal (lunch) intakes of elementary school and middle school were 364.2±61.0 g and 456.1±81.3 g, respectively. The average sodium intakes through lunch of elementary school and middle school were 843.1±224.9 mg and 1,031.5±330.0 mg, respectively. The results of the study showed that sodium intake from school lunches was about 47% of recommended daily intake(RDI, 2,000 mg per day) established by WHO. Therefore, it would be recommended that sodium exposure should be continuously monitored.

Keywords: sodium, school meal, RDI

A-32

WIDE-SCOPE SCREENING OF MANY DIFFERENT ORGANIC POLLUTANTS AND RESIDUES BY GC-TOF-MS AND UHPLC-QTOF-MS IN THE FIELD OF PUBLIC HEALTH

Felix Hernandez[1*], Ramon Díaz[2], Tania Portoles[3], Juan Vicente Sancho[4] Frances Centrich[5], Antoni Rubies[6], Eulalia Muñoz[7]

[1 2 3 4] University Jaume I, Spain
[5 6 7] Agencia de Salud Pública de Barcelona, Spain
[*] Corresponding author–E-mail: felix.hernandez@uji.es; Phone: 34-964-387366; Fax: 34-964-387368

Nowadays, most of public health laboratories apply target methods based on liquid and/or gas chromatography coupled to mass spectrometry (LC-MS and GC-MS) to determine a wide range of organic residues and contaminants (from pesticides in vegetables to veterinary drugs in meat, as representative examples) in many different sample matrices. These target methods are normally directed towards quantification, involve a limited list of analytes and employ MS analyzers like single quadrupole, ion trap and, in the last decade, triple quadrupole. Using target methods can lead to ignore other relevant contaminants that could be present in the sample. In addition, this approach increases the analysis time as a battery of methods have to be applied separately to the same sample as a consequence of the different chemical characteristics of selected analytes. Under these circumstances, there is an urgent need of developing wide-scope universal screening methods for contaminants in the public health field. TOF MS analyzers are one of the most powerful analytical tools for this purpose.

In this work, a wide screening of organic pollutants in different food samples has been developed by combining UHPLC-(ESI)QTOF MS and GC-(EI)TOF MS. The high sensitivity in full spectrum acquisition mode and the accurate mass data provided by TOF MS allow to notably increase the number of compounds to be investigated, with the possibility of searching for a larger number of analytes in a single analysis. Sample preparation was made by Accelerated Solvent Extraction with ethyl acetate as "universal" extraction solvent. Automated Gel Permeation Chromatography was applied for clean-up when necessary (fatty matrices).

Searching of organic contaminants in the food samples analyzed was faced up following different data processing strategies depending on the origin of the full spectrum acquisition data, i.e. GC-TOF MS or LC-TOF MS. The predictable presence of the molecular ion in LC-(ESI)TOF data allowed an automatic and rapid "post-target" searching for more than 1000 LC amenable compounds (including pesticides, antibiotics, veterinary drugs and banned dyes, among others, as well as several metabolites) by extracting the chromatogram, with narrow-mass window, at the exact mass of the molecular ion. More than 200 standards of the most frequently detected compounds were also injected; thus, the information of retention time and in-source fragmentation helped us to their more reliable identification. In the case of GC-(EI)TOF MS data, additional information was obtained by injecting standards before performing the screening. Up to five representative m/z ions were selected from the EI spectrum for each analyte and a plausible chemical structure was proposed for the selected fragments followed by their exact masses calculation. This part was also applied to more than 200 compounds, including PCBs, PAHs, PBDEs, alkyphenols and a notable number of pesticides like insecticides (organochlorine, organophosphorous, carbamates and pyretroids), herbicides (triazines and chloroacetanilides), fungicides and some metabolites. GC-TOF also allowed the investigation of non-target compounds using appropriate processing software to manage MS data and available commercial libraries for electron ionisation spectra.

The potential of combining two powerful techniques, as GC-(EI)TOF MS and UHPLC(ESI)QTOF MS, for wide-scope screening of food samples have been proven in this work by analyzing real-world samples.

Keywords: universal screening, organic contaminants, TOF-MS

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A-33

ILVO'S FOOD-RELATED RESEARCH SERVICES: POWERFUL TOOLS FOR FOOD SAFETY, FOOD PRODUCT QUALITY AND FOOD PRODUCT INNOVATIONS

Hendrik De Ruyck[1*], Lieve Herman[2]

[1 2] ILVO, Melle, Belgium
[*] Corresponding author–E-mail: hendrik.deruyck@ilvo.vlaanderen.be; Phone: + 32 9 272 30 31; Fax: + 32 9 272 30 01

ILVO's Technology and Food Science Unit performs research to improve the chemical and microbiological food safety, the food composition- and transformation-related quality, and the food authenticity of animal and vegetable products along the food chain. The samples analysed are of mostly animal origin. The majority of the service laboratories in the Technology and Food Science Unit are Beltest accredited. Together, they form a powerful tool for both the food sector and the government. More than 50 analyses on food matrices are performed in four different laboratories: the chromatographic laboratory, the laboratory for physical and chemical analyses and ring trial organisation, the microbiological and GMO laboratory, and the laboratory for the detection of antibiotics. The scientific guidance task fulfils an important role in guiding Milk Control Centre (MCC)-Vlaanderen and the Belgian dairy industry. MCC-Vlaanderen is responsible for the determination of the quality and the composition of raw farm milk, the price of which is fixed according to their quality and composition parameters. The scientific guidance contains following aspects: comparative studies, standards, control samples and recombined series. Scientific guidance of the dairy industry by means of comparative studies, recombined series and workshops allow the dairy industry to link themselves to reference methods for the determination of the composition and the quality of raw farm milk on following parameters: total flora, number of coliforms, freezing point, fat and protein content and detection of ß-lactam antibiotics. The Research Unit also has an extended pilot infrastructure at its disposal, which is available for applications for third parties of the food and the feed industry on confidential basis. The different pilot-apparatus for pilot trials are pasteurisation (possibly combined with skimming, bactofugation and homogenisation), cheese-production, UHT-treatment, evaporation and spray-drying, ice-cream production and extrusion. A considerable expansion of the pilot plant is planned for 2010 including apparatus for meat processing, whole meal production, packaging, freezing and cooling etc. Finally, the different partners in the food production chain including farmers and SMEs can consult technological advice about food processing and food quality.

Keywords: analysis, guidance, trials, advice

A-34

NUTRITIONAL PROPERTIES OF FILLETS FROM TRA CATFISH (PANGASIUS HYPOPHTHALMUS) IMPORTED INTO EU

Fabio Caprino[1], Dinesh Velayutham[2], Maria Letizia Busetto[3], Mauro Vasconi[4], Federica Bellagamba[5], Vittorio Maria Moretti[6*]

[1 2 3 4 5 6] Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università degli Studi di Milano, Italia
[*] Corresponding author–E-mail: vittorio.moretti@unimi.it; Phone: +39 (0)2 50315760

Pangasius (Pangasius hypophthalmus) is a catfish farmed in the Mekong delta region and commercialized in more than 70 countries as frozen or thawed fillet. The EU is the main market for pangasius from Vietnam, with about one third of imports in quantity and 40 per cent in value terms (225.000 tons in 2008). Within the EU, Spain is the biggest market. Pangasius has good marketing value also in Italy and it is rather appreciated by consumers due to its low price and low lipid content. Beside these benefits, few information about the real conditions of farming and the nutritional properties of its meat is available. The aim of the present work was to investigate the chemical and nutritional properties of pangasius fillets in order to provide a better information to the consumer. For this aim 83 samples of fillets were collected from the international fish market of Milan and from local retailers and were analyzed for their proximate composition, fatty acid profile, total phosphorus and additives content. Results showed that fillets were characterized by a high moisture (84.5±2.2%) and a low protein (12.6±2.2%) and lipid (1.4±0.7%) content. Moreover, the intramuscular lipids were characterized by a high percentage of saturated (43.0±2.1%) and monounsaturated (38.8±3.4%) fatty acids, and by a low percentage of polyunsaturated (18.2±4.5%) fatty acids. Among polyunsaturated, linoleic acid (18:2n-6) was the most representative fatty acid with a percentage of 8.9±1.6%. The chemical and nutritional properties of pangasius fillets differed from those of other farmed fish species, especially for their low content in n-3 fatty acids (4.0±1.8%).

Keywords: pangasius, fatty acids, additives

A-35
EXTRACTION AND DETERMINATION OF TRANS FATTY ACIDS CONTENT IN COMMERCIAL ICE CREAMS BY GAS CHROMATOGRAPHY

Salvador Maestre[1*], Jose Luis Todolí[2], Soledad Prats[3], Eduardo Paredes[4]

[1 2 3 4] University of Alicante, Department of Analytical Chemistry, Nutrition and Food Sciences, Alicante, Spain
[*] Corresponding author–E-mail: salvador.maestre@ua.es; Phone: +34965903777; Fax: +34965903527

The evaluation of the composition of foods is an important issue from different points of view. For safety reasons the investigation of the presence of contaminants in foods has traditionally received great attention, now the characterization of the nutritive and non nutritive components is been addressed because their relationship with the effects of these components on health of humans.

In the present work the characterization of the lipid fraction of different ice cream is carried out. The work conducted focuses the attention on the fatty acid content of this food item and, specially, on the trans-isomers of the major fatty acid i.e. C18:1 and C18:2 isomers. To this end several ice cream samples acquired from local supermarkets were analyzed, in addition artisanal ice cream samples prepared by local producers were also characterized.

Different extraction schemes, i.e. sample amounts, solvents and solvent mixtures and extraction times, were tested in order to optimize the analysis scheme. In addition solid phase extraction was also employed in order to fractionate the isomers of the previously quoted fatty acids. The identification of the different isomers was carried out by Gas Chromatography using Flame Ionization Detection and Mass Spectrometry Gas Chromatography. The profile of the fatty acids of these samples was compared the main trans-fatty acid components was determined.

Keywords: ice cream; trans-fatty; acid; CG

A-36
ANALYSIS OF BLACK TEA THEARUBIGINS FROM SIX DIFFERENT COMMERCIAL TEAS BY ESI-FTICR MASS SPECTROMETRY

Nikolai Kuhnert[1], Michael Clifford [2], Matthias Witt[3*], Rob van der Heijden[4]

[1] Jacobs University, Bremen, Germany
[2] University of Surrey, Guildford, UK
[3] Bruker Daltonik GmbH, Bremen, Germany
[4] Bruker Daltonics, Wormer, The Netherlands
[*] Corresponding author–E-mail: mwi@bdal.de; Phone: 0049 412 2205 268; Fax: 0049 421 2205 107

Thearubigins isolated from six commercial black teas have for the first time been analyzed by Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometry (MS). Up to 9 000 mass spectral peaks have been detected per sample using the ultra-high resolving power of FT-ICR MS. Due to the high mass accuracy better than 1 ppm molecular formulas assigned to up to 1500 compounds. More than 50 compounds have been named by comparison to the available literature. Not allowing for isomers, these thearubigin samples contain at least 2500 compounds, thus explaining the inability to resolve individual compounds chromatographically.

Data interpretation strategies developed for petrolomics studies (van Krevelen and Kendrick analyses) have been applied to black tea. A novel software program and a protocol have been developed to refine these procedures for the investigation of polyphenols. Using homologous series analysis oxygenation has been identified arising by nucleophilic addition of water to aromatic CH groups as a key feature. A series of reaction schemes have been developed linking known precursors with plausible products that match the available MS data. The accuracy of these predicted structures will be assessed critically by ion trap MS procedures.

Keywords: tea, FT-ICR, mass spektrometry, polyphenols

A-37
COMPARISON OF GC/FID AND DART/MS METHODS FOR ANALYSIS OF VEGETABLE OILS

Jana Kohoutkova[1*], Lukas Vaclavik[2], Jana Hajslova[3]

[1 2 3] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: jana.kohoutkova@vscht.cz; Phone: +420 220443184

Vegetable oils are important commodity in food and beauty industry. Food control authorities experience frequent problems associated with substitution of declared oil by cheaper and more affordable oil.

A conventional method for the identification of oil type is based on the determination of fatty acids by gas chromatography coupled to flame ionization detector (GC/FID). Although this method is very reliable, but labour intensive and time-consuming. In addition, GC/FID method includes several sample preparation steps prior to the instrumental analysis.

A novel alternative approach for the identification of oil type is based on the recently introduced direct analysis in real time ion source coupled to mass spectrometric detection (DART/MS). This kind of ion source is operated in the open air under the ambient conditions. The ionization is performed by reaction of electronic or vibronic excited-state species with reagent molecules and analytes [1]. During the ionization of vegetable oils employing DART/MS, pseudomolecular ions $[M+H]^+$ of present triacylglycerols (TAGs) are instantly observed in the mass spectrum. In the presence of ammonia vapours, $[M+NH_4]^+$ adduct ions are presented, typically with higher abundance compared to $[M+H]^+$. Additionally, ions with lower m/z ratios are produced via loss of one or two fatty acid residues from the glycerol backbone. These mass spectrometric profiles of oils may be used for differentiation of oils with different composition of triacylglycerols [2]. The main advantages of DART/MS, compared to conventional technique (GC/FID), involve the possibility of direct sample examination in the open atmosphere, minimal, or no sample preparation requirements, and, remarkably high sample throughput.

In this study, both methods for the analysis of vegetable oils based on GC/FID and DART/MS are compared.

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Keywords: DART, fatty acid, triacylglycerols

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A-38

CAN WE OVERFEED OUR BABIES WHEN GIVING THEM INFANT FORMULAE AVAILABLE ON THE EUROPEAN MARKET?

Marchela Pandelova[1*], Walkiria Levy Lopez[2], Subrina Kasham[3], Catherine Leclercq[4], Raffaela Piccinelli[5], Karl-Werner Schramm[6]

[1 2 3 6] Helmholtz Zentrum München, Neuherberg, Germany
[4 5] National Research Institute for Food and Nutrition, Rome, Italy
[*] Corresponding author–E-mail: pandelova@helmholtz-muenchen.de; Phone: +49 89 3187 2932

The prevalence of overweight and obesity in children is rising in Europe. During infancy, diet may have a greater impact on nutrient balance and hormone response than energy expenditure. A rapid weight gain during the first four months of life has been significantly associated with an increased risk of being overweight at age 7, regardless of birth weight and weight at 1 year. Obese children suffer serious health problems, like diabetes and heart disease, often carrying these conditions into an obese adulthood. Therefore, energy intake in excess of nutritional needs should be avoided in the infancy period with high priority.

The aim of the present study was to assess the total daily energy intake of infants (aged 0–4 month) exclusively fed with infant formulae available on the EU market. All the products were identified from the European market share data of the year 2007 covering 22 countries. The largest 8 holding companies together constitute over 80% of the EU market of "starting" infant formulae were identified. Finally, 26 products, respectively 11 milk formulae, 6 soy formulae, and 9 hypoallergenic formulae were identified and included in this study. The calculated daily energy intake obtained according to supplier labelling instructions was in tendency higher than the daily energy requirements based on WHO recommendations, considering the standard body weight established by FAO. In addition, the daily energy intake was also calculated based on direct measurements of the amount of formulae contained in the spoon provided by the supplier. It was found that the kcal /day intake based on the direct spoon measurements were even higher than the values recommended by international organizations. Considering individually the three infant formulae typologies, the highest difference was found in milk- and hypoallergenic- infant formulae. In particular, the calculated daily energy intake according to the supplier labeling is about 105 kcal/day (19%) in excess for milk-based products. As well, the calculated daily energy intake according to the measured spoon weight was on average 158 kcal/day (28%) above the international recommendations in hypoallergenic products.

These findings are valid for more than 90% of the infant formulae brands considered, which represent 80% of the EU market. This situation may therefore, affect a large proportion of EU infants who are not breastfed, in case the infants take the food potentially offered to them and accept the complete intake.

Keywords: infant formulae, obesity, EU

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A-39

JELLY MINI-CUPS: EVALUATION OF A CHOKING HAZARD, A CASE STUDY FROM THE GOVERNMENT CHEMIST'S PROGRAMME

Peter Colwell[1*], Michael Walker[2], Ian Axford[3], Jack Crane[4]

[1 2 3] LGC, Teddington, England
[4] State Pathology Department, Belfast, Northern Ireland
[*] Corresponding author–E-mail: pete.colwell@lgc.co.uk; Phone: +44 (0)20 8943 7443

There have been several instances worldwide of children and elderly people choking to death on soft slippery dome shaped jellies that were designed to be placed in the mouth in one bite. This problem was addressed in the European Union by the provisions of Directive 2006/52/EC of the European Parliament and of the Council prohibiting the use of a range of gel forming additives in jelly mini-cups. Jelly mini-cups are defined in the Directive as "jelly confectionery of a firm consistence, contained in semi-rigid mini-cups or mini-capsules, intended to be ingested in a single bite by exerting pressure on the mini-cup or mini-capsule so as to project the confectionery into the mouth".

In the UK the Government Chemist is required to act as the national focus of technical appeal in specified areas where there is an actual or potential dispute between food businesses and regulator. The specified areas are broadly drafted but in practice tend to focus on the results of chemical analysis or their interpretation in the agrifood sector. When a consignment of jelly mini-cups alleged to contravene the above provisions was detained for destruction by a Port Health Authority the importer, in possession of a contrary view from another part of the regulatory system, appealed to the Government Chemist for a ruling.

It was common ground that the product contained E407 carrageenan, one of the banned additives and to reach a conclusion the Government Chemist needed to provide an interpretation of the Directive definition based on objective tests, described in detail in this paper. The Government Chemist found that the samples were covered by the Directive definition of jelly mini-cups and therefore the presence of E407 carrageenan was not permitted in the products which thus failed to conform to the provisions of Directive 2006/52/EC and the Miscellaneous Food Additives Regulations 1995 as amended.

This conclusion was accepted by all parties on the basis of the evidence obtained by tests carried out by scientists from the Government Chemist's Programme in LGC and the consignment was destroyed. The findings were based on the product size (small parts cylinder), weight, shape, solubility in a saliva simulant, compressibility and the slippery nature of its surface.

Keywords: Additives, Jelly, Choking, Mini-cups, Gums

A-40
EVOLUTION OF A RESIDUE LABORATORY NETWORK AND MANAGEMENT TOOLS FOR MONITORING ITS PERFORMANCE

Angelo Queiroz[1*], **Erick Lins**[2]

[1 2] Ministry of Agriculture, Livestock and Food Supply, Brasilia, Brasil
[*] Corresponding author–E-mail: angelo.mauricio@agricultura.gov.br; Phone: +55 61 32182535

Since 2005 the National Residue Control Plan in Brazil has been considerably enhanced, increasing the quantity of samples, substances and species monitored, and also the analytical detection capability. The Brazilian laboratory network is being forced to improve its quality standards in order to comply with the NRCP's own evolution. Many aspects such as the LoQs, the quality management systems within the laboratories and appropriate method validation are in continuous improvement, generating new scenarios and demands. Thus, efficient management mechanisms for monitoring the network performance and its adherence to the established goals and guidelines are required. Performance indicators associated to computerized information systems arise as a powerful tool to monitor the laboratories activity, making use of different parameters to describe this activity on a day-to-day basis. One of these parameters is related to the turnaround times, and this factor is highly affected by the way each laboratory organizes its management system as well as the regulatory requirements. In this work it is shown the global view of the turnaround times related to type of analysis, laboratory, number of samples per year, type of matrix, country region and period of the year, all these data being collected from a computerized system called SISRES. This information gives a solid background to management measures aiming at the improvement of the service offered by the laboratory network.

Keywords: NRCP; laboratory network; performance

A-41
EFFECT OF THE TYPE OF NUTRITION ON THE PUPILS OF PRIMARY SCHOOLS IN ERBIL CITY

Fatin I. Aziz[1*]

[1] School of Govavand, Ministry of Education, Erbil, Iraq
[*] Corresponding author–E-mail: havras@yahoo.com; Phone: +9647504661563

Nutrition is one of the youngest of the sciences, but it is a highly important one. One has only to give a thought to the food situation of the world to-day to realize that nutrition, if not the very life of an individual, and indeed of nations, depends on the adequate production and effective distribution, the wise choice and suitable preparation of food for human consumption. Apart from life itself, good nutrition means good health, and good health is one of the main factors in promoting personal well-being, efficiency in work and play, and, in the long run, a sense of social worthiness. The lack of qualified teachers is one obstacle. More must be done in training teachers to impart up-to-date, accurate knowledge of nutrition in an interesting manner. In the meantime it is recognized that some attention is being given in the training colleges to the theoretical side of nutrition as part of the course of instruction in hygiene (school and personal). It is very necessary that school teachers should be so trained as to be able to give talks on nutrition. In the primary school the class teacher will deal merely with simple facts concerning nutrition and encourage a right attitude to food and good eating habits. Beyond that stage, apart from incidental reference by the class teacher or physical training teacher, the subject naturally falls to be taught in a more scientific way by the specialist teacher of cookery. At the primary school stage both sexes should receive instruction and the subject should be treated in the course of hygiene or health lessons. The idea is to study the effect of the type of nutrition on the teaching of a samples of the primary school pupils of Erbil city during a year.

Keywords: nutrition, children, teaching

A-42

COUPLING OF HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY (HPAEC) AND MASS SPECTROMETRY (MS) FOR ANALYSIS OF OLIGOSACCHARIDES

Leon Coulier[1*], Richard Bas[2], William van Dongen[3]

[1 2 3] TNO Quality of Life, Zeist, the Netherlands
[*] Corresponding author–E-mail: leon.coulier@tno.nl; Phone: +31 30 694 48 88; Fax: +31 30 694 48 94

The analysis of complex mixtures of oligosaccharides is still a challenge in the field of analytical chemistry. Various analytical techniques have been applied to complex mixtures of oligosaccharides in literature, the choice of analytical technique depending on the required level of detail, the type of carbohydrate product and perhaps availability. Due to the nature of carbohydrates and the characteristics of the synthesis of oligosaccharides, mixtures of oligosaccharides may contain hundreds of individual compounds including many isomers. These compounds might differ in the type of monomers, the number of monomers, the order of monomers in the chain and the linkages between monomers.

A very important and often applied analytical method, for either complete oligosaccharide mixtures or fractions obtained from e. g. GPC, is high-performance anion-exchange chromatography (HPAEC). This is a well-established technique for determining underivatized carbohydrates using alkali hydroxide and alkali acetate based eluents. Nowadays, selective and sensitive detection integrated pulsed amperometric detection (PAD) is used, which is directly compatible with the high ionic strength of these eluents. A disadvantage of this method is the high concentration of alkali hydroxide and acetate in the eluents making it very difficult to obtain clean and usable fractions on a preparative scale. Despite the advantages of the PAD detector and its compatibility with HPAEC, it only gives limited additional chemical or mass information of the peaks detected. Especially when analyzing complete mixtures of oligosaccharides, i.e. without prefractionation, it is impossible to verify whether a peak observed with HPAEC-PAD is e.g. a di- or tri-saccharides. Furthermore, molar response factors may differ significantly between (oligo)saccharides analyzed by HPAEC-PAD which is troublesome for quantification, especially when no reference materials are present

We demonstrate here the coupling of HPAEC to mass spectrometry (MS) on an existing LC-MS system. It should be stressed that HPAEC-MS is at the moment still not a ‘standard’ technique which is often applied and commonly available. We describe the experimental set-up as well as application of HPAEC-MS to complex mixtures of oligosaccharides, like galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS) and human milk oligosaccharides (HMO).

Keywords: HPAEC, MS, oligosaccharides, prebiotics

A-43

DETERMINATION OF THE INTERNAL QUALITY OF SUGAR BEET USING NEAR-INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

Hendrik De Ruyck[1*], Herman De Ridder[2], Martine Merchiers[3], Chris Van Waes[4], Bart Vandecasteele[5], Jan De Block[6]

[1 2 3 6] Institute for Agricultural and Fisheries Research (ILVO), Technology and Food Science Unit (T&V), MELLE, BELGIUM
[4 5] Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit (Plant), MERELBEKE, BELGIUM
[*] Corresponding author–E-mail: hendrik.deruyck@ilvo.vlaanderen.be; Phone: + 32 9 272 30 31; Fax: + 32 9 272 30 01

The potential of using near-infrared reflectance spectometry (NIRS) as an analytical technique to perform routine analyses of the internal quality of sugar beet was investigated using a set of 150 beet brei samples. The quality parameters in this research project were α-amino nitrogen, sodium, potassium, ash, moisture, betaine, glutamine and the sugars sucrose, raffinose, glucose and fructose.

Duplicate NIRS spectra within the range of 1100 to 2500 nm were collected using a Foss NIRSystems 5000 spectroscope. Pressed juice samples were also evaluated.

The statistical approach of principal components was applied in order to select a representative calibration subset totalling 50 samples. These calibration samples were analysed using the following ICUMSA (International Commission for Uniform Methods of Sugar Analysis) reference methods: copper method (α-amino nitrogen), oven method (ash), flame photometry (sodium and potassium), drying oven method (moisture), and HPLC (sugars, betaine and glutamine). Calibration models for the different quality parameters were developed by modified partial least squares regression using WINISI III 1.63 software. The statistical values of the NIRS calibrations are summarised below.

Table: Statistical values of NIRS calibrations for the internal quality of sugar beet

Parameter	n	Range	SEC	SECV	SD	SEL	RSQ
α-amino Nitrogen	45	9.29 – 52.93	2.79	3.28	5.06	1.24	0.70
Ash	48	0.42 – 0.65	0.03	0.05	0.06	0.06	0.78
Sodium	49	1.86 – 11.37	1.85	1.98	1.85	–	0.00
Potassium	48	29.64 – 47.25	4.42	4.65	4.54	–	0.05
Moisture	48	62.47 – 80.42	0.43	0.53	1.48	0.66	0.92
Sucrose	48	13.91 – 21.90	0.62	0.71	1.43	0.68	0.81
Glucose	48	0.11 – 1.18	0.05	0.05	0.05	0.03	0.24
Fructose	47	0.08 – 1.25	0.04	0.04	0.04	0.03	0.15
Raffinose	46	0.08 – 0.39	0.03	0.03	0.03	0.02	0.12
Betaine	48	0.13 – 0.29	0.01	0.01	0.03	0.02	0.81
Glutamine	46	0.02 – 0.26	0.02	0.02	0.02	0.02	0.41
Sugar Extractability	45	83.80 – 93.15	0.95	1.20	1.82	–	0.73

n = number of samples SEC = Standard Error of Calibration SECV = Standard Error of Cross Validation SEL = Standard Error of Laboratory reference analyses RSQ = Coefficient of determination
Units of α-amino nitrogen, sodium, potassium: mmol/kg
Units of ash, moisture, sucrose, glucose, fructose, raffinose, betaine, glutamine, and sugar extractability:%

The NIRS technique is an excellent and very fast tool to determine the composition of main organic components. Acceptable NIRS statistical models were obtained for the chemical composition parameters moisture and sucrose.

We concluded that some of the conventional analytical methods can be substituted with NIRS measurements, leading to a significant reduction in the use of dangerous and environmentally harmful reagents as well as lower labour costs.

Keywords: sugar beet, internal quality, NIRS

Iscal Sugar (Ronald Demuynck) and Coco-Vlaanderen (Alain Van Dorpe)

A-44
ANALYSIS OF COLLAGEN IN MEAT EXTRACTS USING LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY

Andre Schreiber[1], Anna Marques[2], Helio Junior[3], Takeo Sakuma[4], Henri Snijders[5*]

[1 2 3 5] Applied Biosystems
[4] MDS Analytical Technologies
[*] Corresponding author–E-mail: andre.schreiber@lifetech.com; Phone: +1-905-660-9006

Collagen is the main protein of connective tissue in animals and the most abundant protein in mammals, including humans. In fact, it makes up about 25% to 35% of the total amount of protein in the body. Hydroxyproline is a major component of the protein collagen playing a key role for collagen's stability. Creatinine is a break-down product of creatine phosphate in muscle. These compounds determine how juicy and tender meat is. Traditionally, colorimetric methods are used routinely in the meat and leather industries. Here we present a method using Liquid Chromatography coupled to tandem Mass Spectrometry (LC/MS/MS) for the analysis of hydroxyproline and creatinine from collagen extracts. The samples were simply diluted and injected onto a Hydrophilic Interaction LC column (HILIC) coupled to an API 3200™ LC/MS/MS system operated in positive polarity. Multiple Reaction Monitoring (MRM) was used for detection because of its high selectivity and sensitivity. The developed method had excellent limits of detection, linear range and reproducibility and was successfully applied to the analysis of meat extracts. The high sensitivity of the developed LC/MS/MS method allowed dilution of meat extracts greatly increasing robustness and reducing the risk of possible matrix effects. This analytical procedure can speed up the sample analysis for hydroxyproline and creatinine, which in turn, improves the whole processing of collagen products.

Keywords: collagen, LC/MS/MS, meat, mass spectrometry

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A-45

APPLICATION OF FOURIER TRANSFORM INFRARED SPECTROMETRY AND LINEAR DISCRIMINANT ANALYSIS FOR INSPECTION OF APPLE QUALITY

Mohammadreza Khanmohammadi[1*], Mehdi Hasaninasab[2], Amir Bagheri Garmarudi[3]

[1] Chemistry department, Faculty of Science, Imam Khomeini International University, Qazvin, Iran
[2] Department of Mechatronics Engineering, Islamic Azad University, Qazvin, Iran
[3] Chemistry department, Faculty of Science, Imam Khomeini International University, Qazvin, Iran.–Department of Chemistry & Polymer Laboratories, Engineering Research Institute, Tehran, Iran
[*] Corresponding author–E-mail: mrkhanmohammadi@gmail.com; Phone: +98 281 3780040–+98 912 3819016

Apples constitute an important part of the human diet, as they are a source of monosaccharides, minerals, dietary fiber, and various biologically active compounds, such as vitamin C, and certain phenolic compounds which are known to act as natural antioxidants. Sugar content and acidity are the properties most likely to match the consumer's perception of maturity. Therefore, the development of a reliable, noninvasive method for the quality evaluation of apples, before harvest and at the packing site, is critical to the success of the apple industry. Quality assessment and classification of food products as well as evaluation of taste attributes is necessary in the modern markets for buyers and producers alike. Conventional analytical techniques used for such measurements are time-consuming, usually require complicated sample preparations, have high running costs and cannot be used in the field. IR spectroscopy is well known for its uniqueness as a nondestructive method in identifying vibrational structure of various materials. The spectra allow measuring complex molecular vibrational modes. The simplicity of sample handling and measurement in FTIR spectrometry makes it a useful alternative for sugar determination. Attenuated total reflectance (ATR) enables the quantitative analysis in IR region. There are several reports indicating the application of FTIR spectroscopy as a useful method for determination of sugar in different samples e.g. fruits, foods, biological samples etc.

Supervised multivariate methods, such as linear discriminant analysis (LDA) are powerful tools to build rules of discrimination that are used later to identify new samples. LDA searches for the variables containing the greatest interclass variance and the smallest intraclass variance, and constructs a linear combination of the variables to discriminate between the classes. The rule is constructed with training set of samples, and further tested with the test set. In this research, it was tried to classify apple samples by ATR-FTIR spectrometry, proposing a treatment of the results by LDA, in order to improve the reliability of the interpretation of the data. The innovative aspect is based in the application of this chemometric tool, which provides a powerful device to extract useful information from the experimental data.

Keywords: apple, ATR-FTIR spectroscopy, chemometrics, quality

A-46

MAXIMIZING THE CHROMATOGRAPHIC RESOLUTION AND DETECTION CONTENT OF COMPLEX PLANT LIPID ANALYSES WITH OPTIMIZED UHPLC SYSTEMS

Jerry Zweigenbaum[1], Mike Woodman[2*]

[1 2] Agilent Technologies, Wilmington, Delaware, USA
[*] Corresponding author–E-mail: michael_woodman@agilent.com; Phone: 1 302 633 8467

Recent UHPLC experiments on columns packed with materials in the 1.5–1.9 µm particle size range have shown great utility in the analysis of complex lipid mixtures. Maximized resolution requires the use of high column efficiency and, typically, shallow gradient profiles. Achieving high resolution while minimizing overall operating pressure and analysis time is also a critical part of method design. The highest resolution separation is a combination of column and mobile phase physics and chemistry with system dispersion (bandspreading) also optimized. In this work we have exploited a broad range of resolution parameters on systems optimized for very high chromatographic resolution. We have developed tools for method design and method translation to make these new technologies easier to use, and to significantly shorten the time required for method optimization.

Refined and unrefined triglyceride mixtures, carotenoids, phytosterols were acquired and subjected to a diversity of solvent and column configurations to explore high peak capacity separations. We use UV, ELSD and MS detection to enhance detection information. Phase particle size and carbon load are examined at various temperatures and gradient slopes.

Keywords: lipid, carotenoid, phytosterol, sitosterol, HPLC

A-47

CHALLENGES OF USING ROUTINE ANALYTICAL METHODS FOR FOOD ANALYSIS: HOW TO MAKE THEM FIT FOR THE PURPOSE OF CERTIFICATION OF CRMS

Marta Dabrio Ramos[1*], Gerhard Buttinger[2], Reinhard Zeleny[3], Hendrik Emons[4]

[1 2 3 4] EC-JRC-IRMM, Geel, Belgium
[*] Corresponding author–E-mail: marta.dabrio@ec.europa.eu; Phone: +32 14 571953; Fax: +32 14 571548

The use of routine analytical methods is widely applied for monitoring of food quality that in many cases is stipulated by legislation at EU-level. Often such methods are characterized by associated uncertainties which, although in many cases are relatively high, are fit for their purpose, thus allowing proper decision making.

Certified reference materials (CRMs) are highly suitable quality assurance tools for the assessment of method performance. Besides the capability of evaluating the bias of a given method, the CRMs can be applied for the estimation of other factors affecting the method performance such as repeatability and intermediate precision. However to fulfill their role, the CRMs themselves should have certified values with appropriate analyte concentration levels with acceptably low uncertainties. The certified uncertainty contains several sources, besides the material characterization, including contributions to cover potentially hidden heterogeneity of the analyte in the material and potential analyte degradation with time upon storage. To quantify these processes, specific analytical methods which satisfactory performance with respect to repeatability must be available in order to make sure that homogeneity and stability can be sufficiently well assessed and that their uncertainty contribution to the final uncertainty is not become too large.

Unfortunately, in many cases, the performance of routine methods is not good enough for these purposes, thereby increasing the difficulty and time needed for production and certification of CRMs in the food area. This situation therefore often involves an additional in-house development and/or improvement of existing analytical methods to reach the necessary trueness and precision. These aspects will be illustrated with practical examples by describing recently concluded or still on-going CRM projects in the food area.

Keywords: CRM, QA/QC, uncertainty, food analysis

A-48
PROFICIENCY TESTING FOR ANALYSIS OF A WIDE RANGE OF FOOD ADDITIVES

Mark Sykes[1*]

[1] Fera, York, UK
[*] Corresponding author–E-mail: mark.sykes@fera.gsi.gov.uk; Phone: +44 (0)1904 462697

The issue of additives in foods remains a much debated topic. Numerous organisations now exist to educate or warn us about the health benefits or dangers of additives in foods. Some additives may be deliberately introduced, to act as preservatives, for example. Alternatively, the presence of some so-called additives may actually be inherent in the foodstuff itself. It comes as no surprise, therefore, that the Additives Proficiency Tests (PT) of the Food Analysis Performance Assessment Scheme (FAPAS®) remain popular, with a range of analytes and matrices.

Proficiency tests for the anti-oxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in oil continue to be run by FAPAS® successfully year after year. This suggests that the analysis is well defined and reproducible. Jam (conserve) is tested for Brix, pH, citric acid, sorbic acid and benzoic acid. Typically, some 80 laboratories participate from 30 countries with generally good satisfactory scores. The acid analytes in jam cover a large concentration range (200–9000 mg/kg). Tomato sauce analysis for Brix, pH, total acidity, sodium and chloride is similarly popular and, generally, successful. Some tests for tomato sauce have included lycopene, with mixed success.

FAPAS® has continued with some tests that, despite mixed results, remain an important component of participants' quality assurance. The analysis of sulphur dioxide, for example, while highly successful in apricots, gives non-normal distributions of results in meat. FAPAS® continues to observe and document these occurrences.

Tests for artificial colours in sweets (confectionary) or illegal dyes in spices or sauces attempt to meet a very specific or contemporary demand for proficiency testing. These types of PT may report results as qualitative or quantitative. Some laboratories' methods may not be capable of quantifying but are simply screening for presence or absence of a colour or dye. Alternatively, some results may have such variation that an assigned value cannot be determined. These PTs also report any colours or dyes that participants have found, despite their not being included in the spiking material. The production of acceptable test materials can be particularly difficult for illegal dyes. It has been virtually impossible to manufacture a dry spice test material that meets the requirements of FAPAS®. It has therefore been necessary to use a liquid food source as the matrix for these tests.

Keywords: Proficiency testing, food additives

A-49

TRACE ELEMENT DETERMINATION USING ICP-MS FOR EUROPEAN NORMS EN:DIN 15765 AND 15763

Shona McSheehy Ducos[1], Michal Godula[2], Meike Hamester[3], Yolanda Fintschenko[4*]

[1 3] Thermo Fisher Scientific, Bremen, Germany
[2] Thermo Fisher Scientific, Prague, Czech Republic
[4] Thermo Fisher Scientific, San Jose, USA
[*] Corresponding author–E-mail: Shona.mcsheehyducos@thermofisher.com; Phone: 49-421-54930

The determination of trace elements and in particular heavy metals in foods is a concern for the food safety community due to the potential toxicity of these metals. European Commission legislation imposes limits on cadmium (Cd), mercury (Hg), lead (Pb) and inorganic tin (Sn) in number of foodstuffs (EC 1881/2006). Subsequently, standard operating procedures have been designed for the appropriate determination of these elements in foods using ICP-MS. The European Norms EN:DIN 15765 for Sn and EN:DIN 15763 for arsenic (As), Cd, Hg and Pb outline the sample preparation protocol based on microwave digestion as well as the analytical procedure used for the metal quantification.

The two European norms were applied to a number of foodstuffs purchased from a local supermarket and four food matrix certified reference materials (CRMs). The ICP-Q-MS (Thermo Scientific XSERIES 2) used for quantification was operated in standard conditions and with the use of collision/reaction cell technology and the results compared. The results obtained for the CRMs were in very good agreement with the certified values, validating the method and the instrumentation for total elemental concentrations in foodstuffs.

Keywords: trace, elements, heavy, metals, ICP

A-50
OPTICAL SENSING TECHNOLOGIES FOR RAPID FOOD SAFETY AND QUALITY INSPECTION

Moon Kim[1*], Kuanglin Chao[2], Diane Chan[3], Won Jun[4], Alan Lefcourt[5], Chun-Chieh Yang[6]

[1 2 3 4 5 6] USDA-ARS, Beltsville, USA
[*] Corresponding author–E-mail: moon.kim@ars.usda.gov; Phone: 1-301-504-8462; Fax: 1-301-504-9466

Public concerns for food safety and foodborne illness have risen in recent years. There is a need to expand efforts to prevent and mitigate any food contamination that can potentially be harmful to human health. Researchers at the Environmental Microbial and Food Safety Laboratory, ARS, USDA is one of the leading groups for the development of opto-electronic sensing technologies and methodologies, successfully demonstrating several cutting-edge systems for detection and inspection for food quality, safety, and sanitation. The optical sensing technologies include Raman, fluorescence, and visible/near-infrared reflectance spectroscopy, and hyperspectral and multispectral imaging. Chemical/biological food properties can often be assessed by spectroscopic methods, while machine vision is already ubiquitous for sorting for physical attributes. New spectral imaging technologies can also deliver high-speed online safety and quality inspection of food and agricultural products on high-throughput processing lines. ARS scientists have developed line-scan hyperspectral imaging methods for high-speed inspection on commercial processing lines, capable of simultaneous multiple inspection algorithms for different safety and quality problems. Adaptable to a broad range of problems and commodities, the line-scan hyperspectral imaging platform will be critically useful for both research and commercial food safety and quality inspection applications. We present recent development and application of the rapid line-scan image-based online safety inspection for apples and chicken carcasses and portable fluorescence imaging techniques for sanitation inspection of food processing surfaces.

Keywords: optical, sensing, food safety inspection

A-51
ENEA'S FACILITIES FOR THE DEVELOPMENT OF NEW AGROFOOD REFERENCE MATERIALS

Rosanna Gatti[1], Paola Sangiorgio[2], Giovanna Zappa[3*], Claudia Zoani[4]

[1 2 3 4] ENEA–Italian National Agency for New Technologies,Energy and the Environment. Biotechnologies, Agro-industry and Health Protection Dept. Casaccia Research Centre – V.Anguillarese,301-00123 Roma–Italy
[*] Corresponding author–E-mail: giovanna.zappa@enea.it; Phone: +39 06 30483436; Fax: +39 06 30486258

The Analytical Chemistry Laboratory of the ENEA BAS Dept. has carried out in Italy pioneer activities in the fields of Reference Materials (RMs) and Quality of Chemical Measurements. By means of the funds arising from National and EU Research Projects, ENEA has developed real plants for the preparation (or production) of RMs, located in the Casaccia (near Rome) and Trisaia (in South Italy) ENEA Research Centres. The plant of the Trisaia R.C. is specifically tailored for agrofood Reference Materials. It has been recently upgraded thanks to the contribution of the joint-stock company Ce.R.T.A. (Regional Centers for Alimentary Technology). The ENEA RM-Plants permit to realize both Matrix-RMs (starting from natural or processed agro-products) and Calibrant-RMs (starting from pure substances). They are designed for obtaining up to 1000 homogeneous and stable aliquots and preparing RMs in different physical-chemical form (i.e: lyophilized, liquids, dry powders). These plants allow to control chemical and biological degradation phenomena during the treatments of raw materials by appropriate settings of temperature, relative humidity and lighting. Furthermore there are foreseen two homogenization steps (one at the wet and one at the dry stage) and the process contaminations are highly reduced by the employment of inert materials. As special performances of these plants, must been pointed out the possibility to prepare: custom-made RMs; Single Use-RMs in pellet form; Double-Phase RMs supplied in its two separated components (liquid and solid phases) to be re-combined before use. Other than RM preparation, the Lab. carries out preliminary characterization, stability tests under thermal and luminous stress and homogeneity studies. Among the materials already prepared, we could quote: mushrooms, tomato (fruit), strawberry (fruit), broccoli, fish feed, milk, fish, meat and environmental matrices as lake sediment, other than single element calibration solutions traceable to SI. Feasibility studies are underway regarding RMs to be certified for nutraceuticals, RMs for checking mycotoxin contamination in foods and RMs to be employed in the evaluation of the toxicity related to nanoparticles.

Keywords: Reference Materials, CRM, Calibrant, Food analysis

A-52

CHEMICAL PROPERTIES OF MILK AND CHEESE IN MILKWEED ABI– PRIZREN KOSOVË

Shkuri Maxhuni[1], Sadije Nikshiq–Kadriu[2], Malësore Pllana–Zeqiri[3], Lavdim Zeqiri [4*], Besnik Hoxha[5]

[1 2 3 5] Faculty of Mining and Metalurgy–University of Prishtina
[4] German Training Center – Mitrovicë
[*] Corresponding author–E-mail: lavdimzeqiri@hotmail.com; Phone: 044596677

In this scientific raport are given some chemical and physical properties of milk analysis in milkweed *ABI* in prizren so here are some experiment with cows milk in an period during 30 days, so here are some analysis of cheese and from this anlysis we have ascertain that during drying of cheese in store will mount quantity of% drying matter, nitrogen and NaCl.

Keywords: Milk, Cheese, Analysis

A-53

APPLICATION OF THE THRESHOLD OF TOXICOLOGICAL CONCERN-CONCEPT AND ADVANCED (BIO-) ANALYTICAL TOOLS IN SAFETY ASSESSMENT OF CHEMICALLY COMPLEX FOOD MATRICES

William van Dongen[1*], Sander Koster[2], Monique Rennen[3], Geert Houben[4]

[1 2 3 4] TNO, Zeist, Nederlands
[*] Corresponding author–E-mail: william.vandongen@tno.nl; Phone: +31 6 51833605

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 high-risk substances in complex mixtures present at toxicological relevant concentrations, rather than time consuming peak-by-peak identification and quantification.

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.

TNO is setting up (bio-)analytical strategies that allow the use of the TTC principle for safety testing of compex food matrices. The first step is to determine the numbers and amounts of substances present at exposure level above certain thresholds specified by the TTC concept. The next step is to exclude the presence of cohort-of-concern, organophosphate, organohalogen substances and potentially genotoxic compounds. In this paper these (bio-)analytical tools are explained and demonstrated for several food matrices.

Keywords: Food; Safety; TTC

A-54
RESIDUE AND FOOD SAFETY RESEARCH AT THE USDA ARS ANIMAL METABOLISM RESEARCH UNIT

David Smith[1*], Janice Huwe[2], Heldur Hakk[3], Nancy Shappell[4], Weilin Shelver[5]

[1 2 3 4 5] USDA ARS, Fargo, USA
[*] Corresponding author–E-mail: david.j.smith@ars.usda.gov; Phone: 701-239-1238; Fax: 701-239-1430

The Animal-Metabolism Agricultural Chemicals research Unit, located in Fargo, North Dakota, conducts a broad range of food-safety research related to the disposition of chemicals in food animals and food animal production systems. Specific studies conducted within the Unit fall under two main projects entitled “Dioxins and other Environmental Contaminants in Food” and “Metabolic Variables Affecting the Efficacy, Safety, and Fate of Agricultural Chemicals”. In order to reduce exposure to dioxins and other environmental contaminants from the food supply, four objectives have been targeted for the environmental contaminants project:

1) develop inexpensive, rapid, sensitive, assays or improved diagnostic tools to screen samples for dioxins, pesticides, chemical residues, and other environmental contaminants;
2) investigate sources which contribute to residues of environmental contaminants in food animals and identify intervention strategies that may reduce contaminants in domestic food animals;
3) investigate the uptake, metabolism, distribution, excretion, and fate after excretion of environmental contaminants in animal systems;
4) update data on levels of dioxins and related compounds in the domestic food supply, for use by Food Safety agencies to ensure the safety and competitiveness of U.S. food animal products.

The Metabolic Variables project is designed to determine the fate of a diverse group of chemicals in food animals and in the environment (excreta, soil, water) after their elimination from food animals. Target analytes include endogenously produced steroid hormones, novel oxyanions and nitro-compounds, and antibiotics. Endogenous steroid hormones are highly potent endocrine-disrupting compounds that may concentrate in intensive food-animal production settings. Novel oxyanions and nitro compounds show promise for food-safety applications in ruminant, non-ruminant, and avian food animals.

Specific objectives for this project include:

1) determine variables such as absorption, tissue and microbial biotransformation, and rates of excretion that positively or negatively influence tissue residues of chlorate salts and novel nitro-compounds such as 2-nitropropanol in food animals;
2) determine the fate of steroid hormones, antibiotics, and developmental compounds in manure management systems of animals and in soils with the goal of gaining an understanding of the impact that residues of such chemicals may have in intensive food animal production settings;
3) 3) develop analytical tools for the accurate measurement and(or) identification of these analytes or their metabolites in a variety of food animal matrices. Meeting these collective objectives should improve the availability of safe food-animal products worldwide.

Keywords: residue, animal, meat, soil, ADME

NOVEL FOODS, GMO, NUTRACEUTICALS, ORGANIC FARMING

(B-1 – B-10)

B-1

NUTRITIONAL ANALYSIS OF THE EDIBLE FRUIT OF CORDIA MYXA IN IRAN (KHUZESTAN, BEHBAHAN)

Ali Aberoumand[1*]

[1] Prof. of Food Science, Natural Resources College,Behbahan University, Behbahan, Iran
[*] Corresponding author–E-mail: aaberoomand@yahoo.com; Phone: 00989166716540; Fax: 00916712229969

Most of nutrient requirements can be met by increasing the consumption of fruits and vegetables to 5–13 servings/day. In addition to meeting nutrient intake levels, greater consumption of fruits and vegetables is associated with reduced risk of cardiovascular disease, stroke, and cancers of the mouth, pharynx, esophagus, lungs, stomach, and colon. The proximate composition and mineral constituents of *Cordia myxa R*. Fruit were evaluated with standard methods. The fruit contained an ashes: 6.7%, crude protein: 8.32%, crude lipid: 2.2%, crude fiber: 25.7% and carbohydrates: 57.08%. The fruit also have high energy value (281.4 kcal/100g) dry weight. mineral ranges (mg/100g dry weight, DW) were: K (7.83), Na (1.62), Ca (0.46), Fe (0.51) and Zn (0.35). Comparing the stem mineral contents with recommended dietary allowances (RDA), the results indicated that *Cordia myxa R*. fruit could be a good supplement for some nutrients such as fibre, protein and Carbohydrates.

The wild fruit could be promoted as a carbohydrate and protein supplement for cereal-based diets in poor rural communities.

Keywords: ICordia myxa fruit, micronutrients

The authors are grateful to Head Department of Food Science Technology of Ramin Agricultural University of Iran. for providing necessary laboratory facilities.

B-2

DETECTION OF GENETICALLY MODIFIED RICE WITH A CONSTRUCT-SPECIFIC REAL-TIME PCR IN THAI RICE

Prasert Wongwathanarat[1*], Khanitha Wongwathanarat[2]

[1] Department of Biotechnology, Faculty of Science and Technology, Thammasat University (Rangsit Center), Pathum-Thani, (12121) Thailand
[2] Office of Biotechnology Research and Development, Department of Agriculture, Thunyaburi, Pathum-Thani. (12110) Thailand
[*] Corresponding author–E-mail: wongwath@alpha.tu.ac.th; Phone: +66 02 5644440-59; Fax: +66 02 5644500

As field trials of genetically modified (GM) rice are carrying out in several countries and an approval for commercial cultivation of GM rice may occur in the near future in some countries such as China, these make Thai GMO detection laboratories to develop a detection and alert system for GM rice. The aim of this work is to watch GM rice in order not to contaminate in Thailand. One hundred and twenty seven unmilled rice and twenty one rice samples were collected from rice mills, wholesale and retail shops in paddy growing provinces during May to August 2008. Genomic DNA was extracted from 2 g. of each ground sample and then purified. Prior to screening of GM rice, the quality of the extracted genomic DNA of each sample and the PCR conditions were examined by amplification of the DNA fragment of the rice phospholipase D gene using the plant-specific primers(KVM159/KVM160). Screening of the GM rice was then verified by the Bt rice construct-specific primers T51F/T51R and probe (T51p) by real-time PCR. It was found that 68 bp DNA fragment of rice phospholipase D gene was amplified from all of the DNA samples. The result indicated that quality of extracted DNA and the PCR conditions were reliable, but none of the GM rice was detected. It suggested that there was still not GM rice contaminated in Thai rice. However, it should be taken in mind that an increase in sample sizes and frequency of sampling should perform with the development of rapid detection methods in order to make precautionary system effectively.

Keywords: GM rice, Real-time PCR

B-3

GRADIENT MANIPULATION AND 1.8 µM LC COLUMNS FOR HIGH RESOLUTION ANALYSIS OF HERBAL SUPPLEMENTS

John W. Henderson Jr.[1*], Thierry Faye[2], Wittek Ulrik[3], Maureen Joseph[4;]

[1] Agilent Technologies, Wilmington, USA
[2] Agilent Technologies, Massy, FR
[3] Agilent Technologies, Waldbronn DE
[4] Agilet Technologies, Wilminton, USA
[*] Corresponding author–E-mail: john_w_henderson@agilent.com; Phone: 302-633-8502

The mobile phase gradient is the chief parameter modified for optimizing RP gradient separations of complex mixtures such as extracts of natural products. Other method parameters that maximize resolution in gradient methods such as column length, particle size, and flow rate and temperature are sometimes ignored due to limitations of the LC system including the column. Licorice Root extract and other herbal supplements will be separated using a UHPLC instrument which provides flexibility to better use a wider range of LC parameters including column length, temperature, and flow rate for optimum resolution. The UHPLC instrument will be used with 1.8µm columns to evaluate how column length, flow rate, and temperature and gradient slope, can be utilized to produce narrower peaks, higher peak capacity, and the best resolution for complex samples requiring gradients.

Keywords: UHPLC, licorice, herbal supplement

B-4
HIGH-OLEIC SUNFLOWER SUPPLEMENTATION OF WHEAT-BASED COOKIES

Biljana Škrbić[1*], Jelena Cvejanov[2], Nataša Đurišić-Mladenović[3]

[1 2 3] Faculty of Technology, University of Novi Sad, Novi Sad, Serbia
[*] Corresponding author–E-mail: biljana@tf.uns.ac.rs; Phone: 021-485-3746

It is well known that sunflower seed represents a source of protein, antioxidants, minerals and unsaturated fatty acids. Due to its superior nutritional quality and the relativly low amount of antinutritional factors, addition of sunflower seed into wheat-based bakery products is very attractive, having positive influence to health status of the general population.

The objective of this work was to study the quality of cookies based on white (refined) and wholegrain wheat flour enriched with high-oleic sunflower seed. Cookies were prepared with the addition of crushed high-oleic sunflower seed in the formulations of cookies at 10 and 30% supplementation levels. The following components were analyzed: basic chemical composition, alpha-tocopherol, macro minerals (potassium, magnesium), trace elements (copper, zinc, iron, selenium and manganese) and heavy elements (arsenic, cadmium, lead, mercury). According to the obtained content, the respective intakes through daily portion of cookies were calculated and compared to dietary reference intakes (DRIs) and provisional tolerable weekly intakes (PTDIs) regarding the heavy elements. Additionally, sensory analysis was performed in order to determine taste, chewiness, factorability, shape, spread ratio. According to the results, it could be seen that the sunflower seed supplementation of cookies caused decrease in the carbohydrate content and increase the protein, fat, ash and alpha-tocopherol content. When compared to DRIs, 100 g of control cookies meets around 48% of the DRIs for alpha-tocopherol. At 10% supplementation level, 62.1% i.e. 62.9% of DRIs for alpha-tocopherol would be met by white and wholegrain cookies, respectively. At 30% levels, higher contributions were estimated, 89.6–95.2% of DRIs, respectively. Results of mineral composition analysis of the supplemented cookies showed significant rise in the content of magnesium, calcium, zinc and selenium. The most prominent was the increase in the selenium content: at 10% supplementation level selenium content increased approximately 4 folds while at 30% level it increased 10 folds in both cookies groups. Considering the content of heavy elements it could be seen that supplementation with sunflower seed would increase the consumers' exposure to mercury for 1.03–1.92% of the PTDI and to cadmium and lead less than 15% of PTDIs. The inatake for arsenic was evaluated to be 30% of the PTDI, indicating the possible negative effect on health status especially taking into account the consumption of other goods from the market basket.

Keywords: sunflower seed, cookies, nutrients, elements

B-5

DETECTION OF GENICALLY MODIFIED ORGANISMS WITH REAL-TIME PCR: A MODULAR APPROACH WITH PARALLELISED REACTIONS

Lars Gerdes[1], Sven Pecoraro[2*]

[1 2] LGL, Oberschleissheim, Germany
[*] Corresponding author–E-mail: sven.pecoraro@lgl.bayern.de; Phone: +49 89 31560585; Fax: +49 89 31560458

The EU established with regulation (EC) No 1829/2003 a legal work (1) to inform customers through the compulsory labelling of GMO (genetically modified organisms). This regulation replaced a *de facto* moratorium on the approval of new GMO. In consequence, the number of GMO applications submitted to the competent European Food Safety Authority (EFSA) grew rapidly since 2004. At present there are more than 60 applications for authorisation of GMO plants as feed or food pending (2). Therefore it is reasonable to assume that the number of approved GMO events in the EU will rise in the near future. One task of the official food and feed control is to monitor the compliance with corresponding EU regulations related to labelling of food and feed by laboratory analysis.

Analysis for constituents of GMO crops is mostly based on the detection of specific DNA sequences. With PCR (polymerase chain reaction) techniques defined parts of DNA can be amplified and subsequently or immediately (Real-time PCR) detected. Real-time PCR detection is the method of choice because of its high specificity, its closed amplification system that minimises carryover risks, and the possibility for quantification of GMO contents. However, the growing number of possible new GMO events that routinely have to be tested for requires from the laboratories increasing efforts to manage staff and financial resources. In particular this is true as analysis for different GMO is widely done consecutively rather than in a parallel format.

The LGL is developing a microtitre plate based analysis real-time PCR format in order to efficiently detect multiple events in parallel. The data from this real-time format is reliable through the use of validated methods (e.g. by the Community Reference Laboratory, CRL and the European Network of GMO Laboratories, ENGL). The open parallel real-time format can easily be extended by inclusion of additional test reactions into the microtitre plate based format. Grouping of related reactions (e.g. by crop species) leads to a modular system. The microtitre plate modules can be prepared in advance and the mixture consisting of PCR mastermix together with primers and probes can be stored for several weeks at -20°C until use. When needed, DNA extracted from routine samples, positive and negative controls are simply added to the reaction wells. This allows a rapid, efficient and parallelised identification of current GMO events and presents a good basis to deal with future challenges in GMO analysis.

[1] EU, http://europa.eu/scadplus/leg/en/lvb/l21170.htm
[2] EFSA, http://registerofquestions.efsa.europa.eu/roqFrontend/questionsList.jsf

Keywords: GMO, detection, real-time PCR, modular

B-6
ENHANCEMENT OF THE WHEAT-BASED COOKIES QUALITY BY THE BARLEY SUPPLEMENTATION

Biljana Škrbić[1*], Snežana Milovac[2], Jelena Živančev[3]

[1 2 3] Faculty of Technology, University of Novi Sad, Novi Sad, Serbia
[*] Corresponding author–E-mail: biljana@tf.uns.ac.rs; Phone: +381-21-485-3746

Barley grain is very desirable ingredient in baked products, being an excellent source of many valuable nutrients such as soluble and insoluble dietary fibers, vitamin B complex, minerals and phenolic compounds. The highest nutritional value has been associated with beta-glucans, the major fiber constituents in barley. The intention of this work was to determine the nutritional profile (carbohydrate, fat, protein, beta-glucan, minerals) of the wheat-based cookies enriched with hull-less barley flour and to assess their adequacy in meeting the recommended dietary intakes (DRIs). Furthermore, the contents of heavy elements (As, Cd, Pb, Hg) in enriched cookies were also taken into account and the potential risk to the consumer health was estimated through comparison of the calculated heavy element intakes with the respective provisional tolerable daily intakes (PTDI). Formulations of cookies were based on: a) white flour, and b) wholegrain flour. For both type of cookies (white and wholegrain), barley flour was added at the 0%-, 30%- and 50%- levels. The cookies supplemented in this way, were compared with the respective controls based on wheat flours (without any addition). In order to evaluate the attractiveness of the supplemented cookies to the consumers, physical characterization and sensory analysis of the obtained products were also performed. Hull-less barley flour added to the cookies contributed to the significant increase of beta-glucan and Cu, Zn, Fe, Se as compared to the controls. However, beside doubtless benefits regarding the nutritional quality, the barley supplementation contributed to the elevated content of heavy elements (lead, cadmium, arsenic) in the newly formulated cookies. Of these elements, the highest exposure through the supplemented cookies consumption would be for arsenic, anticipated to be over 30% of PTDI. The exposure to cadmium and lead by the consumption of the barley enriched cookies was estimated to be over 17% and over 24% of the respective PTDIs. The anticipated intake of mercury through the barley supplemented cookies was low ranging from 0.75-1.34% of the PTDI.

Keywords: cookies, barley, nutritional/safety aspects

B-7

DEVELOPMENT OF A MULTIPLEX POLYMERASE CHAIN REACTION METHOD FOR SIMULTANEOUS DETECTION OF GENETICALLY MODIFIED SOYBEAN AND MAIZE

Kyoung Sik Park[1*], Mi Kyung Kim[2], Dong-Gil Lim[3], Jin Hong[4], Tae Hyung Yoon[5], Ki-Mi No[6], Jayoung Jeong[7]

[1 2 3 4 5 6 7] National Institute of Food and Drug Safety Evaluation, Seoul, Korea
[*] Corresponding author–E-mail: parkks71@kfda.go.kr; Phone: 82-11-9091-3204; Fax: 82-2-385-7081

This study was aimed at developing a novel qualitative multiplex polymerase chain reaction (PCR) for simultaneous detection of genetically modified (GM) soybean and maize within a single reaction. The specific primers designed to detect four respective GM events (A2704-12, MON88017, Bt11, MON863) and two primer pairs for endogenous reference genes (lectin gene in soybean, and *sslIb* in maize) were included in the hexaplex (6plex) PCR system. Each of PCR products for four GM events could be distinguished by agarose gel and capillary electrophoreses based on their different lengths. The detection limit of this multiplex PCR is 0.1% in 25 ng of the DNA template, indicating high levels of sensitivity. In addition to sensitivity test, the specificity and reproducibility of this multiplex PCR were evaluated. This multiplex PCR repeated 3 times consistently amplified two fragments corresponding to a specific inserted gene and an endogenous reference gene in each of the four GM events and also amplified only an endogenous reference fragment in the non-GM organisms. These results indicate that this multiplex PCR method could be an effective qualitative detection method for screening GM soybean and maize in a single reaction.

Keywords: GMO, PCR, simultaneous detection

B-8

DETECTION OF GENETICALLY MODIFIED ORGANISMS IN FOODS AND FEEDSTUFFS CONTAINING MAIZE AND SOYA

Dionysios Baseas[1], Cathrine Salamoura[2], Vangelis Economou[3], George Zakas[4], Panagiota Gousia[5], Vassilios Vozikis[6], Chrissanthy Papadopoulou[7*]

[1 2 3 4 5 7] Food Microbiology Group, Microbiology Department, Medical School, University of Ioannina, Ioannina, Greece
[6] Institute of Food Hygiene, Center of Veterinary Institutes, Thessaloniki-Greece
[*] Corresponding author–E-mail: cpapadop@cc.uoi.gr; Phone: 00306944690511; Fax: 00302651007886

A total of 494 samples of various raw or processed foods, containing either maize (263 samples) or soya (231 samples) were examined. The maize samples included: corn flakes, baby foods, biscuits, raw corn, popcorn, canned sweet corn, corn flour for cooking, corn flour for grocery, fresh milk, eggs from an intensive breeding poultry farm, cornmeal feedstuffs, snacks, bread, rusks and yogurt desserts. The soya samples included: soya milk, minced soya, soya kebabs, isoflavones pills containing soya genistein and daidzein, biscuits, chocolates, soya sauce, fresh milk, raw soya used as animal feed, eggs from intensive breeding poultry farms, soya spaghetti, snacks, bread, rusks, seed oils – oleomargarines and yogurt desserts. The detection and quantification of GMOs in the collected samples was performed applying a Real Time PCR method using the Roche LightCycler instrument and accessories. For the detection of GMO soya and maize the LightCycler GMO Soya Quantification Kit (quantitative detection of Roundup Ready Soya) and the LightCycler GMO Maize Quantification Kit (quantitative detection of Bt-176 Maize) were used respectively (ROCHE Diagnostics).

Three samples of cornmeal feedstuffs were found to contain GMO maize in percentages 2.23%, 6.31% and 7.89% correspondingly and one sample for human consumption (popcorn) in percentage 23.83%. Twelve samples of raw soya used as animal feedstuff contained GMO soya in percentages 4.30%, 2.31%, 62.06%, 75.91%, 9.45%, 60.7%, 20.05%, 94.48%, 55.11%, 36.97%, 74.63% and 0.14%, correspondingly, and eight samples for human consumption (one minced soya, three soya kebabs and four soya spaghetti) contained GMOs in percentages 0.22%, 0.19%, 0.18%, 0.16%, 0.31%, 0.22%, 0.19%, and 0.26%, correspondingly. It should be noted that the detection limit of the employed method is 0.1%. The LightCycler Instrument and the Roche GMO kits used for this study are simple to use, they provide quantitative results rapidly and the automated instrument can process many samples simultaneously. The results indicate that the majority of the examined food samples are considered safe in accordance with the recent EC directives and regulations, ruling that all foods appropriate for human consumption should contain no more than 0.9% GMOs.

Keywords: GMO, maize, soya, food, feedstuff

B-9
DEVELOPMENT OF SIMPLE ANALYSIS METHOD FOR TAR COLORANTS IN NUTRACEUTICALS

You-Gyoung Park[1*], Chang Soo Kim[2], Sang Hyeok Sohn[3], Eun Mi Lee[4], Mi Ae Na[5], Mi Ra Kim[6], In Sun Joo[7], Joon Ho Eom[8], Woo Young Jung[9], Ok Soon Heo[10]

[1 2 3 4 5 6 7 8 9 10] Daejeon Regional Korea Food and Drug Administration, Daejeon, Korea
[*] Corresponding author–E-mail: ygpark@gmail.com; Phone: +82-42-480-8787; Fax: +82-42-480-8790

In Korea Food Additive Code, 9 Tar colorants (Tartrazine, Sunset Yellow FCF, Amaranth, Erythrosine, Allura Red AC, Ponceau 4R, Brilliant Blue FCF, Indigo carmine and Fast Green FCF) are permitted to be used in capsule material or coating agent of nutraceuticals, which helps takers discriminate similar capsules and tablets. Even though they are miscellaneous components in nutraceuticals, the concerns about their content have been increased according to the growing interest on the health effect of nutraceuticals. To date, various methods have been reported to determine the tar colorants in foodstuff, however, only a few of them can be applied for the nutraceuticals with different matrices. In this study, we developed a simple method to release and purify the tar colorants from gelatin matrix using alkaline condition and hydrophobic solvent. We also set the HPLC condition for quantifying 9 colorants simultaneously with a good sensitivity and high resolution. Chromatograms were acquired with 3 analytical channels of photodiode array (PDA) detector, and ammonium acetate buffer and acetonitrile were used in gradient mobile phase. Linearity of all 9 compounds were over 0.999 of R^2 in the concentration range of 0.5–50 μg/ml and detection limits were less than 0.1 μg/ml. Recovery rates of colorants in our simple method were in the range of 92–103% except indigo carmine which is known to its instability.

Keywords: Tar colorant, nutraceutical, HPLC

B-10

THE EFFICIENCY OF PACKAGING FILMS RELEASING PRESERVING AGENTS ON STABILITY OF PACKAGED FOOD

Kristyna Hanusova[1*], Lenka Votavova[2], Kamila Klaudisova[3], Jaroslav Dobias[4], Michal Voldrich[5], Miroslav Marek[6]

[1 2 3 4 5 6] Institute of Chemical Technology, Prague, Czech Republic
[*] Corresponding author–E-mail: kristyna.hanusova@vscht.cz; Phone: +420 220 443 001

Packaging with antimicrobial activity presents important type of active packaging systems. Some of them have already found application in food production in shelf life extending of packaged foodstuffs in consequence of inhibition of the growth of pathogenic or spoilage organisms on food surfaces. This study is aimed to test the efficiency of polymer packaging films coated with lacquer releasing nisin, natamycin, in preservation of selected types of cheese.

Packaging films (polyvinylchloride, polyethylene and coextruded polyamide/polyethylene) coated by commercially available polyvinyldichloride (PVdC) with addition of nisin preparation *Nisaplin*, natamycin preparation *Delvocid* (16.7% w/w) were prepared by company Martin Peroutka, polygrafická výroba, CZ. The coating thickness was 3–5 μm, the films were able to release nisin in the amount up to 850 IU/dm^2 and natamycin up to 1.5 mg/dm^2. The efficiency of all packaging systems against to selected indicator microorganisms in laboratory scale was already proved.

The films were tested for cheese packaging at pilot conditions in cooperation with cheese food producers. These experiments included ripening of the cheese *Blaťácké zlato*, vacuum packaging of portioned cheese of Edam type and wrapping of portioned cheese *Blaťácké zlato* in conditions of a large supermarket. The last application provided the most promising results when the shelf life of cheese wrapped into the film releasing nisin and natamycin was significantly extended. The presentation will inform about obtained results and the possibilities of practical application of antimicrobial films of above mentioned types will be discussed.

Keywords: active packaging, cheese, nisin, natamycin

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FOOD CONTAMINANTS (ENVIRONMENTAL)

(C-1 – C-53)

C-1

DETERMINATION OF 24 PAH'S IN DRINKING WATER BY FAST LIQUID CHROMATOGRAPHY WITH FLUORESCENCE/UV DETECTION

Antoni Rúbies[1*], Mª Carmen Díaz[2], Ascensión Suárez[3], Francesc Centrich[4], Elisabeth Korte[5], Ramon Companyo[6]

[1 2 3 4] Laboratori Agència de Salut Pública de Barcelona
[5] Varian Deutschland GMBH
[6] Departament Química Analítica. Universidad de Barcelona
[*] Corresponding author–E-mail: arubies@aspb.cat; Phone: 00 34 934439400; Fax: 00 34 934413524

One of the purposes of the Laboratory of the Public Health Agency in Barcelona is to set up and validate an automated method for the analysis of organic contaminants in drinking water such as chlorinated pesticides, triazines and polycyclic aromatic hydrocarbons (PAHs). Many methods for the analysis PAHs have already been published; however, only few of them have been developed to determine in one single extract as many organic compounds as possible in drinking water samples.

The first step was to optimize a chromatographic separation for PAHs, using fast LC coupled to fluorescence and diode-array detectors. The optimized method, using a Varian Pursuit LC column (100×4,6 mm, 3 µm particle size), led to a separation of 24 PAH's in 30 minutes. This is a definite improvement as traditional LC methods need around 45 minutes per run to separate a similar amount of analytes. The developed separation will be useful for analysis of PAHs in other types of matrices, such as food or environmental samples.

The second step was the optimization of the automated solid phase extraction (SPE) method. An Auto Trace Workstation (Zymark) was used. The following parameters were tested: SPE sorbents (polymer- and silica-based), drying time, sample pH, addition of organic solvents to the water sample prior to the extraction and elution solvents and volumes. The best and most robust results were achieved with the polymer based sorbent Bond Elut Plexa (Varian, Inc).

Pre-concentration factors of about 600 can be achieved. Absolute recoveries in the range 45 to 96% are obtained. Using standards consisting on spiked water samples submitted to the extraction process, trueness between 88 and 115% is obtained. Reproducibility in terms of relative standard deviation are between 3 and 14%

Keywords: PAHs, drinking water, HPLC-FLD

C-2

RESULTS OF INTERLABORATORY STUDIES ON THE DETERMINATION OF PCDD/Fs AND PCBs IN FEED AND FOOD ORGANIZED BY THE CRL FOR DIOXINS AND PCBs BETWEEN 2006 AND 2009

Alexander Kotz[1*], Rainer Malisch[2], Kerstin Wahl[3], Johannes Hädrich[4], Nicole Bitomsky[5]

[1 2 3 4 5] CRL for Dioxins and PCBs, Freiburg, Germany
[*] Corresponding author–E-mail: alexander.kotz@cvuafr.bwl.de; Phone: +49 761 8855 139

The Community Reference Laboratory (CRL) for Dioxins and PCBs in Feed and Food (Freiburg, Germany) organized five interlaboratory studies and proficiency tests (PTs) on the determination of PCDD/F and PCB in different food and feed matrices between 2006 and 2009. National Reference Laboratories (NRLs) for Dioxins and PCBs from EU member states, official and private laboratories participated in these studies.

Two ring tests with the aim of harmonization of extraction methods and establishing recommendations for the pre-treatment and extraction of different mineral feed additives were performed with sepiolite (2006), fullers earth and manganese oxide (2007). Summarizing conclusions on extraction methods and solvents for mineral feeds, premixtures and compound feed were drawn from these studies.

Additionally three proficiency tests on food and food additives, guar gum, fish oil (2008) and pork sausage (2009), were organized. The proficiency test on the determination of PCDD/Fs, PCBs and PCP in guar gum was organized by the CRL for Residues of Pesticides–Single Residue Methods, Stuttgart, Germany and the CRL for Dioxins and PCBs in Feed and Food, Freiburg, Germany, in 2008. The concentration range and congener pattern in the PT samples reflected the range of contamination of guar gum originating from India in 2007. In 2008 and 2009 two proficiency tests on the determination of PCDD/Fs, dioxin-like PCBs and indicator PCBs in fish oil and pork sausage were organized. The fish oil and pork sausage sample contained WHO-PCDD/F-TEQ and WHO-PCB-TEQ concentrations in the range of maximum levels and action levels defined for these matrices in Commission Regulation (EC) No. 1881/2006 and Commission Recommendation 2006/88. For the evaluation of the data of proficiency tests a scoring system for the successful participation in these tests was developed.

Keywords: PCDD/F, PCB, PT, food, feed

C-3

ESTIMATING HUMAN EXPOSURE TO FLUORINATED, CHLORINATED AND BROMINATED CONTAMINANTS FROM FISH CONSUMPTION: INTEGRATING CONTAMINANT DATASETS

S.P.J. van Leeuwen[1*], P.E.G. Leonards[2], J. de Boer[3]

[1 2 3] Institute for Environmental Studies (IVM) VU University, Amsterdam, The Netherlands
[*] Corresponding author–E-mail: Stefan.van.Leeuwen@ivm.vu.nl; Phone: +31 20 59 89 545; Fax: + 31 20 59 89 553

Fish and fish products contribute substantially to the human exposure to fluorinated, chlorinated and brominated persistent contaminants. In recent years, several organohalogen contaminant surveys on wild and farmed fish consumed in The Netherlands were carried out[1-4]. This resulted in a substantial amount of data on the concentrations of polychlorinated dibenzo-p-dioxins and –furans (PCDD/Fs), polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), p,p'-DDT, -DDD and -DDE, polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and perfluorooctane sulfonate (PFOS). In this study, these datasets were integrated and combined contaminant exposures were estimated. The objective of this study was to specify (*i*) the main contributing fish species to the human exposure in The Netherlands; (*ii*) the main contributing contaminants, and (*iii*) to determine the contribution of recently introduced farmed species like pangasius and tilapia.

Datasets on levels of above mentioned contaminants if popular (farmed) fish species were combined with fish consumption data from the Netherlands and fish sales data from new species (i.e. farmed shrimps, pangasius and tilapia). The absolute exposure amounts decrease in the following order: ∑indicator-PCBs (1.1 ng/kg bw per day) > PFOS (1.0 ng/kg bw per day) > ∑3 DDTs (0.45 ng/kg bw per day) > ∑8PBDEs (0.27 ng/kg bw per day) > HCB (0.09 ng/kg bw per day) ≈ α-HBCD (0.06 ng/kg bw per day) >> Dioxins and dl-PCBs (0.26 pg total-TEQ/kg bw per day). PFOS shows a distinct exposure pattern as compared to the other contaminants (e.g. because there is no contribution from salmon). The exposure of dioxins and dioxin-like PCBs (as compared to other contaminants) is closest is to the WHO tolerable daily intake (TDI) of 2 pg/kg bw per day, leaving only a small margin of exposure. From a species point of view, herring and farmed salmon are the main contributors to the contaminant exposure from fish, followed by cod, plaice and mussels. The contribution of farmed tilapia, pangasius and shrimp was very low (<1% for all species).

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Keywords: pangasius, exposure, aquaculture, POPs, PFOS

C-4
DETECTION AND IDENTIFICATION OF UNKNOWN CONTAMINANTS IN FOOD USING LC/QTOF MS

Jerry Zweigenbaum[1*]

[1] Agilent Technologies, Wilmington, USA
[*] Corresponding author–E-mail: j_zweigenbaum@agilent.com; Phone: 1 302 633 8661

The detection and identification of unknown contaminants in food is a major concern in today's global market. The ability to do so is complex because of the many factors involved. These include preparing the sample in a way that will lend to detection. For example an extraction procedure may be required or a direct analysis may be needed. Secondly an appropriate analytical technique must be used; a metal contaminant might require ICP/MS, an organic GC/MS, LC/MS or DART/DESI. Finally, there must be some process to distinguish contaminants from material that is normally in a complex matrix. Thus successful determination of contaminants in complex food samples requires a comprehensive strategy and process. This presentation will describe one possible component of an overall strategy to address this need. The use of large databases to screen for contaminants using accurate mass measurement has been developed and can help the analyst determine if a contaminant in the database is present. However, the detection and identification of contaminant not in a database represents a difficult problem. Using LC/QTOF MS, a process to identify targeted compounds (a list of compounds being sought that have been shown to be detected by the LC/MS technique), non-targeted compounds (those in a large database) and unknowns (neither targeted or in a database) will be described. As part of the strategy to determine if unknown contaminants are present in a food sample, there must be a process to define what a "normal" sample contains thus allowing distinction between those materials that should be present and those that shouldn't. This too is a difficult task given the possible variation within a group of commodities and from one region to another. A process to make this assessment will also be described. Examples of these processes, the advantages, and the pitfalls will be included.

Keywords: Identification, unknowns, food, LC/QTOF MS

C-5
DIOXINS GO QUECHERS – A NEW APPROACH IN THE ANALYSIS OF WELL-KNOWN CONTAMINANTS

Jens Luetjohann[1*], Lindan Zhang[2], Stefan Bammann[3], Juergen Kuballa[4], Eckard Jantzen[5]

[1 2 3 4 5] GALAB Laboratories GmbH, Max-Planck-Str. 1, 21502 Geesthacht, Germany
[*] Corresponding author–E-mail: luetjohann@galab.de; Phone: 0049 4152 889 432; Fax: 0049 4152 889 413

Polychlorinated dibenzodioxins and –furans (PCDD/PCDFs) are formed during combustion and as by-products of industrial processes. These compounds are highly resistant to breakdown processes, and consequently persist in the environment, followed by uptake into the food chain. The major part of human exposure to dioxins results from the consumption of food of animal origin including meat, fish, eggs and milk products. Maximum levels (MLs) for dioxins and dioxin-like PCBs in these foods have been set by the Commission Regulation 1881/2006/EC, the methods of sampling and performance criteria for methods of analysis by the Directive 2002/69/EC. Conventional analytical methods for the analysis of PCDD/PCDFs in food (e.g. EPA 1613) contain laborious and time-consuming extraction processes, several clean-up steps followed by HRGC-HRMS analysis. The general duration of sample analysis is estimated between four to five days to meet the requirements regarding low limits of quantification, high recovery rates and analytical precision. Some steps in the sample preparation can be accelerated by pressurized liquid extraction or automated sample preparation systems raising extra costs for expensive equipment. The aim of this study was to combine the flexibility, the universality and velocity of the well-known QuEChERS methodology for the analysis of pesticides with efficient primary and secondary clean-up steps generating a sample extract qualified for HRMS analysis without the need of cost-intensive extra devices. Meat, fat and dairy samples were extracted in a matrix-solid phase dispersion (MSPD) process with acetonitrile in a repetitive manner. For humid samples there was no need for a drying process. After solvent change a fast destructive column cleanup including customized acidic silica was applied. Without solvent change a secondary cartridge cleanup step (aluminum oxide and/or carbon black) was performed. The final extract was applied to HRGC-HRMS analysis (DFS, Thermo Fisher). The total runtime of confirmatory PCDD/PCDF analysis did not exceed 48 hours. The method was validated according DIN EN 32645 and met the requirements of EC legislation regarding accuracy, precision and recovery rates. The LOQs for 2,3,7,8-TCDD/-TCDF were in the range of 0.02 ppt.

Keywords: PCDD, PCDF, QuEChERS, HRGC-HRMS

C-6
DETERMINATION OF ENDOCRINE ACTIVE SUBSTANCES

Anna Poliwoda[1], Agnieszka Żminkowska[1], Piotr P. Wieczorek[1]

[1 2] Opole University, Faculty of Chemistry, Pl. Kopernika 11, 45-040 Opole, Poland
[*] Corresponding author–E-mail: Anna.Poliwoda@uni.opole.pl; Phone: + 48 77 4527115; Fax: + 48 77 4527115

An endocrine active substances are synthetic chemicals that when absorbed into the body either mimic or blocks hormones and disrupts the body's normal functions. Chemicals that are known human endocrine disruptors include diethylstilbesterol (the drug DES), dioxin, PCBs, DDT, and some other pesticides as well as bisphenol A, nonylphenol and some derivatives used as coatings of cans for foodstuff packaging purposes. Many chemicals, particularly pesticides, some antibiotics and plasticizers, are suspected endocrine disruptors based on limited animal studies. Recently, many plant and animal species are showing signs of ill health due to exposure to endocrine disrupting chemicals. Therefore the necessity to determine the presence and concentration level of those compounds in various food samples is required. Unfortunately, the complexity of food samples forces the need of pretreatment stage proceeding. It means that either the isolation of the target substance and/or removing of the interfering molecules before the final analysis have to be proceed. Conventional methods for the determination of these analytes, included liquid-liquid extraction or solid-phase extraction as sample pre-concentration and clean-up steps. Recently, also liquid membrane extraction such as supported liquid membrane extraction (SLM) and microporous membrane liquid-liquid extraction (MMLLE) and molecularly imprinted polymers (MIPs) have been introduced as successful alternatives to improve the selectivity of the analytical extraction processes in comparison to traditional sample preparation methods. They offer efficient alternatives to classical sample preparation techniques.

In this work the potential of sample pretreatment method with the use of liquid membrane extraction technique, for determination of endocrine disrupters (alkylphenols) in environmental and food samples is presented. The extraction was performed from donor phase with pH about 8 to a basic acceptor, through an organic liquid membrane with tri-n-octylphosphine oxide as a carrier. The influence of donor, membrane and acceptor phase composition on SLM extraction efficiency of analytes was studied. After extraction the analyte (enriched acceptor phase) was manually injected into a HPLC-DAD system for further analysis. The complexity of the study material limits insignificantly the enrichment step compared to an aqueous sample analysis. The minimum quantifiable concentration of the investigated analytes was at ppb level.

The work was supported by the research grant no 0536/R/T02/2007/03 from the Ministry of Science and higher Education, Poland.

C-7

QUANTIFICATION OF NITRATE AND NITRITE IN SPINACH AND LETTUCE BY REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/UV

Edgar Pinto[1], Catarina Petisca[2*], Luís Filipe Amaro[3], Olívia Pinho[4], Isabel Ferreira[5]

[1 2 3 5] REQUIMTE- Serviço de Bromatologia, Faculdade de Farmácia da Universidade do Porto, Rua Anibal Cunha 164, 4099-030 Porto; Portugal
[4] REQUIMTEFaculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[*] Corresponding author–E-mail: catarinapetisca@fcna.up.pt; Phone: +351222078929; Fax: +351222003977

Vegetables tend to concentrate nitrate ions, thus, they are a major source of human exposure to this compound. Nitrate concentrations vary significantly, ranging from 1 to 10,000 mg kg^{-1} fresh weight, while nitrite levels in fresh vegetables are low (<2 mg kg^{-1}). However, nitrite levels in vegetables may increase during post-harvest storage by the action of indigenous bacteria and/or the presence of nitrate reductase, especially when they are left at room temperature or higher. Nitrate content in vegetables is concerned with food safety. The European Commission /EC) established maximum levels of nitrate in lettuce (Lactuca sativa L) and spinach (Spinacea oleracea). The vegetables producers should gradually modify their farming methods by applying the codes of Good Agricultural Practice (GAP) recommended at national levels, so as to comply with the maximum levels to reduce nitrate levels. A rapid and cost-effective RP-HPLC method with UV detector was validated for quantification of nitrate and nitrite in spinach and lettuce. The HPLC separation conditions were optimized with respect to chemical composition of the mobile phase, flow rate, chromatographic resolution and analysis time. The chromatographic separation was achieved using a HyPurity C18, m chromatographic column with 25 cm and elution with 0.01M n-octylamine to pH 6.5. Linearity was obtained over the tested concentration range of 0.3–15 mg/L of nitrate and nitrite. The linear regression equations of nitrate and nitrite standard curves were calculated as y = 24041x + 1941 and y = 33772x -2000, respectively. The correlation coefficients were both greater than 0.999, which showed a very good linearity. The detection limit of nitrate and nitrite, defined as a signal-to-noise ratio of 3, was the same, 0.05 mg/L. The method showed good sensitivity and can detect trace levels of nitrate and nitrite. Reproducibility of the measurements was evaluated by intra-day and inter-day analysis calculated from the results of 3 replicates. Repeated trails all obtained CV values less than 2%, pointing out high degrees of reproducibility. The recoveries of nitrate and nitrite spiked into vegetable samples were higher than 96%. Extraction of nitrate and nitrite into hot water prior to filtration and measurement is the most usual process; however, several interferents appear in the chromatogram. Extraction in presence of activated charcoal of freeze-dried and frozen samples was compared. The results of analysis of samples of Lactuca sativa L, Spinacea oleracea, Tetragonia tetragonioides showed that the method was fast, reliable and sensitive.

Keywords: nitrite, nitrate, ion-pair HPLC, vegetables

C-8
HEAVY METAL RESIDUE LEVELS IN SOME IMPORTED MOLLUSKS MARKETED IN ROMANIA

Mara Georgescu[1*], Constantin Savu[2], Ovidiu Savu[3]

[1 2 3] University of Agronomical Sciences and Veterinary Medicine of Bucharest, Romania, Veterinary Medicine Faculty
[*] Corresponding author–E-mail: dr_georgescu_mara@yahoo.com; Phone: +40751109241

Four types of imported mollusks marketed in Romania were subjected to heavy metal residues detection. Mercury, cadmium, lead, copper and zinc were analyzed by flame atomic absorption spectrofotometry in 194 oyster and 220 mussel samples from various hypermarkets in Bucharest. The overall contamination levels proved to be smaller for mussels (16.8% and 2.72% samples exceeding the limits set by EC Regulation no. 1881/2006 for lead and copper respectively, whilst no exceedings were found for cadmium, mercury and zinc) in comparison to oysters (61.8%, 11.8%, 10% and 100% samples with exceeding levels of cadmium, lead, zinc and copper respectively).

Oysters which have accumulated cadmium and copper above the European legislation limits appear to be imported and marketed in Bucharest to an unusual high degree. The average cadmium level was found to be 1.45 µg/g (0.16–3.81 µg/g) and 0.27 µg/g (0.11–0.97), in oyster and mussel samples, respectively. Copper levels were higher than the European limits in all the oyster samples, with an average level of 18.6 µg/g (5.6–31.4 µg/g). The average level in mussels was much lower (1.62 µg/g).

The average lead levels were not generally high in neither of the analyzed mollusks (0.74 µg/g and 1.46 µg/g in oyster and mussels samples respectively), but the spread of data was rather significant (0.3–6.8 µg/g and 0.1–3.6 µg/g for oysters and mussels, respectively). Unlike for the rest of the heavy metal residues, the percent of samples with exceeding levels of lead was higher for mussels (16.8%), than for oysters (11.8%).

The average mercury levels were quite low for both oyster (0.16 µg/g) and mussel (0.23 µg/g) samples, none of the samples exceeding the legal maximum level (0.5 µg/g). Zinc residues contamination also appeared to be low, with 10% oyster samples exceeding the limits set by national regulations (50 µg/g). The average zinc levels were low in both oyster (41 µg/g) and mussel (19 µg/g) samples.

This study has shown that there is a significant contamination with cadmium and copper in oysters marketed in Bucharest. Also, the presence of lead residues in mussels is to be taken into consideration. Consequently, there is a strong need for monitoring heavy metal residues in imported mollusks and for identification of possible correlations with specific suppliers or certain areas of harvesting.

Keywords: oysters, mussels, heavy metal residues

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C-9

FAAS/CALCINATION VERSUS GFAAS/WET DIGESTION, FOR THE ANALYSIS OF CADMIUM RESIDUES IN MUSSEL SAMPLES

Constantin Savu[1], Mara Georgescu[2*], Ovidiu Savu[3]

[1 2 3] University of Agronomical Sciences and Veterinary Medicine of Bucharest, Romania, Veterinary Medicine Faculty
[*] Corresponding author–E-mail: dr_georgescu_mara@yahoo.com; Phone: +40751109241

This study aimed to investigate the precision of two analytical methods (FAAS and GFAAS), associated with different methods of sample preparation (calcination and wet digestion) for the detection of cadmium residues in mussel samples. FAAS/calcination and GFAAS/wet digestion were used for the analysis of cadmium residues in 54 samples of naturally contaminated mussels and in 20 samples of uncontaminated mussels, enriched with this heavy metal.

Cadmium level results for all 54 naturally contaminated mussel samples obtained using GFAAS/wet digestion, were higher than those obtained by FAAS/calcination, with a difference range for average values of $1.8x10^{-3}$ – $5.9x10^{-3}$µg/g (10 repetitions for each sample). Precision of FAAS/calcination and GFAAS/wet digestion was assessed by calculating the average, the standard deviation (SD), the relative standard deviation (RSD), the confidence interval (CI), the relative average deviation from mean (RADM) and the repeatability (r), for all 54 samples (10 repetitions each). The average values for the mentioned parameters calculated for 54 mussel samples were the following: SD = $2.32x10^{-3}$ and $6.07x10^{-4}$, RDS = 1.51 and 0.38, CI = 0.15509 ± $2.3x10^{-3}$ and 0.16038 ± $6x10^{-4}$, RADM = 0.2932 and 0.1, r = $6.5x10^{-3}$ and $1.60071x10^{-3}$ for FAAS/calcination and GFAAS/wet digestion, respectively.

The recovery degrees of cadmium from the 20 mussel samples enriched with 0.1 ppm cadmium each, ranged from 98.482% to 99.179% for GFAAS/wet digestion and from 96.77% to 97.251% for FAAS/calcination.

The comparative analysis of all parameters revealed that GFAAS/wet digestion is associated with more precise results and with a better repeatability than FAAS/calcination. Additionally, higher recovery degrees for GFAAS/wet digestion indicate that this method seems to be more appropriate for the analysis of cadmium residues in mussel samples, in comparison with FAAS/calcination.

Keywords: FAAS/calcination, GFAAS/humid digestion, cadmium, mussels

Funded by CNCSIS project IDEI (ID_736/2007, Project Director: Professor PhD Constantin Savu).

C-10

NEW STREAMLINE SOFTWARE FOR SCREENING TO DETERMINE 250 PESTICIDES IN ORANGE OIL BY LC-MS/MS

Charles Yang[1*], Jonathan Beck[2], Jamie Humphries[3]

[1 2] Thermo Fisher Scientific, San Jose, USA
[3] Thermo Fisher Scientific, Austin, USA
[*] Corresponding author–E-mail: charles.yang@thermofisher.com; Phone: 9256280929

Introduction: A software program has been developed with built-in workflows for routine analysis in environmental and food residue analysis. The software incorporates a methods database that can be customized by the user to include unique compounds, in addition to pre loaded contaminants commonly encountered in environmental and food samples. A NIST format LC-MS/MS library of these commonly found contaminants helps confirm the compounds being analyzed. Data collection, analysis and report generation are carried out using the same software program. To demonstrate capabilities of the software we analyzed a mixture of 250 pesticides spiked into orange oil samples using both negative and positive ionization modes.

Method: Orange oil was spiked with 250 pesticides at 1 and 10 ng/mL (ppb). 10 µL of the spiked orange oil was injected directly onto a Hypersil GOLD aQ 50 × 2.1 mm 3µ. A simple HPLC gradient was used with a retention time of 18 mins. Using TraceFinder software we were able to use Timed SRM (TSRM) to create the instrument method, collect the data and process it.

Preliminary Data: To build the method for analysis of this mixture of compounds, the included database of compounds was used since it included all of the compounds in our experiment. Parameters for each analyte, including precursor mass, product ion masses, collision energy, tube lens values were specified by the database, eliminating the need for time consuming infusions of each individual compound. Furthermore, the information in the database is carried through the software to the data analysis and reporting section of the software, making the data processing setup and data review very efficient. Individual flagging of each analyte can be customized, for example a response above or below a specified level, or ion ratio failures. The use of the TSRM allowed overlapping retention time of analytes versus a run with all analytes in one list, therefore, increasing scan time per analyte as well as being able to screen for more analytes then the 250 mentioned.

Keywords: Pesticides, Orange, Screening, Food, Environmental

C-11
PERFLUORINATED COMPOUNDS IN SWISS GROUNDWATERS

Otmar Zoller[1*], Heinz Rupp[2], Silvia Zeltner[3], Ronald Kozel[4], Miriam Reinhardt[5]

[1 2 3] Swiss Federal Office of Public Health, Bern, Switzerland
[4 5] Swiss Federal Office for the Environment, Bern, Switzerland
[*] Corresponding author–E-mail: otmar.zoller@bag.admin.ch; Phone: +41 31 322 95 51; Fax: +41 31 322 95 74

The greatest portion of consumer exposure to perfluorinated compounds (PFC) is likely to result from the intake of contaminated foods, including drinking water. The contribution of drinking water may be of importance.

Groundwater is the most important drinking water resource in Switzerland. In a pilot study of the NAQUA National groundwater monitoring, the occurrence of the following eleven PFC was determined:

PFPeA	Perfluoropentanoic acid
PFHxA	Perfluorohexanoic acid
PFHpA	Perfluoroheptanoic acid
PFOA	Perfluoroctanoic acid
PFNA	Perfluorononanoic acid
PFDA	Perfluorodecanoic acid
PFUnA	Perfluoroundecanoic acid
PFBS	Perfluorobutanesulfonate
PFHxS	Perfluorohexanesulfonate
PFOS	Perfluorooctanesulfonate
PFOSA	Perluorooctanesulfonamide

We used direct injection of a 1 mL sample onto a C8-analytical column and detection with LC-ESI-MS-MS (ABS API5000). Using this setup the limit of quantification (LOQ) was 0.5 to 1 ng/L for all compounds. The omission of a preconcentration step helped to minimize blank and/or background contamination problems. The use of the most similar isotopically labelled compounds as internal standards was important for correct quantification.

49 monitoring sites in aquifers typical for Switzerland were sampled 2 to 3 times with an interval of 3 to 6 months during a time span of 14 months.

PFDA, PFUnA and PFNA were not detected at all and the detection of PFOSA is not unequivocal confirmed. All other compounds could be detected at several sites.

At 27 sites (55%) no PFC could be detected. At 11 sites (22%) the sum of PFC was between 0–10 ng/L and at 8 sites (16%) between 10–50 ng/L. Only at two sites the sum was between 50–100 ng/L and at 1 site the sum was at 225 ng/L.

Concentrations at monitoring were stable over time.

All sampling sites with distinct PFC contamination were situated in urban areas. We could distinguish 4 different contamination profiles. The contamination at the site with the highest concentration seems to be a local incident of yet unknown origin.

In summary the contamination level of groundwater with PFC in Switzerland is low and restricted to urban areas. At the current state of knowledge, the concentrations detected in the pilot study pose no health risk for the consumer and the groundwater can be used as drinking water resource without reservation.

Keywords: groundwater, perfluorinated-compounds, PFOS, PFOA, LCMSMS

C-12

ASSESSMENT AND OPTIMIZATION OF A PRESSURIZED LIQUID EXTRACTION- METHOD TO DETERMINE THE 15+1 EU PRIORITY POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN MEAT SAMPLES.

Patricia López[1*], Szilard Szilágyi[2], Jose Angel Gómez-Ruiz[3], Donata Lerda[4], Thomas Wenzl[5]

[1 2 4 5] European Commission, DG Joint Research Centre, IRMM (Geel, Belgium)
[3] Instituto de Fermentaciones Industriales, Consejo Superior de Investigaciones Científicas CSIC, (Madrid, Spain)
[*] Corresponding author–E-mail: patricia.lopez-sanchez@ec.europa.eu; Phone: 0032-14-571924; Fax: 0032-14-571343

A pressurized liquid extraction (PLE) procedure to determine the 15+1 EU Polycyclic aromatic hydrocarbons (PAHs) in meat samples was assessed and optimized for absolute recovery of the analytes. Sample clean-up consisted of gel permeation chromatography and solid-phase extraction. Gas chromatography-mass spectrometry was applied for the measurement of the sample extracts [1]. The performance criteria specified in Commission Regulation (EC) No. 333/2007 for the determination of benzo[*a*]pyrene in food (e.g. recovery between 50-120%) were applied as target values [2]. The experiments were performed with spiked meat samples.

The influence of the spiking procedure, of the water content, and of the nature of the extraction solvent on the recovery of both the 15+1 EU-priority PAHs and the isotopically labelled internal standards was investigated.

The effect of four operational parameters (extraction temperature, static extraction time, number of extraction cycles and flush time), on the efficiency of PLE was assessed and then optimized by a two-step computer based experimental design. At first, a Plackett–Burman screening design was performed to identify parameters with significant effect. Subsequently, a response surface model (RSM) was applied to estimate optimum parameter settings.

The PLE performance of the tested extraction solvents (toluene, *n*-hexane and a mixture of *n*-hexane:acetone (1:1)) for the extraction of fresh food samples was comparable in terms of absolute recoveries. The application of freeze-dried samples did not show any improvement when hexane:acetone (1:1) was applied as extraction solvent. Compared to the extraction of fresh samples, higher recoveries were achieved for $^{13}C_3$-pyrene in the extraction of freeze-dried samples applying solely *n*-hexane as extractant.

Finally, the PLE performance was compared to Soxhlet extraction. No significant differences were found in terms of absolute recovery. Nevertheless, preference is given to PLE due to its lower solvent-consumption, shorter extraction time and higher selectivity, which allowed reducing the amount of co-extractives and interferences.

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Keywords: 15+1-EU-PAHs, meat-samples, pressurized-liquid-extraction

C-13

SIMULTANEOUS MONITORING OF MATRIX INTERFERENTS DURING THE ANALYSIS OF PERFLUORINATED COMPOUNDS IN TISSUE WITH A NOVEL DUAL SCAN-MRM APPROACH

Peter Hancock[1*], Paul Silcock[2], Anna Kärrman[3], Keith Worrall[4], Bert van Bavel[5]

[1 2 4] Waters, Manchester, UK
[3 5] Örebro University, Örebro, Sweden
[*] Corresponding author–E-mail: peter_hancock@waters.com; Phone: +44 7788 411812

Perfluorinated compounds (PFCs) have been determined over the last ten years in an array of matrices by various techniques including liquid chromatography tandem quadrupole mass spectrometry (LC/MS/MS)[1]. More recently UltraPerformance LC (UPLC) has been introduced as a technique utilised in the analysis of PFCs and has offered rapid analysis whilst preserving separations[2]. The ability of laboratories to successfully measure PFCs in various matrices has improved greatly, with some of the success attributed to the continuous improvement in data quality with advances in instrumental technology[3,4].

Advances in LC/MS/MS instrumental performance have largely been focussed on the sensitivity of Multiple Reaction Monitoring (MRM) mode to satisfy the need for increasingly lower detection limits. While this is clearly a priority for this type of instrumentation there has previously been limitations in acquiring important qualitative information from a sample in a single injection. This information can be of high value when analysing ultra trace level contaminants in difficult sample matrices such as tissues when trying to further improve the quality of methods.

The potential of a novel acquisition mode, Dual Scan-MRM, in LC/MS/MS instrumentation applied to the analysis of PFCs will be discussed. Full scan background matrix data was simultaneously acquired with quantitative MRM data using Dual Scan-MRM. This was utilised in combination with rapid 5 minute UPLC separation for the analysis of salmon liver from unknown locations in Norway.

Dual Scan-MRM acquisitions allowed correlations between background matrix components and analytical problems to be observed, particularly for indigenous bile acids in salmon liver. Additional evidence for these compounds were obtained using product ion scanning which indicated the presence of deoxytaurocholate isomers co-eluting with PFOS.

Further work is required to manage the negative effects of matrix in PFC analysis but continuously monitoring sample background using a Dual-Scan MRM approach can lead to more information about the challenges posed by each individual sample. This is a novel intra-sample quality control (QC) check that has the potential to help improve quality within PFC analysis and is made possible by next generation instrumentation.

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Keywords: Perfluorinated compounds (PFCs), Dual Scan-MRM

C-14

ACCURATE ANALYSIS OF FOODSTUFFS USING VAPOR GENERATION ATOMIC ABSORPTION SPECTROSCOPY AND ICP-OES

Klaus Mittendorf[1*], Yolanda Fintschenko[2], Hazel Dickson[3]

[1 2 3] Thermo Fisher Scientific
[*] Corresponding author–E-mail: klaus.mittendorf@thermofisher.com; Phone: +492771330333; Fax: +492771330334

Demand for the analysis of food is constantly increasing in the modern world. Consumers are becoming much more aware of food safety and are more interested in the contents of the food that they eat. This is adding to the pressures that suppliers are facing to test their products and in many cases display the results on the label. Legislation is also changing to meet the consumer demand and due to the many 'incidents' involving food safety. Regulations governing the content of metallic elements in food is vast in the case of some elements and sparse for others, with variations occurring depending on product, country and origin. With all this complex legislation and pressure there are several options available to the analyst when it comes to the analysis of such samples.

Growing concerns about the possible presence of the toxic chemical, mercury, in the food supply chain have led to tighter restrictions on its presence in the food we eat which has led to the enforcement of regulations that state a maximum concentration of mercury in fish of approximately 0.5 mg/kg wet weight. Coupled with the VP100 vapour generation accessory, the iCE 3000 Series AA spectrometers are capable of reaching detection limits of 0.01 mg/kg in solid. The advantages of multi element analyses cannot be denied for high throughput laboratories, where AAS and ICP-OES are often used in conjunction with each other in order to cover the linear dynamic range required for the analysis of foodstuffs. Using the iCAP 6000 Series ICP-OES, two multi element methods are presented which demonstrate the analysis of majors, minors and trace element contaminants in a variety of foodstuffs.

This paper will discuss some of the many world wide legislations and how AAS and ICP-OES can be used for compliance.

Keywords: ICP-OES, AAS, Mercury, Fish

C-15

RAPID DETECTION OF MELAMINE AND CYANURIC ACID USING A NOVEL HIGH CAPACITY ION TRAP MASS SPECTROMETER

Leith J. Fremlin[1], Matthias Pelzing[2], Michal Bohac[3*]

[1 2] Bruker Daltonics Division, Preston, Australia
[3] Bruker Daltonics CZ
[*] Corresponding author–E-mail: michal.bohac@bdal.cz; Phone: +420 544 526 988; Fax: +420 544 526 989

Novel Aspect: We present the rapid detection and quantitation of melamine and cyanuric acid by novel high capacity ion trap mass spectrometer.

Introduction: Adulteration of food and beverages with industrial chemicals has become an issue of late, as evidenced in September 2008 with the contamination of infant milk formula in China with melamine. There is a requirement for rapid and sensitive methods to detect and quantify such chemicals in complete matrices. LC-MSMS methods are superior to GC-MS methods in that they have an easy sample preparation and do not require sample derivatization. The high capacity ion trap mass spectrometer is a highly robust system capable of operating in full scan, MS^n and MRM modes. Here we report a recently developed method for the extraction, detection, and quantitation of melamine and cyanuric acid residues.

Methods: Chromatographic separations were carried out using a Dionex UltiMate 3000 using an Acclaim Mixed-Mode WAX-1 (Dionex, Sunnyvale, CA, USA) column (2.1 × 150 mm, 5 µm) maintained at 30°C and operated in a HILIC mode. For the simultaneous detection of melamine and cyanuric acid the mobile phase gradient consisted of water / 0.1% formic acid (10%) and acetonitrile (90%) to 50%:50% over two minutes before returning to initial conditions at a flow rate of 300 µL/min, and a run time of five minutes. 10 µL injections were made. The ion-trap mass spectrometer was optimized for the detection of melamine in MRM mode.

Preliminary results: Using the novel high capacity ion trap mass spectrometer it was possible to establish a calibration curve for melamine ranging from 1 to 1000 ppb. The limit of detection was established to be 0.05 ppb which resulted in a signal to noise of 50:1. Melamine was spiked into infant milk formula (1 ppm and 2.5 ppm) and extracted as per the conditions above. Despite the low detection limits, these concentrations were chosen on the basis of infant milk contamination recommendations by the FDA. Furthermore, it was possible to detect and quantify melamine contamination in a number of samples of contaminated infant milk power from China well above the limits determined by the FDA.

Keywords: melamine, ion trap, Adulteration, contamination

C-16

APPLYING ACCURATE MASS TIME OF FLIGHT SPECTROMETRY TO ROUTINE SCREENING OF CONTAMINANTS IN FOOD AS AN ALTERNATIVE TO TARGETED APPROACHES

Cornelia Petronela Ene[1], Ruxandra Subasu[2], Shaun Bilsborough[3*]

[1] Veterinary Institute, Bucharest, Romania
[2] Agilrom Scientific, Bucharest, Romania
[3] Agilent Technologies, Cheadle, UK
[*] Corresponding author–E-mail: shaun_bilsborough@agilent.com; Phone: +447917232567

The requirements for screening many hundreds of compounds in complex matrices presents a key analytical challenge.Targeted methods using triple quadrupole mass spectrometry provide low limits of detection. However, such approaches require the analyst to optimise the methodology for a limited number of compounds to maintain instrument performance. Non-targeted approaches allow for rapid screening of samples for many thousands of compounds without the need for any prior knowledge from the analyst of suspected contaminants. In this paper, we discuss the application of both techniques in the screening of complex food matrices for contaminants.

The successful detection and identification of food contaminants using the non-targeted approach presented two challenges. Firstly, to locate and extract the relevant data resulting from the analysis of a complex mixture containing numerous compounds present across a wide dynamic range and secondly, to identify those contaminants. To address this, rapid resolution chromatography was employed to allow fast analysis while maintaining high resolution thereby avoiding the risk of chemical interference leading to a loss in mass accuracy. Dynamic range issues were overcome through the use of analogue to digital conversion for ion detection providing 5 orders of magnitude within the same scan. Compounds were identified using an Accurate Mass Retention Time (AMRT) database, which contained over 7,000 compounds pertaining to environmental and toxicological studies. Additionally, any compound that was found but was not present in the database could be investigated further through empirical formula determination using the accurate mass measurement provided by time of flight mass spectrometry. Indeed, the development of analog to digital ion detection with fast digitization rates has provided the further advantage of accurate mass MSMS analysis leading to increased confidence in identification. All Sudan reds dyes could be readily detected at the low ppb level using QTOF mass spectrometry, including Para-Red which showed a lower response in comparison to the other Sudan dyes. In comparison, sub-ppb level detection and quantitation was possible using triple quadrupole mass spectrometry, showing the higher quantitative performance of the MRM functionality afforded by this type of instrument. However, as Para-Red was an ‘unexpected’ contaminant it was not part of the target list for the triple quadrupole analysis and was therefore not detected.

We conclude by proposing the use of both targeted and non-targeted methodologies for food safety applications, by firstly screening samples for all contaminants and then targeting those compounds identified for quantitation using a triple quadrupole method.

Keywords: screening, contaminants, spectrometry, LCMS, identification

C-17

MERCURY SPECIATION IN BIOLOGICAL PATTERN BY DOUBLE ISOTOPE DILUTION AND GC-ICP-MS

Patricia Navarro[1], Stéphanie Da Fonseca[2], Vincent Perrot[3], Thierry Guérin[4], Olivier F. X. Donard[5], Mathilde Montperrus[6*], David Amouroux[7]

[1 3 5 6 7] Laboratoire de Chimie Analytique BioInorganique et Environnement (LCABIE), Institut Pluridisciplinaire de Recherche sur l'Environnement et les Matériaux, UMR 5254 CNRS-UPPA, Pau, France
[2 4] Laboratoire d'Etudes et de Recherches sur la Qualité des Aliments et des Procédés agro-alimentaires (LERQAP), Agence Française de Sécurité Sanitaire des Aliments (AFSSA), Maisons-Alfort, France
[*] Corresponding author–E-mail: mathilde.monperrus@univ-pau.fr; Phone: +(0)5 40 17 50 42

Mercury is a contaminant encountered in all compartments of the environment. At the time being, government agencies assess the safety of food items based solely on the total concentration of this element but organomercury species, particularly methylmercury, are considerably more toxic than inorganic species. Thus speciation analysis i.e. the determination of the chemical forms (species) of these elements present a croissant interest in environmental issues. The goal of this project is to develop an accurate method, based on isotopic dilution, which can be used as a reference method by government agencies to determine the speciation of Hg in seafood.

In this work, various analytical methods are developed for mercury speciation by gas chromatography–inductively coupled plasma mass spectrometry (GC-ICP-MS). These developments are based on the use of multiple isotopic labeled species in order to correct the transformations reactions that the analyte species may suffer during the chemical analysis. All these analytical methods are applied to two certified references materials (DOLT-4 dogfish liver and BCR-464 tuna fish). The results show that, the most efficient method is to spike the isotopic labeled species before the extraction by tetramethylammonium hydroxyl (TMAH) and derive them by propylation. The quantification by isotopic dilution is efficient in terms of accuracy and fidelity but demonstrates that species interconversions are not perfectly controlled during the sample preparation. Consequently, all the results obtained by isotopic dilution are also calculated by isotopic pattern deconvolution methodology, a numerical method able to quantify both the species concentrations and the transformation rates. A perfect match between these two methods is obtained. The optimized method is then applied to the speciation of mercury in elvers and eels tissues. Interesting results are obtained showing the accuracy and the robustness of such methods when they are applied to real matrices.

Keywords: mercury, speciation, GC-ICP-MS

C-18

SPECIATION OF ARSENIC IN SEAFOOD BY IEC/ICP-MS FOLLOWING MICROWAVE ASSISTED EXTRACTION: METHOD VALIDATION

Axelle Leufroy[1], Véronique Sirot[2], Jean-Charles Leblanc[3], Laurent Noël[4], Diane Beauchemin[5], Thierry Guérin[6*]

[1 4 6] Laboratoire d'Etudes et de Recherches sur la Qualité des Aliments et des Procédés agroalimentaires, Agence Française de Sécurité Sanitaire des Aliments, Maisons-Alfort, France
[2 3] Unité Appréciation Quantitative du risque en Physico-Chimie, Agence Française de Sécurité Sanitaire des Aliments, Maisons-Alfort, France
[5] Department of Chemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6
[*] Corresponding author–E-mail: t.guerin@afssa.fr; Phone: +33 1 49 77 27 11

Seafood has been identified as a source of major exposure to arsenic through human consumption, and it is well known that the toxicity of arsenic (As) is dependent on its speciation i.e. chemical forms (species). Therefore, to assess food safety, it is important to know not only the total concentration of As in food, but also the nature and abundance of the species that are present.

This work describes a reliable and reproducible method that is capable of determining arsenic speciation in several fishes and other seafood samples. First, the process involves microwave-assisted extraction (MAE) into water, using low microwave power in order to preserve the carbon-arsenic bonds. Then, the extracts are analyzed by a relatively rapid HPLC/ICP-MS method that was previously optimised and involves the baseline separation of 7 As species within 12 min in a single chromatographic run on a Dionex IonPac AS 7 anion-exchange column with a nitric acid gradient eluent.

The method was validated following the French AFNOR guidelines NF V03-110: linearity, limits of detection and of quantification, specificity, accuracy and precision on 4 different Certified Reference Materials (CRMs) were evaluated.

Finally, the method was used to determine the arsenic content in the fish and seafood samples of the 2nd French Total Diet Study.

Keywords: Arsenic, speciation, seafood

C-19
KINETICS OF BENZO(A)PYRENE PHOTOLYSIS IN MODEL SYSTEMS AND ATTEMPTED IDENTIFICATION OF SOME OXIDIZED PRODUCTS

Alena Bednáriková[1], Božena Skláršová[2], Emil Kolek[3], Peter Šimko[4*]

[1 2 3 4] VUP Food Research Institute, Bratislava, Slovak Republic
[*] Corresponding author–E-mail: simko@vup.sk; Phone: 421 2 50237135

Polycyclic aromatic hydrocarbons (PAHs) are a group of harmful micropollutants containing two or more fused aromatic rings. Many of these PAHs exhibit mutagenic or carcinogenic activity, and are harmful to living organisms even in very low doses. Photolysis plays an important role in the degradation of PAHs in the environment.

In this report, we studied the photolysis of benzo(a)pyrene (BaP), which is one of the most prevalent PAHs with five condensed rings, in different liquid media. Initial pseudo-first-order rate constants and half lives were calculated with linear regression of ln (c/c_0) versus time.

Photolysis decreased the nonpolar fraction and strongly increased the polar and bound fraction, so it caused the formation of partially oxidized intermediate compounds that are more susceptible to biodegradation than the parent compound.

Keywords: benzo(a)pyrene, photolysis

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C-20

QUANTITATIVE ANALYSIS OF POLYBROMINATED DIPHENYL ETHERS DI- TO DECA-BROMINATED IN FISHMEAL AND FISH FEED BY GAS CHROMATOGRAPHY/ION TRAP TANDEM MASS SPECTROMETRY AND ISOTOPE DILUTION.

Lucía Blanco[1*], Juan Vieites[2]

[1 2] Centro Técnico Nacional de Conservación de Productos de la Pesca (ANFACO-CECOPESCA), Carretera Colexio Universitario nº 16, 36310, Vigo, Pontevedra, Spain
[*] Corresponding author–E-mail: lucia@anfaco.es; Phone: 34 986 469303; Fax: 34 986 469269

Polybrominated diphenyl ethers (PBDEs), used as additive flame retardants, are contaminants of concern because of their presence in environment and the toxicological effects. Increasing concern on risk assessment of brominated compounds has made governments establish some restrictions in the use of these compounds in electronics, although exposure will continue during the coming decades. Recently, Tetrabromodiphenyl ether and Pentabromodiphenyl ether have been included in Annex A (chemicals to be eliminated) of the Stockholm Convention[1].

Feeding stuffs used in aquaculture are made mainly from fish oil and fish meal, being fish fat one of the main components where persistent contaminants are accumulated, due to bioaccumulation and biomagnification in the aquatic environment. In order to evaluate the safety of these raw materials, development of a method for the determination of PBDEs in these sample matrices was carried out in this study. We have also applied automated cleanup and fractionation procedures of the FMS Power Prep system to obtain an efficient and fast method.

Gas chromatography (GC) separation using a 15m DB-5HT narrowbore GC column, followed by ion trap tandem mass spectrometry detection in EI mode (ITD MS/MS) for the analysis of 26 native polybrominated diphenyl ethers (PBDEs) and 11 $^{13}C_{12}$-labeled congeners, including decaBDE-209, in fishmeal and fish feed, using isotope dilution, was developed.

GC and ITD MS/MS parameters were optimized in order to obtain the best sensitivity for the PBDE analysis. External electronic ionization mode was used.

$[M-2Br]^+$ cluster was the most abundant species followed by the molecular ion cluster $[M]^+$ for most di-decaBDE congeners, although we have found differences in the proportions of the main ions produced if the emission current changed. Values around 0.30 for the stability parameter q resulted in the best fragmentation conditions. CID voltages applied ranged from 2 to 5.5 V.

An automated purification procedure recently described[2] in FMS Power Prep system was succesfully applied to quantification of PBDEs in fish feed samples. Average recoveries of 43-96% were obtained for the $^{13}C_{12}$-labeled congeners spiked in the samples prior to preparation. The method detection limits ranged from 0.1–3.4 pg/g in fish feed. Reference Material from NIST SRM 1947 was used to test the whole procedure, obtaining satisfactory results.

[1] Stockholm Convention on Persistent Organic Pollutants (POPs). COP4-Geneva 9 May 2009.Available from: http://chm.pops.int/Convention/Pressrelease/COP4Geneva9May2009/tabid/542/language/en-US/Default.aspx
[2] Wyrzykowska B, Tabor D, Gullet BK. *Anal. Chem.* 2009, DOI: 10.1021/ac900105a.

Keywords: PBDEs, ITD MS/MS, fishmeal, feedingstuffs

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C-21

CERTAIN ENVIRONMENTAL CONTAMINANTS IN FRESHWATER FISH IN SERBIA

Sasa Jankovic[1*], Jasna Djinovic[2], Tatjana Radicevic[3], Srdjan Stefanovic[4], Radivoj Petronijevic[5], Slavica Veskovic-Moracanin[6]

[1 2 3 4 5 6] Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia
[*] Corresponding author–E-mail: sasa@inmesbgd.com; Phone: +381-11-2650-655; Fax: +381-11-2651-825

Nutritional benefits of fish are mainly due to the content of high-quality proteins, vitamins, macro and microelements and omega-3 polyunsaturated fatty acids. However, increased fish consumption may simultaneously increase the contaminant intake to the levels of toxicological concern.

Mercury, DDT (sum of DDE, DDD and DDT) and polychlorinated byphenils' concentrations were measured in the muscle tissue of fish species farmed in Serbia's fish ponds–carp (*Cyprinus carpio*) and trout (Salmo *irideus*). These species are of major importance in the diet of the Serbian population. Total number of samples was 30.

For mercury analysis, samples were prepared by microwave digestion (ETHOS Milestone) with nitric acid and hydrogen peroxide. Analyses were carried out on atomic absorption spectrometer Varian "SpectrAA 220" with VGA 77 hydride system. Cold vapour technique was applied for mercury, using 30% tin chloride as reductant. Analytical quality control was achieved by using certified reference material BCR 186. Replicate analyses were in the range of certified values.

Mean mercury concentration was 0.013 $\mu g g^{-1}$.

Gas chromatograph (GC) Varian, model 3800 equipped with a ^{63}Ni electron capture detector (ECD) and Varian VF 5-ms column were used for analysis of DDT and polychlorinated byphenils.

Analytical quality control was achieved by using certified reference material ERM-BB446 (IRMM, Belgium). Replicate analyses were in the range of certified values.

Content of DDT in carp and trout was in the range 0.001 $\mu g g^{-1}$–0.021 $\mu g g^{-1}$ with the mean value of 0.003 $\mu g g^{-1}$.

Concentration of six target individual congeners (IUPAC numbers 28, 52, 101, 138, 153 and 180) was in the range 0.001 $\mu g g^{-1}$–0.029 $\mu g g^{-1}$ with mean value of 0.004 $\mu g g^{-1}$.

All samples contained mercury, DDT and polychlorinated byphenils below the maximum level fixed by the European Commission Decision and Serbian national Regulation.

On the basis of these results we can conclude that the consumption of carp and trout from Serbia's fish ponds does not pose health risk according to the limits set in both national and European Regulation.

Keywords: mercury, DDT, PCB, fish

This work was supported by the project TR-20122, sponsored by the Ministry of Science and Technological Development of the Republic of Serbia.

C-22

VALIDATION OF A MULTI-RESIDUE METHOD TO DETERMINE ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS IN BOVINE FAT BASED ON THE SANCO/2007/3131 DOCUMENT

Fernando Lopes[1*], Cláudia Kowalski[2], Igor Olivares[3], Elizabete Saglioni[4], Anuska Modro[5]

[1 2 3 4 5] Ministry of Agriculture, Livestock and Food Supply (LANAGRO-SP), Brazil
[*] Corresponding author–E-mail: fernando.lopes@agricultura.gov.br; Phone: 55 19 3252 0155

The persistence and extended use of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) have resulted in their distribution in the environment and bioaccumulation in the food chain, mainly in food with high lipid content. This way, maximum residue limits (MRL) for each residue were established to assure the quality of these products.

In such a way, the goal of this study was to validate a multi-residue method to determine seventeen OCP and seven PCB in bovine fat. The methodology was done by solid phase extraction with alumina and analyzed by gas chromatography with electron capture detector (GC-ECD) following the SANCO/2007/3131 to perform the validation.

The validation parameters evaluated were: linearity was studied spiking the fat extract with the standards in five levels (0.5, 0.75, 1.0, 1.5 and 2.0 MRL) and six replicates of injection; the repeatability was done with the extract spiked in 2 levels (0.5 and 1.0 MRL) and six replicates. The reproducibility considered in the same repeatability experiments but with two others analysts, in two different days; the recovery and accuracy were calculated from repeatability data, estimating the percentage of analytes extracted, estimating a correction factor; the limit of detection of the equipment (LDE) was determined by the 3 signal/noise relation and the limit of quantification (LOQ) by the lowest level of spiked extract; the selectivity was analyzed with 10 blank samples, checking matrix interference.

Significant correlation was found in the range tested: the correlation coefficient found were between 0.9979 (α-Hexaclorocyclohexano) and 0.9998 (PCB 180). The repeatability values were between 6.92 (Aldrin) and 16.03% (α-hexachlorocycloexano) to 0.5 MRL level and between 4.74 (4,4'-DDE) and 11.41% (*trans*-chlordane) to 1.0 MRL; besides, the recoveries achieved to 0.5 MRL were between 75.54 (Hexachlorobenzeno) and 114.80% (PCB 153) and to 1.0 MRL were between 77.30 (Dieldrin) and 104.02% (PCB 153). The reproducibility were between 5.87 (4,4'-DDT) and 19.60% (α-hexaclorocyclohexano) to 0.5 MRL and between 5.04 (Mirex) and 14.58% (Hexachlorobenzeno) to 1.0 MRL. The LDE were between 0.064 (Hexachlorobenzeno) and 0.305 μg kg^{-1} (Dieldrin). Also, the accuracy and the LOQ calculated were in agreement with the SANCO document and selectivity experiments did not showed matrix interference in the retention time of the OCPs and PCBs.

Therefore, the method showed efficiency to separate thirteen OCPs and five PCBs and can be currently employed as a routine method to a monitoring program, controling these residues in bovine fat.

Keywords: pesticides, contaminants, organochlorine, validation, PCB

CNPq (Brazil) and Ministry of Agriculture, Livestock and Food Supply

C-23

ANALYSIS OF PERFLUOROALKYL COMPOUNDS IN BIOTA BY SOLVENT MICROEXTRACTION AND LIQUID CHROMATOGRAPHY/ION ISOLATION-BASED ION TRAP MASS SPECTROMETRY

Soledad Rubio[1*], Stefan van Leeuwen[2], Ana Ballesteros-Gómez[3], Noelia Luque[4]

[1 3 4] University of Córdoba, Spain
[2] VU University of Amsterdam, The Netherlands
[*] Corresponding author–E-mail: qa1rubrs@uco.es; Phone: 34-957-218644; Fax: 34-957-218644

Perfluoroalkyl compounds (PFCs) are ubiquitous in the environment and biota owing to their widespread use and many are chemical and biological resistant. The presence of PFCs in many fishes, sea birds and marine mammals is a consequence of the role as sink that oceans play and although no regulations have been set yet concerning PFCs in biota, a considerable effort is being addressed to develop accurate and reproducible quantification methods able to deal with the challenges involved.

This research presents a novel analytical method for the simultaneous monitoring of perfluoroalkyl carboxylic acids (PFACs) and perfluoroalkyl sulfonates (PFASs) in biota by combining a fast, simple and efficient solvent microextraction and a selective and sensitive monitoring mode based on ion isolation ion-trap MS. The method involves the vortex-shaking of 0.2 g of liver/muscle from fish and marine birds and 800 µL of tetrahydrofuran (THF):water (75:25, *v/v*) for 7 min, subsequent centrifugation for 13 min and direct quantitation of PFCs in the extract against solvent-based calibration curves. Selection of solvent composition was based on Hildebrand solubility parameters and their components (i.e. dispersion, dipole-dipole and hydrogen bonding forces). The THF:water mixture used as extractant offered an adequate balance between polar and non-polar forces and the possibility of establishing different types of interactions with the polar groups of PFCs (i.e. ion-dipole and hydrogen bonding).These properties made it possible to extract fast and efficiently both ionic and nonionic PFCs with hydrocarbon chain lengths between C_4 and C_{14} using low solvent volumes. Recoveries in sample ranged between 85 and 111%. It was proved that the method worked not only with ion-trap MS, as here proposed, but also with other MS techniques as triple quadrupole.

The ion isolation monitoring mode, proposed for the first time for ion-trap MS quantitation, proved to be effective in avoiding space-charge effects caused by coeluting matrix components while keeping the sensitivity of full scan MS operation. The complete analysis cycle included the accumulation of the ions in the trap, the isolation of a specific or different *m/z* ions and the production of a spectrum by resonantly ejecting the ions from the trap to detector. Detection limits of the method (sample wet weight) were in the range 0.8–6 ng/g for PFACs and 0.4–0.8 ng/g for PFASs. The method was validated using a reference material made up of flounder muscle and by comparison with triple quadrupole MS measurements and it was applied to the determination of PFCs in liver and muscle samples from sea birds and fishes. Only PFASs were found in samples at quantifiable levels (2.9 and 13.1 ng/g) while PFACs were below the respective quantitation limits. This method allows fast and simple microextraction with minimal solvent consumption, while delivering accurate and precise data. It may serve as a true alternative to the current popular extraction and clean-up methods.

Keywords: perfluoroalkyls, biota, microextraction, ion-trap MS

C-24

CONTENT OF POLYCYCLIC ATROMATIC HYDROCARBONS AND HEAVY METALS IN CZECH HONEY

Ivana Borkovcová[1*], Petra Batelková [2], Olga Čelechovská[3], Lenka Vorlová[4], Klára Bartáková[5]

[1 2 3 4 5] Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
[*] Corresponding author–E-mail: borkovcovai@vfu.cz; Phone: +420 541 562 715; Fax: +420 541 562 711

Protective properties in honey are expected by consumers. Honey from the Czech provenance is known for its high quality product and it can belong to organic food. The aim of this study is to determinate some exogenous contaminant such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Hg, Cd, Pb, As). Source of these pollutants is bee's pasture but total content depends on bee's organism too.

10 random samples of honey obtained directly from the Czech bee-keepers and 10 random samples of honey from shops with the Czech Republic declared as a country of origin were analysed.

Prior HPLC analyse target PAHs were isolated by three another kinds of procedures. The first was directly from samples into organic solvents with addition of anhydrous sodium sulphate by ultrasonic extraction combined with mixing at 10 000 rpm^{-1}. The second and third were from their aqueous solutions (liquid/liquid extraction or solid phase extraction). Determination was provided by reverse phase HPLC with gradient elution and fluorescence detection.

The content of 15 priority pollutants according to the US EPA was monitored, namely naphtalen (NAPT), acenaphten (ACENAPT), fluoren (FLU), anthracen (ANT), fluoranthen (FLT), pyren (PY), benzo-a-anthracen (BaA), chrysen (CHRY) benzo-b-fluoranthen (BbF), benzo-k-fluoranthen (BkF), benzo-a-pyren (BaP), dibenzo-a,h-anthracen (DBahA), benzo-g,h,i-perylen (BghiPE) and indeno-1.2,3-cd-pyren (IPY).

Concentrations of individual PAHs found in honey samples were low, they were in range 0.02–2.22 $\mu g.kg^{-1}$.

Cadmium and lead were determined by electrothermal atomisation HR-AAS (High Resolution Continuum Source Atomic Absorption Spectrometry) on the apparatus ContrAA 700 after mineralization. Arsenic was determined by hydride technique in graphite atomizer. The measurement of mercury was done by AAS on an Advanced Mercury Analyzer AMA – 254.

Concentration of mercury was in range from 3.24 to 11.31 $\mu g.kg^{-1}$, concentration of cadmium was 0.95–32.35 $\mu g.kg^{-1}$, content of lead was from 22.8 to 177.85 $\mu g.kg^{-1}$. Concentration of arsenic was only in three samples over detection limit and it was 3.51 to 4.35 $\mu g.kg^{-1}$.

In conclusion this study confirms high quality and safety of Czech honey. The good hygiene and manufacturing practice and bee's detoxicant mechanisms influence this properties.

Keywords: PAHs, Heavy Metals, Honey

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C-25

ASSESSMENT OF DIETARY EXPOSURE TO PCDD/F AND DIOXIN-LIKE PCB IN INFANT FORMULAE AVAILABLE ON THE EU MARKET

Marchela Pandelova[1*], Subrina Kasham[2], Bernhard Henkelmann[3], Catherine Leclercq[4], Raffaela Piccinelli[5], Karl-Werner Schramm[6]

[1 2 3] Helmholtz Zentrum München, Neuherberg, Germany
[4 5] National Research Institute for Food and Nutrition, Rome, Italy
[6] Helmholtz Zentrum München, Neuherberg, Germany; TUM, Freising, Germany
[*] Corresponding author–E-mail: pandelova@helmholtz-muenchen.de; Phone: +49 89 3187 2932; Fax: +49 89 3187 3371

Exposure of PCDD/F (polychlorinated dibenzo-p-dioxins and dibenzofurans) and dioxin-like PCB (dl-PCBs, i.e. polychlorinated non-ortho and mono-ortho biphenyls) occurs predominantly via animal fats in the diet. In infants, breast milk and formula milk are the major food sources. Recently in some EU countries an increasing percentage of mothers do not breastfeed their babies and only about 50% of mothers initiate breastfeeding. As a result the baby food market has grown up significantly and with it, the assortment of products offered. Since the first year of life is a very vulnerable and sensitive period in the human development, the composition of baby foods and their consumption pattern are crucial. Under the EU CASCADE project (www.cascadenet.org), representative market baskets of commercial baby foods for the first 9 months of life of an "average EU baby" fed with infant formulae were designed. The main objective was to assess the dietary exposure to endocrine disrupters in infant fed with commercial baby foods. With regard to infant formulae baskets, overall 62 different products were sampled from 8 different countries (France, Germany, Italy, Portugal, Slovakia, Spain, Sweden, UK) and 6 pooled samples of infant formulae– "starting" (aged 0–4 months) and "follow on" (after 4 months) of milk formula, soy formula and hypoallergenic formula respectively, were prepared. The PCDD/F and PCB analysis were performed on HRGC/HRMS and the results were expressed in WHO-TEQ pg/g sample. The mean daily dietary exposure to PCDD/F and dioxin-like PCB (WHO-TEQ pg/kg bw per day) was calculated based on the estimated average amount of "infant formulae" (ml/day) consumed by infants. The calculated mean dietary exposure to dioxin-like PCB from the six typologies of infant formula in the first 9 months of life was less than 0.04 WHO-TEQ pg/kg bw per day in all cases considered. On the other hand, the dietary exposure to PCDD/F was higher than the TDI minimum safety value of 1 WHO-TEQ pg/kg bw per day when considering all three typologies of starting infant formulae in the first two months of life. It reached 2.9 WHO-TEQ pg/kg bw per day for hypoallergenic-based formula in the first month of life.

Keywords: infant formulae, PCDD/F, PCB

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C-26
DETERMINATION OF CADMIUM IN POPPY SEEDS AND IN POPPY SEEDS CONTAINING PRODUCTS

Jan Knápek[1*], Romana Buchtová[2], Dagmar Vošmerová[3]

[1 2 3] Czech Agriculture and Food Inspection Authority, Brno, Czech Republic
[*] Corresponding author–E-mail: jan.knapek@szpi.gov.cz; Phone: 543 540 255; Fax: 543 540 241

Cadmium (Cd) is a heavy metal found as an environmental contaminant, both through natural occurrence and from industrial and agriculture sources. Cadmium has primarily toxic effects on kidney function, especially on proximal tubular cells where it accumulates over time and may result in renal dysfunction. Poppy, despite low cadmium concentrations in the soil, reaches the highest cadmium concentrations in the seeds. Poppy seeds therefore must be considered as one of the most cadmium contaminated foods. Concentration of cadmium in poppy seeds, semi-finished products and baked goods with poppy seeds was determined by graphite furnace atomic absorption spectrometry (GF-AAS). Homogenous samples of poppy seeds and products containing poppy seeds were digested by means of microwave digestion system MLS 1200 MEGA by using of nitric acid and hydrogen peroxide as reagents.The measurements were carried out using a Perkin-Elmer AAnalyst 600 atomic absorption spectrometer with Zeeman background correction. Detection limit was 0.003 mg kg^{-1}. A total of 202 samples of poppy seeds (from years 2004–2009) and 15 samples of semi-finished and baked goods were analysed. The average content was 0.64 mg kg^{-1} (median 0.64 mg kg^{-1}) for poppy seeds and 0.085 mg kg^{-1} (median 0.087 mg kg^{-1}) for semi-finished and baked goods.

Keywords: cadmium, poppy seeds, AAS

C-27
HEAVY METALS CONTAMINATION: SHELLFISH AS VENICE LAGOON BIOINDICATOR

Federica Gallocchio[1], Giulia Segato[2], Roberto Angeletti[3], Giancarlo Biancotto[4], Cristiana Benetti[5], Monica Lorenzetto[6], Evelina Chierin[7], Giovanni Binato[8*]

[1 2 3 4 5 8] Istituto Zooprofilattico Sperimentale delle Venezie
[6] Centro Regionale Epidemiologico Veneto
[7] Regione Veneto
[*] Corresponding author–E-mail: gbinato@izsvenezie.it; Phone: +39 049 8084336; Fax: +39 049 8830572

The term heavy metal refers to different chemical elements being part of different groups from transition metals to non metals. They are natural components of the Earth's crust usually present in trace amount. Examples of heavy metals include mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), thallium (Tl). Some heavy metals (e.g selenium, zinc) at trace levels are essential to organisms but at high concentration they can lead to poisoning. Heavy metal environment contamination can arise from many sources (industrial processes and wastes, mainly) and can affect organisms from environmental or dietary exposure.

Some types of organisms present a particular behaviour towards these contaminants. Owing to their high bioconcentration factor, molluscs like shellfish can be chosen as sentinel organisms for the detection of marine pollutants and therefore to assess the quality of marine ecosystems.

The Venetian Lagoon, the enclosed bay of the Adriatic Sea in which the city of Venice is situated, which represents the largest wetland in the Mediterranean Basin with a surface area of around 550 km^2, can be affected by different human activities such as agriculture, industrial wastes and rivers mouths from Northern Italy. Molluscs fishing and breeding on lagoon water is an important economic source considering that almost 90% of the Italian production of shellfish (clams, mussels, oysters) comes from the Venetian Lagoon, hence the importance of establishing water pollution level which may highlight any critical situation in specific areas. In this aim the analyses of metallic pollutants performed according to local Regional Monitoring Programs, are focused to verify the levels of contamination of shellfish (and their compliance according to Regulation CE/1881/2006) and therefore the contamination of breeding areas.

In this study the results of analyses of Cd, Hg, Pb performed during the period 2007–2008 on shellfish samples of the Venetian Lagoon (*Mytilus galloprovincialis*, *Tapes philippinarum*, *Chamelaea gallina*) are presented and discussed. Analyses were performed by Atomic Absorption Spectrometry, with electro thermal atomisation for Pb and Cd (ETA-AAS) and direct determination for Hg (TDA-AAS).

Keywords: heavy metals, shellfish, venetian lagoon

Regione Veneto

C-28

PLANTS – THE POSSIBLE SOURCE OF POLYBROMINATED DIPHENYL ETHERS IN FOOD CHAIN

Hana Stiborova[1*], Jana Vrkoslavova[2], Tereza Zemanova[3], Jana Pulkrabova[4], Petra Hradkova[5], Jana Hajslova[6], Martina Mackova[7], Tomas Macek[8], Katerina Demnerova[9]

[1 2 3 4 5 6 7 9] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[8] Institute of Organic Chemistry and Biochemistry, Prague, Czech Repubic
[*] Corresponding author–E-mail: hana.stiborova@vscht.cz; Phone: 604209879

Polybrominated diphenyl ethers (PBDE) are often used in plastics, electronics, insulation panels and textile products to prevent or delay combustion. These xenobiotics have been recently detected at exponentially increasing concentrations in number of environmental samples, diverse biota, human blood serum and breast milk. PBDEs have a biomagnification potential and their concentration is increasing in higher trophic levels. The most probable route for the exposure of the general human population to PBDEs is though the inhalation and the diet. Due to their chemical properties the highest amount of their accumulation was detected in sewage sludge, sediments and soil. Chemical characteristics rank sewage sludge among popular fertilizers applied on agricultural soils – it contains high proportions of organic matter and organic and inorganic plant nutrients. In the Czech Republic 175 thousand tones of sewage sludge were generated in 2006. Out of that volume 86% were land-applied e.g. on agricultural use (27%), for composting (51%) and for landfilling (8%). The median levels of PBDEs in sewage sludge in the Czech Republic measured in monitoring study in 2006 achieved 192 ng/g for Octa and PentaBDE and 445 ng/g for DecaBDE. Based on these results estimated release of BFRs into the environment via land-application is 29.2 kg/a for Penta and OctaBDE and 67.6 kg/a for DecaBDE.

The objective of this study was to find out whereas the plants are able to uptake, translocate and metabolize PBDEs. As model plants *Nicotiana tabacum* and *Solanum nigrum* were chosen. After six months of cultivation of above mentioned plants in pots with the contaminated sewage sludge following congeners were detected in significant concentrations: 31.5 ng/g dw of the lower brominated congeners of PBDEs (BDE 28, BDE 37, BDE 47, BDE 49, BDE 66, BDE 85, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183) in the pots with *Solanum nigrum*, 148.5 ng/g dw of the lower brominated congeners of PBDEs in the samples with *Nicotiana tabacum* and 117.3 ng/g dw of BDE 209 in the pots cultivated with *Nicotiana tabacum*. Obtained results also indicate that the accumulation of certain congeners is plant and tissue specific.

Keywords: polybrominated diphenyl ethers, plants, accumulation

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C-29

METHOD DEVELOPMENT FOR CONTROL DETERMINATION OF MERCURY IN BRAZILIAN FRESH FISH SAMPLES BY SOLID DIRECT ANALYSIS USING THE DIRECT MERCURY ANALYZER DMA-80

Daiane Torres[1], Maristela Martins[2], Erika Silva[3], Helena Queiroz[4*]

[1] State University of Campinas, Campinas, Brazil
[2 3 4] Ministry of Agriculture, Livestock and Food Supply, Campinas, Brazil
[*] Corresponding author–E-mail: helena.queiroz@agricultura.gov.br; Phone: 55 19 32520155; Fax: 55 19 32524835

Consumption of fish has several advantages for human health, such as the high contents of Omega 3 and Omega 6. However, the piscivorous species may bioacummulate both organic and inorganic contaminants, which enter the food chain. Mercury toxicity is well-known because of its accumulative and persistent character in the environment, and fish is one of the major sources of human contamination through dietary.

The aim of this work is to develop a simple and rapid method for the determination of total mercury in fish samples by using the direct mercury analyzer DMA-80 (Milestone), for control and regulation purposes. First, an aliquot of previously weighed sample is dried and then thermally and chemically decomposed in an oxygenated decomposition furnace. The remaining decomposition products are then carried to an amalgamator that selectively traps mercury. Upon heating release from amalgamator, flowing oxygen carries the mercury vapor through two sequential absorbance cells, with lengths in a rate of 10:1, for the first and the second ones, respectively. Peak height was used for signal evaluation. Some experimental parameters have been studied and optimized. In this hand, the sample mass was about 100 mg, weighted immediately before measurement. The relative standard deviation was lower than 7.0% for most of solid samples. Two calibration curves against aqueous standards were carried out. The first one from 2.0 to 15.0 ng of Hg (the low linear range), and the second one from 30.0 to 375 ng of Hg (the high linear range), for which correlation coefficient better than 0.997 were achieved. Mercury reference solutions were prepared in 5.0% *v/v* nitric acid medium. Lyophilized fishes were also analyzed, however this procedure did not present any advantage over the direct analysis of the fresh fish, increasing the total analytical process time. The recovery test was applied to a blank fish sample and the obtained recovery value was 110%. A fish homogenate reference material, IAEA-407 (fish homogenate), has been analyzed and the mercury concentration obtained agrees with the certified value, according to the *t*-test at a 95% confidence level. The limit of quantification (LOQ) in the sample was 6.2 ng of Hg, which means 62.3 μg kg^{-1} in the employed conditions. This LOQ is in accordance with performance criteria required by the commission regulation (EC) No 333/2007. Simplicity and high efficiency, without the need of any sample preparation procedure, are some of the qualities of the proposed method.

Keywords: mercury, fish, food control, validation

CNPq and Ministry of Agriculture, Livestock and Food Supply

C-30
QUALITY ASSURE AND QUALITY CONTROL FOR ANALYSIS PCDD/Fs AND PCBs IN FOOD AND FEED

Yunxia Yang[1*]

[1] Istituto Zooprofilattico Sperimentale delle regioni Lazio e toscana, Rome, Italy
[*] Corresponding author–E-mail: yunxia@vet.unipi.it; Phone: 0039-3803455341

The contaminations of Polychlorinated dibenzo-p-dioxin (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in food supply is a threat to the health of humans, so they are required to detect in the food and feed in EU. But as we know, it is very difficult to analysis these substances in the food and feed because of the ultra-trace and multi-compound of them. This paper showed how to assure the validity and accuracy for the analysis PCDD/Fs and PCBs in food and feed from the profile of the analysis technique, including each part of analysis procedure such as sample collection and preparation, extraction, clean-up, concentrate, chromatographic separation and so on through analysis the food samples of animal origin, at the same time, the paper gave the data validation standard operating procedures for PCDD/Fs and PCBs analysis by high resolution gas chromatography/high resolution mass spectrometry in order to provide the intercalibration among the laboratories.

Keywords: QC/QA, PCDD/Fs, PCBs, food

C-31

INVESTIGATION OF THE SEMIOCHEMICALS OF CONFUSED FLOUR BEETLE TRIBOLIUM CONFUSUM DUVAL AND GRAIN WEEVIL SITOPHILUS GRANARIUS (L.) IN STORED WHEAT GRAIN AND FLOUR

Nagat Abuelnnor[1], Norman Ratcliffe[2*], Ben de Lacy Costello[3], Peter Spencer-Phillips[4]

[1 2 3 4] Department of Applied Sciences, University of the West of England, UK
[*] Corresponding author–E-mail: Norman.Ratcliffe@uwe.ac.uk; Phone: 0117 3282501; Fax: 0117 3282904

Grain and food products are attaked by pests such as insects, mites and microorganisms during storage. The resulting post-harvest losses are approximately 10% worldwide annually (Hodges *et al.*, 1996; Neethirajan et al., 2007). Infestation by insects encourages growth of fungi including those that produce mycotoxins, and results in contamination of commodities with insect bodies and waste products that may be toxic, repulsive or allergenic (Freeman, 1976). Thus, detection of insect infestation is important as an early warning for taking suitable control measures, and to ensure provision of safe and wholesome food to consumers.

The aim of the study presented here was to assess the use of volatile organic compounds (VOCs) as markers for the early detection of Confused Flour Beetle *Tribolium confusum* and Grain Weevil *Sitophilus granarius* in wheat grain and flour, using solid-phase micro-extraction (SPME) with gas chromatography-mass spectrometry (GC-MS).

Insects were reared on wheat grain, white and wholemeal wheat flour in glass jars covered with gauze, and incubated at 25°C under a 14 h light and 10 h dark photoperiod and 70% relative humidity. Either 100 adult beetles or 100 larvae or three grams previously infested with a Confused Flour Beetles or three grams of wheat grain previously infested with Grain Weevils were placed in 10 ml glass vials sealed with a Teflon cover with rubber septum. The vial was heated at 28°C and solid-phase micro-extraction (SPME) was performed for either 4 or 16 h. The experiments was repeated 4 times with new adults, larvae, wheat flour and wheat grain. GC-MS analysis showed the presence of many VOCs. For instance, some VOCs were found only in the adult of Confused Flour Beetles such as 2-methyl-1,4-benzoquinone, 2-ethyl-1,4-benzoquinone, 2-methyl-1,3-benzenediol and 1-nonadecene, whilst different VOCs were found only in larvae. Also, 2-methyl-propanoic acid and 3-methyl-butanoic acid were found only in the Grain Weevil samples. The study of other insect and mite pests may establish specific markers for each species, and allow detection of larval stages which is difficult using conventional methods. A full understanding of insect semiochemicals may also allow new biological control measures to be devised.

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Keywords: Confused Beetle, Grain Weevil, Semiochemicals

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C-32

MULTIRESIDUE SCREENING FOR PESTICIDES IN WATERS FROM CATCHMENT AREAS OF WIELKOPOLSKA REGION UNDER INTENSIVE AGRICULTURAL ACTIVITIES USING GC/MS/MS AND UPLC/MS/MS

Dariusz Drozdzynski[1*], Stanislaw Walorczyk[2]

[1 2] Institute of Plant Protection–National Research Institute
[*] Corresponding author–E-mail: d.drozdzynski@ior.poznan.pl; Phone: +48 61 8649182

A comprehensive survey on pesticides (123 compounds) in the catchment areas of Wielkopolska region of Poland particularly vulnerable to pesticides contamination due to intensive agriculture, topography and soil properties was carried out. For this purpose, a method based on water sample enrichment on graphitized carbon black (GCB) columns (brand name Carbograph, Alltech, US) was developed. After elution from the column, the extract was analyzed concurrently by both GC/MS/MS (74 compounds) and UPLC/MS/MS (49 compounds). The method performance was assessed through analysis of spiked water samples. At the 0.1 µg/L spiking level, the recoveries were in the range 70-120% with associated RSDs $\leq$ 20% for 99 compounds (80%), whereas they were below 70%. for 24 compounds (20%). Consistently low but repeatable recoveries were obtained for the most of organochlorine and pyrethroid insecticides and for some of phenoxyacidic and sulphonurea herbicides. Satisfactory average recoveries but RSDs > 20% were obtained for HCH-alfa, diazinon, MCPA and dichlorprop. But, the detection at the maximum permissible limit of 0.1 µg/L set by Directive 2000/60/EC for drinking water was achieved for all targeted pesticides. Of 44 samples analyzed in 2008, 35 samples contained pesticide residues, in which 33 different pesticides were determined in the concentration range between 0.01 and 1.4 µg/L. Most often found pesticides were those currently used in agriculture such as carbendazim, isoproturon, atrazine, metamitron, tebuconazole and epoxiconazole. Of the pesticides from the EU priority list only atrazine, simazine and isoproturon were found.

Keywords: GC/MS/MS, UPLC/MS/MS, pesticides, water

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C-33

OPTIMIZATION OF GC×GC-TOF/MS FOR THE SIMULTANEOUS DETERMINATION OF PCBs, PBDEs AND PAHs IN FOOD SAMPLES

Kamila Kalachova[1*], Jana Pulkrabova[2], Lucie Drabova[3], Tomas Cajka[4], Jan Poustka[5], Vladimir Kocourek[6], Jana Hajslova[7]

[1 2 3 4 5 6 7] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: kamila.kalachova@vscht.cz; Phone: +420 220 44 3272

Since polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and polycyclic aromatic hydrocarbons (PAHs) belong, according to European Food Safety Authority (EFSA) among the food contaminants that should be monitored; the quick, rugged, sensitive and inexpensive analytical methods are currently required. The comprehensive two-dimensional gas chromatography coupled to the time-of-flight mass spectrometry (GC×GC-TOF/MS) represents a powerful tool for a simultaneous determination of these different types of analytes as well as for non-target screening of contaminants in various complex matrices such as fresh and smoked fish within a single run.

The main aim of this study was to optimize the chromatographic separation of selected analytes to obtain the best resolution and detection limits. Therefore several chromatographic capillary column combinations with different polarities were tested–BPX-5 and BPX-50 in the 1st dimension and BPX-50, Rt-LC-35 and HT-8 in 2nd dimension and critical pairs of analytes identified. The high attention was paid mainly to the most difficult groups of PAHs which were as follows: benzo[a]antracen with cyklopenta[c,d]pyren and chrysen, benzo[j]fluoranthen with benzo[k]fluoranthen and benzo[b]fluoranthen and dibenzo[a,h]anthracen with indeno[1,2,3-cd]pyren and benzo[g,h,i]perylen. In following experiments, the programmable temperature vaporization (PTV) injection technique was optimized to decrease detection limits (LODs). Obtained LODs were 0.5–3 pg injected, 1–10 pg injected and 0.5–5 pg injected for PCBS, PBDEs and PAHs, respectively.

It should be noted that before the optimization of GC×GC, also a rapid sample preparation method including a pressurized liquid extraction (PLE) was developed. This technique, in comparison with a routinely used rather time consuming Soxhlet extraction, requires less time and low volume of extraction solvent. Moreover, extraction and clean up can be performed in one fully automated step operation.

Keywords: GC×GC-TOFMS, PLE, PCB, PBDE, PAH

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C-34

OPTIMIZATION OF ANALYTICAL METHOD FOR PERFLUORINATED COMPOUNDS IN FRESH AND CANNED FISH

Petra Hradkova[1*], Jan Poustka[2], Jana Pulkrabova[3], Veronika Hlouskova[4], Monika Tomaniova[5], Dorte Herzke[6], Sandra Huber[7], Rolland Kallenborn[8], Jana Hajslova[9]

[1 2 3 4 5 9] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[6 7] Norwegian Institute for Air Research,The Polar Environmental Centre, Hjalmar Johansens gate 14
[8] Norwegian Institute for Air Research (NILU), Instituttveien 18, NO-2027 Kjeller, Norway
[*] Corresponding author–E-mail: petra.hradkova@vscht.cz; Phone: +420220443272

PFCs belong to one of the most important group of “emerging” contaminants. To assess health risks associated with dietary intake, in 2008, EFSA (European Food Safety Authority) recommended to member states to monitor two major representatives of this group – PFOS (perfluorooctane sulfonate) and PFOA (perfluorooctanoic acid) in food stuff. These two chemicals are usually used as indicator substances for potential occurrence of other PFCs. Due to their properties such as persistency, mobility in the environment and bioaccumulation, PFCs have been found in animals and human tissues. Since marine and freshwater fish intake is a major source of humans' exposure, it is necessary to monitor levels of PFCs in these biota samples as well as in other fish products.

In presented study, interlaboratory comparison of two analytical procedures was carried out. Altogether 40 fish and fish product samples (tuna, herring, salmon, cod, and sardines) collected in the Czech and Norwegian markets were examined for a wide range of perfluorinated carboxylic acids, sulfonates, sulphonamides and telomers alcohols. Both of the extraction procedures were based on using organic extraction solvent (*i*) methanol (*ii*) acetonitrile and dispersive solid phase clean-up step (*i*) activated charcoal and (*ii*) ENVIcarb. Samples were separated by a high performance liquid chromatography (HPLC) using an analytical column (*i*) Atlantis T3 (100mm × 2.1 mm; 3 µm) (Waters, USA) (*ii*) ACE 3 RP C18 (150 mm × 2.1 mm; 3 µm) (ACE, UK).

The identification/detection of target analytes was performed employing (*i*) Quattro Premier XE (Waters) tandem quadrupole mass spectrometer operated in MRM mode and (*ii*) Q-Tof Micro (Waters) in MS mode.

Keywords: PFC, fish products, extraction

This study was funded by the Research Support Fund of the National Training Fund within the project EMERCON (Identification and quantification of emerging organic contaminants in the Czech aquatic ecosystem and food market supply. With focus on perfluorinated alkylated compounds (PFC), no. A/CZ0046/2/0026.

C-35

ALTERNATIVE LIQUID CHROMATOGRAPHY MASS SPECTROMETRY APPROACHES IN ANALYSIS PERFLUORINATED COMPOUNDS (PFCs) IN BIOTIC MATRICES

Petra Hradkova[1*], Jan Poustka[2], Jana Pulkrabova[3], Kamila Kalachova[4], Monika Tomaniova[5], Jana Horakova[6], Jana Hajslova[7]

[1 2 3 4 5 6 7] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: petra.hradkova@vscht.cz; Phone: +420220443272

Liquid chromatography coupled to various triple-quadrupole mass spectrometers is currently one of the most popular detection technique typically used for quantitative determination of perfluorinated compounds (PFCs). These emerging organic pollutants have unique properties (stability, persistence, oleophobicity and waterphobicity), containing both polar and non-polar chain. Nowadays they are intensively monitored in both food and environmental samples. The major representatives of sulfonates (PFAS) and carboxylated acids (PFCA) groups are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the main degradation products of the others PFC–these compounds are detected at the highest levels in environmental compartments and various food.

In this study, 3 different LC-MS systems (liquid chromatography coupled with different ion analyzators (*i*) triple quadrupole (*ii*) high resolution time of flight detector and (*iii*) linear ion trap – Q-trap were compared as a potential and/or suitable instrumentations for the analysis of 16 PFCs. These detectors were used for routine analysis of PFCs where the time of separation was compressed until to 10 minutes. The other goal–using Q-trap was attempted to reduce detection and quantification limit. The last but not least objective was to separate branched isomers of PFC (tested for PFOS isomers) and tried to determine them individually according to their retention times and different fragmentation patterns.

Keywords: PFOS, Q-trap, branched isomers

This study was funded by the 7th FP within the project PERFOOD (PERFluorinated Organics in Our Diet), grant agreement no. 227525.

C-36

SIMPLIFIED SAMPLE PREPARATION PROCEDURE FOR SIMULTANEOUS ISOLATION OF ORGANOHALOGEN POLLUTANTS AND PAHS FROM FISH SAMPLES

Jana Pulkrabova[1*], Kamila Kalachova[2], Lucie Drabova[3], Jan Poustka[4], Jana Hajslova[5]

[1 2 3 4 5] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: jana.pulkrabova@vscht.cz; Phone: 00420220443272

To protect consumers´ health, maximum levels in feed and food have been set in Commission Regulation (EC) No 1881/2006 for various food contaminants including some persistent halogenated organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs). With regards to the lack of credible data in some areas, legislative requirements and considering EFSA's request for more data on POPs occurrence in EU diets, representatives and hydroxylated metabolites of the following three groups are selected as target analytes: (*i*) dioxin-like polychlorinated biphenyls (PCBs)–congeners that exhibit similar adverse health effects as PCDDs/PCDFs; (*ii*) brominated flame retardants (BFRs) such as polybrominated diphenylethers (PBDEs) and hexabromocyclododecane isomers (HBCD) – these "emerging" halogenated POPs are suspected for various toxic effects including endocrine disruption; (*iii*) PAHs – ubiquitous environmental/ processing contaminants with carcinogenic potency. Analytical methods for determination of these contaminants such in environmental and food matrices are typically based on multistep procedures that include Soxhlet extraction with a subsequent clean up and fractionation steps prior to relatively slow gas chromatography (GC) runs using either an electron capture (ECD) or a mass spectrometric (MS) detection in case of halogenated analytes, PAHs are commonly analysed separately using a liquid chromatography coupled to a fluorescence detector (LC-FLD), but for several non-flurescence PAHs including in the EFSA opinion, a GC-MS analysis is needed. In this study, the fast extraction and clean-up procedure of all mentioned pollutants from a fish fillet by a pressurised liquid extraction (PLE) has been tested. The main goal was to optimize an appropriate extraction/clean up procedure for analysis of PCBs, PBDEs and PAHs in a single run. Repeatability of this simplified sample preparation procedure for all target analytes, expressed as relative standard deviation (RSD, n=6) ranged from 3 to 19% and recovery on the level 1 µg/kg ranged from 71 to 121%.

This study is a part of the 7FP EU project CON*ff*IDENCE (CONtaminants in Food and Feed: Inexpensive DEtectioN for Control of Exposure), its aim is to support the needs that were identified by the Scientific Committee on the Food of the European Commission in the area of POPs. For the application of two novel complementary bioanalytical screening and the GC×GC/TOFMS comprehensive profiling a simple and fast sample preparation strategy is needed.

Keywords: PLE, POPs, PAHs, sample preparation

This study was carried out within the EU project CONffIDENCE (FP7-211326-CP) and the project MSM 6046137305 supported by the Ministry of Education, Youth and Sports of the Czech Republic.

C-37
RAPID METHOD FOR ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN OLIVE OILS USING MIP-SPE AND GC×GC TOFMS

Lucie Drabova[1*], Jana Pulkrabova[2], Jaromir Hradecky[3], Kamila Kalachova[4], Vladimir Kocourek[5], Monika Tomaniova[6], Jana Hajslova[7]

[1 2 3 4 5 6 7] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: lucie.drabova@vscht.cz; Phone: +420 220 444 372

Several RASFF notifications were released on polycyclic aromatic hydrocarbons (PAHs), especially on a carcinogenic benzo[*a*]pyrene (B[*a*]P), in various food products during recent years. Most often the contamination has been reported in vegetable oils and particularly in olive oils from many countries worldwide. Contamination of vegetable oils (including seed oils and olive residue oils) with PAHs usually occurs during technological processes like direct fire drying, where combustion products may come into contact with oil seeds or the oil.

The maximum limit recently set by the Regulation No. 1881/2006/EC on benzo[a]pyrene (B[a]P) for oils and fats intended for direct human consumption is 2 µg/kg. Nevertheless, the Commission (see Recommendation 2005/108/EC) asked the Member States to monitor all the 15 priority PAHs identified by the Scientific Committee on Food (SCF).

A new extraction procedure for the determination of 15+1 EU priority PAHs in vegetable oils has been developed within this study. The rapid and simple sample preparation procedure consists of extraction/clean-up step employing solid-phase extraction (SPE) on molecularly imprinted polymer (MIP) column followed by two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC TOFMS). Isotope dilution technique was used for quantitation of target analytes. GC/MS and GC×GC TOFMS techniques were compared on the basis of parameters such as time of analysis and method validation characteristics.

This validated analytical method was used for examination of PAH levels in 20 olive oils randomly sampled on the Czech market. The sum of 8 carcinogenic PAHs (B[*a*]A, Chr, B[*b*]F, B[*k*]F, B[*a*]P, DB[*ah*]A, B[*ghi*]P, I[*cd*]P) ranged from 1.8 to 18.7 µg/kg, from 2.2 to 3.5 µg/kg and 3.7 to 14.2 µg/kg in virgine olive oils, olive oils and in olive pomace oil samples, respectively. Nevertheless, none of the samples exceed the maximum limit set by the Reg. 1881/2006/EC for benzo[a]pyrene.

Keywords: PAH, oil, MIP, GC×GCTOFMS

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

C-38
RAPID GC/MS ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN VARIOUS TYPES OF TEA

Lucie Drabova[1*], Jana Pulkrabova[2], Jaromir Hradecky[3], Kamila Kalachova[4], Vladimir Kocourek[5], Jana Hajslova[6]

[1 2 3 4 5 6] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: lucie.drabova@vscht.cz; Phone: +420 220 444 372

Tea is an old worldwide popular commodity. It is valued for its specific aroma and flavor as well as for health-promoting properties. However, under certain conditions tea leaves may contain pesticide residues or some contaminants such as polycyclic aromatic hydrocarbons (PAHs), which may be contain there due to improper drying process of tea leaves. During the recent years, an increasing public concern and scientific investigation have been focused on presence and control of PAHs in herbal products of plant teas to assess the potential health hazards more thoroughly.

The aim of the presented study was to develop an efficient and sensitive method for determination of 16 polycyclic aromatic hydrocarbons (16 EU PAHs) classified as a priority for different food groups by Scientific Committee on Food), in tea samples. The sample preparation consists of ethyl acetate extraction followed by clean-up using solid phase extraction (SPE) on molecularly imprinted polymer (MIP) column for selective isolation of target compounds. Either gas chromatography-mass spectrometry (GC/MS) or two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC TOFMS) was employed for identification/quantitation of PAHs.

A wide range of different types of tea (black tea, green tea, Mate tea and fruit tea samples) available at Czech market were examined within this study. According to our expectation, the highest levels of PAHs were found in Mate and black tea samples while relatively low contamination was reported in fruit tea samples.

Keywords: PAH, tea, MIP, GC×GCTOFMS

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

C-39

DETERMINATION OF CADMIUM IN WHEAT FLOUR CERTIFIED REFERENCE MATERIAL CANDIDATE BY ID-ICP-MS AND ICP-MS AND THE FOLLOWING APPLICATION IN THE PROFICIENCY TEST (CNAS T0402)

Haifeng Li[1*]

[1] National Institute of Metrology
[*] Corresponding author–E-mail: lihf@nim.ac.cn; Phone: 86-010-64294060; Fax: 86-010-64294060

Cadmium (Cd) is a toxic element which might accumulate in the human body and do harm to the health of people. Cd in wheat flour is so seriously concerned for the huge amount of flour in daily diet and for the possibility of ingested by food-chain especially in recent few decades. Wheat sample was collected in 2006 as certified Reference Material (CRM) candidate (now as CRM GBW100035) from contaminated sites in Henan Province, P.R China. It were dried and then crashed and passed into 80 mesh(≤180 μm) sieve. After repackaged into brown glassy bottles, samples were irradiated by Co^{60} and stored and tested for the homogeneous and stable study by F-test. Value was assigned by Isotope Dilution Inductively Coupled Plasma Mass Spectrometry (ID-ICP-MS) and inductively Coupled Plasma Mass spectrometry (ICP-MS). Collision cell technology was chosen with the optimum measurement condition including RF power 1400W-1500W, carrier gas flow 1.0 L/min–1.1 L/min, H_2 or He gas flow 3.0 mL/min–3.5 mL/min, pumping rate 0.1 rps. Content of Cd was shown by mean ± uncertainty as: 0.074±0.003 mg/kg (dry base). In 2008, a proficiency test was carried out in which about 67 laboratories from different parts of China participated. Robust statistics method was used to analyze the data in which there were 7 laboratories with 2＜abstract value of Z score ≤3 and 3 laboratories with the abstract value of Z score＞3. Result in PT test can be shown by Median ± Standard Deviation (0.070±0.010 mg/kg) and mean ± Standard Deviation (0.074±0.010 mg/kg), which showed good agreement with the certified value. Meanwhile, cluster analysis (CA) was used to study the similarity of the data from different laboratories. Compared with robust statistics method, data showed that CA method has good advantage in the process and depth of analysis. According to the similarity of results, laboratory can be gathered together to form several groups which is useful for further resolution of the problems existing in the PT and which might promote the participator to know more about themselves so as to improve the traceability and technology about contaminates analysis in food.

Keywords: Chemistry;Analysis;inorgnic;traceability;heavy metal

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C-40

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN COOKING OILS BY LOW-TEMPERATURE CLEANUP

Thongsuk Payanan[1], Puttaruksa Varanusupakul[2], Natchanun Leepipatpiboon[3*]

[1 2 3] Faculty of Science, Chulalongkorn University, Bangkok, Thailand
[*] Corresponding author–E-mail: natchanun.l@chula.ac.th; Phone: 662 218 7608

Low-temperature cleanup is a simple, compact, fast, and cost-efficient sample preparation technique using only small amount of organic solvent. The technique allows for convenient treatment of multiple samples simultaneously. Parameters affecting the extraction efficiency include freezing temperature, extraction time, and extraction solvent. The method was developed to target sixteen priority polycyclic aromatic hydrocarbons in pure and used cooking oils. Optimum extraction was achieved when using a mixture of acetonitrile and acetone and freeze between -18°C to -25°C for 24 hours. At these temperatures, fat was frozen out and organic solvent can be simply separated for further cleanup by Alumina-N cartridge. Determination of polycyclic aromatic hydrocarbons was done with high performance liquid chromatography/fluorescence detector using gradient elution. Run time was achieved within 55 minutes. Method performance was evaluated with spiked oil. Average recoveries range from 45 to 119% with corresponded relative standard deviations of 1.1 to 24.4%. Method accuracy was determined with benzo(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene, benzo(g,h,i)pyrene, and indeno(1,2,3-c,d)perylene certified reference materials in CRM T0631 olive oil. Linearity and working ranges were determined from 0.25 to 6.0 µg/kg for benzo(a)pyrene and the correlation coefficient values better than 0.99 were obtained for all compounds. Limit of detections and limit of quantitations were graphically evaluated to be from 0.12 to 3.12 and 0.25 to 6.25 µg/kg, respectively. The validated method was successfully tested on used cooking oil samples.

Keywords: PAHs, low-temperature cleanup, cooking oil

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C-41

CHARACTERIZATION OF PERFLUORINATED FOOD CONTACT SUBSTANCES BY LCMS USING TARGETED AND UNTARGETED ANALYSIS

Gregory Noonan[1*], Timothy Begley[2], Yichuan Xu[3], Gregory Diachenko[4]

[1 2 3 4] US Food and Drug Administration, College Park, USA
[*] Corresponding author–E-mail: Gregory.Noonan@fda.hhs.gov; Phone: 1-301-436-2250; Fax: 1-301-436-2634

A broad range of fluorochemicals are used to impart oil and water barrier properties on paper and paperboard packaging commonly used to package high-fat content and convenience foods. The fluorochemicals are generally applied to paper and paperboard as solution mixtures and not as a single well characterized moiety. These solutions are often complex mixtures which are poorly characterized and contain reaction products and by-products with varying physicochemical properties.

This research has utilized liquid chromatography mass spectrometry to better characterize the starting material, evaluate commercially available packaging materials, and study the migration of fluorochemicals into foods and food simulants. Due to the commercial availability, the work has focused on a number of grease-proofing agents including; di-perfluoro-alkyloxy-amino-acid (diPFAoAA), and 2 polyfluoroalkyl phosphate surfactants (di-PAPS). The initial analytical work, utilizing manual evaluation of the LCMS data, has focused on identifying clearly discernable components of the mass chromatograms. In all of the products tested, reaction by-products, not explicitly identified as food contact substances, were detected. These by-products include, diethanolamine, perfluorinated carboxylic and sulfonic acids, perfluorinated acrylates and perfluorinated “hydrocarbons”. Additionally, many of the by-products are detectable in commercial packaging, clearly showing that the by-products are not removed during application and treatment of paper and paperboard. Further work is underway utilizing mass spectral deconvolution software to further identify other substituents of the fluorochemical mixtures and treated packaging.

Given the varying physicochemical properties of the fluorochemical products and their by-products, determining the extent of migration of the food contact substance becomes quite complex. Migration studies show that the main fluorochemical products (dimers and monomers) and certain by-products do migrate into food simulants. However, for a number of the fluorochemical grease-proofing agents the use of common fatty food-simulating liquids does not accurately predict the amount of migration that might occur with foods. Indeed, the addition of emulsifiers to the fatty food stimulant Miglyol Oil significantly increases the migration of di-PFAoAA and di-PAPs. Additionally, initial data on di-PFAoAA indicates poor stability at room temperature and 40°C in Miglyol Oil, but degradation or reaction products have not been determined. LCMS and deconvolution software are currently being utilized to determine possible degradation products of di-PFAoAA in Miglyol Oil.

Keywords: Packaging, Perfluorinated, Mass Spectrometry

C-42

THE INFLUENCE OF THE BARBECUE PROCESS ON THE CONCENTRATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN FOOD

Kelly Servaes[1*], Katrien Renders[2], Bert Van den Bosch[3], Guido Vanermen[4]

[1 3 4] Flemish Institute for Technological Research (VITO), Mol, Belgium
[2] Test-Aankoop/Test-Achats, Brussels, Belgium
[*] Corresponding author–E-mail: kelly.servaes@vito.be; Phone: +32 14 33 50 39; Fax: +32 14 31 94 72

A number of polycyclic aromatic hydrocarbons (PAH) have been characterized as genoxtoxic carcinogens. Consumption of food – which can be contaminated with PAHs through environmental sources, industrial processing or domestic food preparation like smoking, grilling and cooking – is one of the major routes of exposure in humans.

In this study, we have investigated the influence of different methods of barbecuing on the formation of PAHs in meat. Thereto, three pieces of meat with a varying fat content (steak, sausage and spare ribs) have been prepared by a chef in five different ways: on a classical gas barbecue, a charcoal barbecue, a charcoal barbecue with use of a perforated aluminium dripping pan, a charcoal barbecue with coconut charcoal briquettes and a gas barbecue with roofed burners. The preparation of each kind of meat was repeated for the five different barbecuing methods.

After preparation, the meat has been analyzed for the presence of PAHs. The samples were saponified by refluxing with an ethanolic KOH-solution. After addition of water, the PAHs were extracted with cyclohexane. Clean-up of the cyclohexane phase was performed on a combined silica-alumina column. Sixteen compounds of interest, the 16 PAHs prioritized by the Scientific Committee on Food and the joint FAO/WHO Expert Committee on Food Additives, were determined by means of gas chromatography-high resolution mass spectrometry (GC-HRMS): benzo[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, cyclopenta[cd]pyrene, dibenzo[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 and benzo[c]fluorene. The different compounds were quantified by means of the internal standard method.

The concentration of the individual PAHs in the barbecued meat varied between < 0.5 µg/kg and 84 µg/kg. Total amounts of the selection of sixteen PAHs were calculated and varied between 4 µg/kg and 197 µg/kg.

From the results we can conclude that barbecuing with roofed burners results in lower PAH concentrations compared to a classical charcoal barbecue and a classical gas barbecue. These observations can be explained by the fact that by horizontal grilling fat can drip into the open flame giving rise to the formation of PAHs. In case of horizontal grilling the concentration of PAHs can be significantly reduced by using an aluminium dripping pan. This dripping pan prevents the contact between fat and the open flame. Furthermore, the PAH formation was shown to be dependent on the fat content of the meat. Meat with a higher fat content resulted in a higher concentration of PAHs after preparation.

Keywords: PAH, food processing, GC-HRMS

C-43

ASSURANCE OF SPECTROPHOTOMETRIC DETERMINATION OF NITRITES CONTENT IN BEEF-BURGER PRODUCED BY SOME EGYPTIAN FACTORIES

Ehab Hashim[1*], Abd-Alateef Seddek[2], Mohamed-Wael Abd Al-Azeem[3], Yaser Abd Al-Galeel[4]

[1] Veterinary Services Department, Egypt
[2 3 4] Faculty of Veterinary Medicine, South Valley University, Qena, Egypt
[*] Corresponding author–E-mail: vetehab_44@hotmail.com; Phone: 002 010 925 83 83; Fax: 002 047 3 88 99 88

In many countries, people may suffer from many health hazards, which may be resulted from consumption of low quality food products. Meat products (like beef burger), when produced in a factory has not good manufacturing practices, leading to consumers health hazards. Nitrite ($NaNO_2$),as one of important chemical meat additives used in such products, has established much attention in Egypt in recent years, because of growing concern for safety by consumer protection societies and authorities. Nitrosamines formation (Dimethylnitrosamines-DMNA-and Diethylnitrosamines-DEN), which have carcinogenic, mutation effect and immunosuppressive, explains why it is necessary to continuously monitor nitrite contents in meat products.This study was conducted to determine the nitrite levels in beef burger (as a final product of three factories A, B and C) and to evaluate the methodological approaches analysis of nitrite levels used by the quality control laboratories of these plants, based on the international acceptable permissible limits (APL). The analytical method of choice for the quantitative determination of nitrite in the meat product under analysis, was spectrophotometry. In this study, a total of 75 representative samples of beef burger (25 samples were collected from each plant) were examined for detection of nitrite levels. The obtained results revealed that the nitrite contents were ranged from 46 ppm to 146 ppm, 47 ppm to 144 ppm and 50 to 141 ppm, with a mean values 81.08 ppm, 92.16 ppm and 83.44 ppm, for the samples collected from factories A, B and C,respectively. This study concluded that 4.0%, 8.0% and 4.0% of the examined samples were above the APL, although this agreed with that obtained by the quality control laboratories in two plants (A and C) but the second one (B) did not agree.

Keywords: Beef burger, Nitrite, Spectrophotometer

C-44

DEVELOPMENT, APPLICATION AND COMPARISON OF LC-MS/MS AND LC-HRMS APPROACHES FOR MULTI-RESIDUE MONITORING OF ABOUT THIRTY COMPOUNDS OF PERFLUORINATED COMPOUNDS IN FISH

Hanane Kadar[1*], Bruno Veyrand[2], Fabrice Monteau[3], Sophie Durand[4], Philippe Marchand[5], Jean-Philippe Antignac[6], Bruno Le Bizec[7]

[1 2 3 4 5 6 7] LABERCA, ENVN, Nantes, France
[*] Corresponding author–E-mail: kadar@vet-nantes.fr; Phone: 33 2 40 68 78 80

Perfluorinated compounds are considered as emerging chemical contaminants that focused high interest from international scientific community. These analytes, synthesized by human since half a century, are used in a large amount of commercialized products for their stain, oil repellent and surfactant properties. Nowadays, they are present in our daily lives through several products (clothes, detergent, carpet, food packaging…). They are released in environment during all their life cycle, from production to use. Most of the studies focused on the environmental impact of these compounds. Consequently, analytical methods are essentially performed on fish liver or blood and few methods are reported in literature for perfluorinated compounds in fish muscle.

The aim of our work consists in evaluation of food exposure by perfluorinated compounds, especially through fish consumption, which is supposed to be one of the principal route of human exposure. For this purpose, we have developed a method to analyze about thirty compounds from a wide range of chemical families (carboxylic, sulfonic and sulfinate acids, fluorotelomer acid saturated and unsaturated…). Prior to analysis, a liquid solid extraction is performed, followed by a dispersive solid phase extraction with graphitized carbon and centrifugation of the final extract. For LC-MS based measurement, two different approaches have been compared in term of specificity and sensitivity, i.e. LC-MS/MS on a quadripole instrument (QqQ, Agilent 6410) and LC-HRMS on an orbital trap instrument (LTQ-Orbitrap, Thermo). The performance of the method has been evaluated in accordance with the EU regulations, demonstrating the advantage of LC-HRMS through the mass defect characteristic of the pseudo-molecular ions monitored after negative electrospray ionization, but the advantage of LC-MS/MS to fulfill regulatory identification criteria.Finally, this method has been applied to french rivers' fishes permitting to collect the first exposure data with such extended range of monitored PFCs compounds.

Keywords: perfluorinated compounds, fish, LC-MS/MS, LC-HRMS

C-45
MOLECULAR IDENTIFICATION OF BACTERIAL COMMUNITIES INVOLVED IN DEGRADATION OF READY-TO-USE SALAD VEGETABLES

Zwielehner Jutta[1], Hippe Berit[2], Haslberger Alexander G[3*]

[1 2] Department for Nutritional Science, University of Vienna, Vienna, Austria
[3] Department for Nutritional Science, University of Vienna, Vienna
[*] Corresponding author–E-mail: alexander.haslberger@univie.ac.at; Phone: +43 6991 2211212; Fax: +43 1 8795896

For ready to eat salads often questions are raised about the safety and microbiological quality but indigenous microbial communities may also exert antagonistic effects on pathogens.

We used PCR-DGGE fingerprinting, cloning and sequencing to assess the microbial diversity associated with degradation of ready-to-use packed mixed leafy vegetables. Abundance of bacteria and lactic acid bacteria was assessed with TaqMan qPCR.

We identified several sequences in the bandpattern that increase in abundance during progression of the deterioration, and some sequences whose abundance decrease. Degradation was found to be associated with a decrease in species diversity as shown in PCR-DGGE fingerprinting.This decrease of diversity was particularly pronounced for lactic acid bacteria in the course of decomposition.

Analysis with qPCR indicates an increase of bacterial abundance with degradation. Abundance of lactic acid bacteria increased during the first five days of degradation. After this time span the lactic acid bacteria reach a plateau and even decrease slightly.

Keywords: ready-to-eat, PCR-DGGE, qPCR

C-46

PYRENE AND FLUORANTHENE AS A SUITABLE INDICATORS OF THE OCCURRENCE OF HEAVY PAHS IN FOOD

Marta Ciecierska[1], Mieczysław Obiedziński[2*], Piotr Jankowski[3]

[1 2 3] Warsaw University of Life Science (SGGW), Faculty of Food Technology, Warsaw, Poland
[*] Corresponding author–E-mail: mieczyslaw_obiedzinski@sggw.pl; Phone: +48(22) 59 37 672

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous chemical contaminants. Food contamination can derived from environmental sources, industrial processing and from certain home cooking practices. Polyarenes generally occur in complex mixtures, including light and heavy PAHs. Until recently benzo[a]pyrene was regarded as an indicator for the occurrence of PAHs in food, especially heavy and carcinogenic PAHs. It is generally known, that in the majority of foodstuffs, the content of this compound as well as the other heavy PAHs are relatively low, and often they are found at trace amounts, what causes their determination problematical. On the contrary, in the quality profiles of PAHs contamination, light PAHs are always found to be predominant and the levels of their content are much higher.

Considering that in the mechanism of formation of carcinogenic polyarenes, such as benzo[a]pyrene, benzofluoranthenes or dibenzopyrenes, the parent substances are fluoranthene and pyrene, the hypothesis was proposed that their determination could be a suitable PAHs indicator in screening tests of food products contamination. This thesis was verified, for selected groups of foodstuffs, by the multivariate analysis of correlation of pyrene, benzo[a]pyrene and 15 heavy PAHs (listed by the EU Scientific Committee on Food) contents and correlation of fluoranthene, the sum of benzofluoranthenes and 15 heavy PAHs contents. Moreover, the multivariate analysis of correlation of pyrene, benzo[a]pyrene, 16 US EPA PAHs content and the sum of carcinogenic PAHs from EPA list was also performed. The materials investigated were different groups of foodstuffs, both from the plant products and animal-origin foodstuffs (breads, vegetable oils, coffees and teas, meat products, vegetable and animal fats), available in the Polish market. Methodology applied for the PAHs determination consisted of fat extraction, PAHs isolation using GPC (gel permeation chromatography) and consequently qualitative-quantitative compounds determination by HPLC-FLD/DAD or GC/MS.

The study revealed that in analyzed foodstuffs the correlation degree of pyrene, benzo[a]pyrene and the sum of 15 heavy PAHs content was strong. The correlation coefficients of the sum of benzofluoranthenes and 15 heavy PAHs contents towards fluoranthene were also highly significant. It was also proved that the correlations of benzo[a]pyrene, 16 US EPA PAHs content and the sum of carcinogenic PAHs from EPA list towards pyrene were also high. Therefore, it was confirmed that pyrene and fluoranthene can be a suitable indicators for the occurrence of benzo[a]pyrene, benzofluoranthenes and other heavy PAHs in foods, especially in simplified and screening tests of food products contamination.

Keywords: pyrene, fluoranthene, PAHs indicators, food

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C-47
VESICLE-BASED SUPRAMOLECULAR SOLVENT FOR THE EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN FOOD

Soledad Rubio[1*], Ana Ballesteros-Gómez[2], Francisco José López-Jiménez[3]

[1 2 3] University of Córdoba, Spain
[*] Corresponding author–E-mail: qa1rubrs@uco.es; Phone: 34-957-218644; Fax: 34-957-218644

Polycyclic aromatic hydrocarbons (PAHs) are a well-known group of pollutants widely studied and internationally regulated because of their carcinogenic activity. An important way for human intake of these compounds is food. PAHs pollute food directly from air or water (vegetables, meat, fish) or during its preparation (smoked meat of fish) or cooking (roasting, charcoaling or grilling).

Recently, the European Union has published a study where sixteen PAHs are considered as carcinogenic pollutants in food and expresses the need for their regulation [1]. Up to now, European laws have only set limit levels for benz[a]pyrene (BaP) in a variety of foods including meat, fish, molluscs and infant foods. Current legislation considers BaP as an indicator of PAHs contamination, however, the study above cited considers that total occurrence of PAHs cannot be accurately predicted from BaP measurements. In this context, the development of methods that permit to obtain fast and accurate information about the presence of carcinogenic PAHs in food, at the low levels they commonly occur, is mandatory.

In this research, a new analytical method intended to meet the criteria for future regulatory decisions has been developed. It is based on the fast, low cost and efficient supramolecular solvent-based microextraction of the target carcinogenic PAHs from food and their direct quantitation in the supramolecular extract by liquid chromatography and fluorescence detection. The supramolecular solvent used for extraction was made up of vesicles of octanoic acid and tetrabutylammonium octanoate and it spontaneously formed in aqueous solutions containing octanoic acid and tetrabutylammonium hydroxide at molar ratios around 2. The procedure involved the vortex-shaking of 200 µL of supramolecular solvent and 200 mg of sample in 1.5 mL-eppendorf tubes containing four glass pearls (3 mm of internal diameter) for 10 min at 2670 rpm. Then, the mixture was centrifuged at 15000 rpm for 20 minutes and an aliquot of the extract was injected in the chromatographic system. Recoveries of PAHs in samples ranged between 89 and 108 for a spiking level of 5-fold the quantitation limit of the method. Detection limits for PAHs were 0.2 µg/kg for benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, 5-methylchrysene, benzo[ghi]perylene, 0.9 µg/kg for benzo[j]fluoranthene, 1 µg/Kg for indeno[1,2,3-cd]pyrene and 2.5 µg/kg for benzo[c]fluorene. The method was applied to the determination of PAHs in smoked meats, fishes and molluscs proving its suitability for a wide range of foods. No interferences were detected for any of the matrices investigated.

[1] Findings of the EFSA Data Collection on Polycyclic Aromatic Hydrocarbons in Food. European Food Safety Authority (EFSA). Parma, 2008.

Keywords: supramolecular solvents, microextraction, food, PAHs

C-48
MEASUREMENT OF TOXIC METALS IN THE LOW AMOUNT OF SALT

Amir Sasan Mozaffari Nejad[1*], Ghanbar Laey[2]

[1] Department of Food Science, Islamic Azad University, Young Research Club, Damghan branch, Damghan, Iran
[2] §Department of Plant Sciences, Faculty of Agriculture, Islamic Azad University, Damghan Branch
[*] Corresponding author–E-mail: sasan_mozafari@hotmail.com; Phone: 00989111289266;
Fax: 00981232234191

Considering the noticeable daily salt consumption and its impact on health of individuals and society as its role in the food industry, especially the salt quality control measure and the least amount of toxic metals important to many.

In this study of 10 salt production workshop in Tehran and randomly sample the set values of some qualitative characteristics of metals, copper, cadmium, lead, mercury and arsenic using international standards were assessed.

Range changes (and average) figures obtained for the degree of purity 45/98–86/87 percent (18/95), material insoluble in water 11/21–06/0 percent (03/1), sulfate 55/0–22/0 percent (35/0) and humidity 11/0–01/0 percent (0/05) is. Range changes and the average concentration of metals measured in samples of copper salt 270/0–0 (112/0), cadmium 008/0–0 (003/0), lead 037/0–0(009/0), mercury 044/0–008/0 (021/0) and arsenic 017/0–0 (008/0) mg kg is.

Keywords: toxic metals, salt, Iran

C-49

BEHAVIOR OF DECABROMODIPHENYL ETHER (BDE-209) IN THE SOIL-PLANT SYSTEM: UPTAKE, TRANSLOCATION AND METABOLISM IN PLANTS AND DISSIPATION IN SOIL

Shuzhen Zhang[1*], Honglin Huang[2], Peter Christie[3], Sen Wang[4]

[1 2 4] Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China
[3] Agri-Environment Branch, Agriculture Food and Environmental Science Division, Agri-Food and Biosciences Institute, Newforge Lane, Belfast BT9 5PX, UK
[*] Corresponding author–E-mail: szzhang@rcees.ac.cn; Phone: 86-10-62849683; Fax: 86-10-62923563

Decabromodiphenyl ether (BDE-209) is the principal component in commercial flame retardants. Concerns over BDE-209 have increased due to its increasing occurrence in the environment and in humans. In this study the behavior of BDE-209 in the soil-plant system was investigated. Accumulation of BDE-209 was observed in the roots and shoots of all six plant species studied. Root uptake of BDE-209 was positively correlated with root lipid content ($p < 0.001$, $r^2 = 0.81$) and shoot accumulation of BDE-209 showed a negative correlation with root lipid content ($p < 0.05$, $r^2 = 0.55$). An inverse relationship was observed between BDE-209 concentration in roots and its translocation factors (TFs, C_{shoot}/C_{root}). Nineteen additional debrominated (tri- to nona- PBDEs) and 6 hydroxylated congeners were detected in the soil and plant samples, indicating debromination and hydroxylation of BDE-209 in the soil–plant system. Observation of a relatively higher proportion of lower (penta- through tri-BDE) brominated products in plant tissues than in the soil samples implies that BDE-209 can be further debrominated within plants. Dissipation of BDE-209 in soil is mainly attributable to microbial degradation and there was a significant negative correlation between the residual BDE-209 concentration in soil and the soil microbial biomass measured as the total microbial PLFA ($p < 0.05$, $r^2 = 0.77$). The results of this study provide important information about the behavior of BDE-209 in the soil-plant system which is crucial for elucidating the behavior and fate of BDE-209 in the environment.

Keywords: BDE-209, plant uptake, metabolism

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C-50

COMPARISON OF TWO DIFFERENT ANALYTICAL METHODS FOR DETERMINATION OF PERFLUORINATED COMPOUNDS (PFCs) IN FRESH FISH AND FISH PRODUCTS

Kamila Kalachova[1*], Petra Hradkova[2], Jana Horakova[3], Dorte Herzke[4], Sandra Huber[5], Rolland Kallenborn[6], Jana Pulkrabova[7], Monika Tomaniova[8], Jan Poustka[9], Jana Hajslova[10]

[1 2 3 7 8 9 10] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[4 5] Norwegian Institute for Air Research, The Polar Environmental Centre, Hjalmar Johansens gate 14 NO-9296 Tromsø, Norway
[6] Norwegian Institute for Air Research (NILU), Instituttveien 18, NO-2027 KJELLER, Norway
[*] Corresponding author–E-mail: kamila.kalachova@vscht.cz; Phone: +420 220 443 272

Perfluorinated compounds (PFCs) have become emerging food and environmental contaminants since they have been used in many common products such as waterproof clothes and shoes, packaging materials and fire-fighting foams, from which they can release to the surroundings. Till now they have been found in a wide range of concentrations both in abiotic and biotic matrices including human samples as plasma, blood and breast milk. In May 2009 perfluorooctane suphonate (PFOS) was included in the list of Stockholm convention and European Food Safety Agency (EFSA) recommended that further data on PFAS levels in food and in humans would be desirable, particularly with respect to monitoring trends in exposure. Therefore quick, sensitive, reliable and cheap method is required for monitoring not only of PFOS but also for other PFCs that have to be studied more in details.

The main aim of this study was to compare two different analytical methods used for detection of ionic PFCs in fresh fish and fish products. Two extraction techniques – shaking with methanol and ultrasonication using acetonitrile, followed by dispersive solid phase extraction clean up step with activated charcoal and ENVI-Carb were realized on PFCs interlaboratory study fish sample. In addition to sample preparation steps following measurements using liquid chromatography (LC) coupled to (*i*) time-of-flight mass spectometry (TOFMS) and (*ii*) tandem mass spectrometry with triple quadrupole type of ion analyser (MS/MS), both operated in negative electrospray ionisation mode (ESI-), were compared and detection limits estimated.

Keywords: PFCs, fish, extraction, LC-TOFMS, LC-MS/MS

This study was funded by the Research Support Fund of the National Training Fund within the project EMERCON (Identification and quantification of emerging organic contaminants in the Czech aquatic ecosystem and food market supply). With focus on perfluorinated alkylated compounds (PFC), no. A/CZ0046/2/0026.

C-51
MELAMINESENSOR: A RAPID TEST FOR QUICK DETECTION OF MELAMINE IN MILK

Vincent Chabottaux[1*], Noan Nivarlet[2], Céline Bonhomme[3], Benoit Granier[4]

[1] UNISENSOR S.A., Rue du Dossay n°3, B-4020 Liège, Belgium
[2 3 4] UNISENSOR S.A., Rue du Dossay n°3, B-4020 Liège, Belgium
[*] Corresponding author–E-mail: vincent.chabottaux@unisensor.be; Phone: +32 (0) 4 252 66 02 ; Fax: +32 (0) 4 252 90 55

Melamine (2,4,6-triamino-1,3,5-triazine ; $C_3H_6N_6$) is a small synthetic organic molecule composed by 66% of nitrogen. This molecule is mainly used in the production of plastics, paint and adhesives. In food, melamine can be found accidentally following contamination from plastic packages. But unfortunately, because of the high level of nitrogen, it has become a bad practice to adulterate diluted milks with melamine in order to increase the apparent level of protein.

In China, the country where this practice was more common and where higher concentration in milk has been found, the presence of melamine lead to kidney stones and other urinary tract problems. At least four babies died and more than 50.000 children have fallen ill after being fed with milk tainted with melamine.

We have therefore developed the first rapid screening test for melamine detection in milk. It is an indirect competitive lateral flow assay that does not require any sample cleaning or preparation. When the sample is free of melamine, a color development occurs at the “test” line, indicating the absence of melamine in the milk sample. On the contrary, the presence of melamine in the sample will not cause the colored signal to appear at the “test” capture line. The dipstick test takes 5 minutes of incubation to get the result. The sensitivity is 250 ppb (ng/ml) for visual reading but it can be improved to 150 ppb with the help of an optical reader. The product was already tested with success in china dairy plans and no important variations have been found with milk from different origins or with powder milk.

Keywords: Melamine, Milk, Dipstick

C-52
DETERMINATION OF PAHs IN SMOKED SAUSAGES BY GC/MS

Ludmila Mravcová[1*], Milada Vávrová[2], Josef Čáslavský[3], Helena Zlámalová Gargošová[4], Iva Steinhauserová[5], Josef Kameník[6], František Mikeš[7]

[1 2 3 4] Institute of Chemistry and Technology of Environmental Protection, Faculty of Chemistry, Brno University of Technology, Purkyňova 118, Czech Republic
[5] Department of Hygiene and Technology of Meat, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic
[6 7] KMOTR–Masna Kromeriz, a.s., Hulinska 2286, 767 60 Kromeriz, Czech Republic
[*] Corresponding author–E-mail: mravcova@fch.vutbr.cz; Phone: +420-541 149 531

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants which can be toxic and carcinogenic. Most PAHs in the environment are formed during incomplete combustion of carbon-containing materials. For the nonsmokers the main sources of exposures are air inhalation and food. Food may be contaminated with PAHs by two different routes, from the environment and during food processing (barbecuing, frying, roasting, smoking).

Our study was focused on the monitoring of PAH levels in smoked sausages. Samples were taken from company KMOTR-Masna Kroměříž a.s. The production of dry fermented sausages (Poličan, Paprikáš) consists of several steps. The last step is smoking and ripening in conditioned smoke rooms under controlled conditions (temperature, humidity, wind speed) during one month. In the production facility three types of smoking rooms are available (differ from each other in the year of construction). At the beginning of ripening period passive samplers of SPMD type were placed in one of each type of smoke rooms. Sausages of “Poličan” type were sampled at the beginning, after one week and one month after the end of smoking (before expedition of the finished product).

The treatment of sausages samples includes: Soxtec extraction for 6 hours with n-hexane, GPC on Bio-Beads S-X3 for efficient lipid removal. Exposed SPMDs were dialyzed in n-hexane (2×24 h), dialysate was concentrated using rotary evaporator and GPC as the clean-up step was also used.

GC/MS was used for identification and quantification of PAHs in obtained fraction. The mix of 5 deuterated PAHs was used as an internal standard.

The obtained results enabled estimation of the influence of various types of smoke rooms on the content of PAHs in the final product. The lowest concentration of PAHs was detected in the newest smoke room.

Keywords: PAHs, smoked sausages, SPMDs

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C-53

NEW CAPILLARY COLUMN FOR THE GC/MS SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS INCLUDING CHRYSENE AND TRIPHENYLENE

Johan Kuipers[1*], Max Erwine[2]

[1 2] Varian Inc.
[*] Corresponding author–E-mail: johan.kuipers@varianinc.com; Phone: +31610460596

Polycyclic Aromatic Hydrocarbons (PAHs) comprise of a large group of chemical compounds known to be cancer-causing agents. Some PAHs have been demonstrated to be carcinogenic and mutagenic. The scope of PAHs to be analyzed is shifting under influence of international advisory committees and changing legislation giving need for the separation of critical PAHs previously not monitored.

For GC/MS analysis some coeluting PAHs exhibit identical MS fragmentation patterns which sets the need for more optimized capillary column selectivity and dedicated liquid phase chemistry to obtain unambiguous PAH identification and prevent the reporting of false positives.

A new dedicated selective liquid phase for PAH analysis was developed with a unique ability to isolate chrysene from triphenylene and the simultaneous separation of the three benzo(b,j,k)fluoranthenes. Also other critical peak pairs such as the Indeno(1,2,3-cd)pyrene, benzo(b)triphenylene and dibenz(a,h)anthracene can be separated.

The liquid phase and GC columns shows a highly thermally stable profile with low bleed characteristics at 350°C which enables the elution and detection of the high boiling dibenzopyrenes also included in the EFSA (European Food Safety Authority) PAH priority list.

This paper discusses the possibilities offered by this new liquid phase for more accurate reporting of regulated EFSA PAHs. Several applications are shown illustrating the efficient separation and identification of over 54 PAHs including all (15+1) EFSA and 16 US EPA priority PAHs and interfering isomers for food related matrices.

Keywords: PAH, GC/MS, Chrysene/Triphenylene,

Dr. Claudia Schulz, Ansgar Ruthenschror, Eurofins WEJ Contaminants

NANOPARTICLES

(D-1 – D-7)

D-1
THE INHIBITORY EFFECT OF NANOCID® AGAINST STAPHYLOCOCCUS AUREUS AT DIFFERENT TEMPERATURES

Hassan Gandomi[1*], Ali Khanjari [2], Ali Misaghi[3], Hamed Ahary [4], Mosayeb Vahedi [5], Fereshteh Ghadami[6], Jafar Rahmanian[7]

[1 2 3 5 6] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, The University of Tehran
[4] Science and Technology park, The university of Tehran
[7] Research and Development Center, Pars Nanonasb Co.
[*] Corresponding author–E-mail: gandomih@ut.ac.ir; Phone: +98-2161117042

Background and Objective: Silver is a metal known for its broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, protozoa and certain viruses, including antibiotic-resistant strains. In the present scenario, nanoscale materials have emerged up as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties. The aim of this study was to evaluate the effect of different concentrations of silver nanoparticles (NANOCID L2000®, Pars Nanonasb Co., Tehran, Iran) on the growth of *Staphylococcus aureus* at three different temperatures (35, 20 and 5°C) during 10 days.

Materials and methods: Different concentrations of nanosilver (12.5, 25, 50, and 100 ppm) were prepared in 50 ml BHI broth in Erlenmeyer flasks, inoculated with bacterial suspension at the end concentration of 5×10^5 CFU/ml, and incubated for 10 days at three different temperatures (35, 20, and 5°C). Bacterial samples were taken every day, serially diluted and plated, in duplicate, on BHI agar. After 24–48 hours incubation at 35°C, the colony count was carried out and the number of the bacteria was calculated as CFU/ml.

Results and Discussion: All concentrations of nanosilver had an inhibitory effect on bacterial growth at all temperatures examined. At 35°C, concentrations containing 12.5, 25, and 50 ppm nanosilver induced 1 log reduction in bacterial growth comparing to the control group at the first day and the bacterial count reached to the control by the second day. At 100 ppm no growth was observed at the first day and the bacterial count was 2 logs lower than the control by the day 10. At 20°C, in concentrations containing 50 and 100 ppm nanosilver not only there was no bacterial growth but a bactericidal effect also was observed, as no bacteria were detected by the third day in these concentrations. At 5°C, although no bacterial proliferation was seen at control, the bacterial count in treatment groups reduced by the first day and this effect was dose dependant. The bactericidal effect from higher concentrations of nanosilver was greater at 20°C comparing to 5°C. Nanosilver used in this study showed a strong inhibitory effect against *Staphylococcus aureus* and this effect increased when the temperature decreased or the concentration of nanosilver increased.

Keywords: Silver nanoparticle, *Staphylococcus aureus*, Temperature, Antibacterial effect

D-2
GROWTH RESPONSE OF SALMONELLA TYPHIMURIUM TO SILVER NANOPARTICLE AT DIFFERENT TEMPERATURES

Ali Misaghi[1*], Ali Khanjari [2], Hassan Gandomi[3], Hamed Ahary [4], Mosayeb Vahedi [5], Fereshteh Ghadami[6], Jafar Rahmanian[7]

[1 2 3 5 6] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, The University of Tehran
[4] Science and Technology park, The university of Tehran
[7] Research and Development Center, Pars Nanonasb Co.
[*] Corresponding author–E-mail: misaghia@vetmed.ut.ac.ir; Phone: +98-2161117042

Background and Objective: Nanosized inorganic particles, of either simple or composite nature, display unique physical and chemical properties. Different types of nanomaterials like copper, zinc, titanium, magnesium, gold, alginate and silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms. The aim of this study was to investigate the behavior of a food borne pathogen, *Salmonella typhimurium*, in BHI broth containing different concentrations of silver nanoparticles (NANOCID L2000®, Pars Nanonasb Co., Tehran, Iran) at different temperatures.

Materials and methods: Different concentrations of nanosilver (12.5, 25, 50, and 100 ppm) were prepared in 50 ml BHI broth in Erlenmeyer flasks, inoculated with bacterial suspension at the end concentration of 1.5×10^5 CFU/ml, and incubated for 10 days at three different temperatures (35, 20, and 5°C). Bacterial samples were taken every day, serially diluted, and plated, in duplicate, on the BHI agar. After 24–48 hours incubation at 35°C, the colony count was carried out and the number of the bacteria was calculated as CFU/ml.

Results and Discussion: In this study a dose dependant inhibitory effect of nanosilver was observed and this effect decreased with increasing the temperature. At 35°C, the growth of the bacteria in all treatment groups was similar to the control during the first day while the bacterial count reduced at concentration of 100 ppm by the day 2. At 20°C, concentration of 100 ppm nanosilver reduced the bacterial number from 1.5×10^5 to 1.7×10^4 by the first day and the bacterial count at 50 ppm was 4 logs lower comparing to the control. The greatest antibacterial effect of nanosilver was seen at 5°C whereas at 100 ppm no bacteria was detected after 10 days and the bacterial count was 5×10^1 CFU/ml in BHI containing 50 ppm nanosilver comparing to 1.5×10^5 CFU/ml in control. The results showed that silver nanoparticle has a strong antibacterial effect and this effect increases with decreasing the temperature or increasing the concentration of nanosilver.

Keywords: Nanosilver, *Salmonella typhimurium*, Temperature, Bacterial inhibition, Food borne pathogen

D-3
MAGNETIC HYDROPHILIC MICROSPHERES P (HEMA-CO-GMA) FOR DNA ISOLATION FROM MOUSE FAECES

Štěpánka Trachtová[1*], Tanja Obermajer[2], Alena Španová[3 4], Bojana Bogovič Matijašić[5], Irena Rogelj[6], Daniel Horák[7], Bohuslav Rittich[8 9]

[1 3 8] Brno University of Technology, Faculty of Chemistry, Institute of Food Science and Biotechnology, Purkyňova 464/118, CZ-612 00 Brno, Czech Republic
[2 5 6] University of Ljubljana, Biotechnical Faculty, Zootechnical Department, Chair of Dairy Science, Groblje 3, SI-1230 Domžale, Slovenia
[4 9] Masaryk University, Faculty of Science, Institute of Experimental Biology, Tvrdého 14, CZ-611 37 Brno, Czech Republic
[7] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovský Sq. 2, CZ-162 06 Prague, Czech Republic
[*] Corresponding author–E-mail: xctrachtova@fch.vutbr.cz; Phone: +420777641104

Probiotic lactic acid bacteria (LAC) play an important role in health maintenance of the human gastrointestinal tract. A mouse model is widely used for the study of LAC influence on the organism's health. Polymerase chain reaction (PCR) has become a powerful diagnostic tool for the analysis of microorganisms in different types of samples. Faecal samples are a difficult specimen for the application of PCR due to the presence of PCR inhibitors. For this reason an optimal DNA extraction method is crucial for the identification of microorganisms in faecal samples. The problem can be solved by reversible adsorption of whole DNA on magnetic particle surface in the presence of high concentrations of PEG 6000 and sodium chloride. Magnetic non-porous hydrophilic poly(2-hydroxyethyl methacrylate-*co*-glycidyl methacrylate)–P(HEMA-*co*-GMA) microspheres containing carboxyl groups were used for this purpose (Rittich et al. 2009). In this work the proposed method was used for DNA isolation from mouse faeces and identification of the probiotic *Lactobacillus gasseri* K7 strain by PCR (Majhenič et al. 2003). The DNA absorption on microsphere surface and the release of adsorbed DNA were optimised. The quality of eluted DNA and the presence of target DNA were examined by PCR and q-PCR using genus-specific *Lactobacillus* and a specific *Lactobacillus gasseri* K7 primer set (Dubernet et al. 2002, Majhenič et al. 2003). The next four DNA extraction procedures were used for comparison. It was shown that DNA extracted using P(HEMA-*co*-EDMA) microspheres gives the same results in PCR as DNA extracted using other tested procedures. The amount of isolated DNA was smaller in comparison with the tested procedures but target DNA could be detected. By means of q-PCR no inhibition was detected using undiluted DNA extracts as DNA matrix.

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Keywords: Magnetic particles, Lactobacillus gasseri K7

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D-4
ANALYSIS OF NANOPARTICLES IN FOOD AND FOOD PACKAGING

Ruud Peters[1*], Bert Brouwer[2], Elly Wijma[3], Xue Jiang[4], Stefan Weigel[5], Hans Bouwmeester[6]

[1 2 3 4 5 6] RIKILT, Wageningen, The Netherlands
[*] Corresponding author–E-mail: ruudj.peters@wur.nl; Phone: +31 317 486 348; Fax: +31 317 417 717

The potential benefits for consumers and producers of the application of nanotechnology are widely recognized. Products based on nanotechnology or containing engineered nanoparticles (ENPs) are already manufactured in the field of electronics, consumer products and pharmaceutical industry, and are beginning to impact the food associated industries. Detection and characterization of ENPs in food and food packaging materials is an essential part of understanding the potential benefits as well as the potential risks of the application of ENPs. Currently, there are no adequate analytical methods to characterise the ENPs in food and therefore the current usage levels of ENPs in the food and feed area is unknown. Clearly a variety of ENPs are claimed to be used in food packaging materials, some food supplements and even some food products; e.g.silica, silver, titanium, copper, gold and zinc. This indicates that direct and indirect consumer exposure to ENPs is likely. Here we present the development of analytical methods to characterise nanosilica and nanosilver particles in food and the presence of these ENPs in some food commodities.

We developed an analytical method for the detection and characterisation of silver and silica ENPs in food. Sample preparation, e.g. separation of ENPs from the matrix is achieved using a series of physical preparation and separation steps. While this does not guarantee the conservation of the actual aggregation state of the ENPs, it does enable size separation and detection of the ENPs in the resulting dispersions. Size separation is achieved using hydrodynamic chromatography (HDC) on a 800 $\times$ 25 mm column packed with non-coated, non-porous silica spheres. While detection of silver and gold ENPs can also be done using UV detection at specific wavelengths, we applied HDC on-line coupled to ICPMS for element specific detection of silver and silicon. Using the HDC/ICPMS combination silver and silicon ENPs can be sized in the range of 10 up to 200 nm and detected in the mg/kg product range. While silver ENPs can be detected with high sensitivity resulting in detection limits <0.1 mg/kg product, silicon (as expected) suffers from background interference limiting the sensitivity <10 mg/kg.

Silver ENPs could easily be detected after addition to soft drinks, fruit or vegetables and the analytical method for silver was validated by analysing food samples spiked in the range of 0.02 to 2 mg/kg. While the analysis of actual food samples showed that no silver ENPs were present, silver ENPs were found in some “nano-silver” food supplements that are marketed on the internet. Natural silica nano-particles were found in mineral waters and even exist in most tap waters. Silica ENPs were found in most food samples containing the food additive E551, an anti-caking agent. These include mainly powdered food items or additives such as pancake mixtures, seasoning mixtures, soup powder, coffee creamer, etc. While the total silica concentrations in these products are in the order of 2 g/kg, concentrations of silica ENPs are substantially lower indicating the presence of larger silica agglomerates. On the other hand, the size distribution of the silica ENPs in these products is a good reflexion of that in E551, a good indication of the source of the silica ENPs.

Keywords: Nanoparticles, HDC-ICPMS, Silver, Silica, Food

D-5

DETECTION OF HYDROGEN PEROXIDE USING A MWCNTS/ POLY(OXYETHYLENE)-SEGMENTED IMIDE/SILVER NANOPARTICLES MODIFIED SCREEN-PRINTED CARBON ELECTRODE

Chia-Yu Lin[1], Rui-Xuan Dong[2], Ying-Chiao Wang[3], Wei-Cheng Tsai[4], Jiang-Jen Lin[5], Kuo-Chuan Ho[6*]

[1 2 3 6] National Taiwan University, Taipei city, Taiwan
[4] National Chung Hsing University, Taichung, Taiwan
[5] National Taiwan University, Taipei city, Taiwan & National Chung Hsing University, Taichung, Taiwan
* Corresponding author–E-mail: kcho@ntu.edu.tw; Phone: +886-2-33663020

Due to its redox properties, silver nanoparticles (Ag_{NPs}) have been proposed as electrocatalysts for the detection of some biologically and environmentally important compounds, such as H_2O_2. Since the scale and distribution of Ag_{NPs} plays a vital role in the catalytic ability for H_2O_2, the control of the growth of Ag_{NPs} is important. Several materials have been proposed as the growth-control agent, such as multi-walled carbon nanotubes (MWCNTs).

In this study, MWCNTs/poly(oxyethylene)-segmented imide/Ag_{NPs} (MWCNTs/POE-imide/Ag_{NPs}) modified screen-printed carbon electrodes (SPCEs) were fabricated and their electrocatalytic properties to H_2O_2 was investigated. POE-imide was selected not only as it can be used as the dispersion agent for MWCNTs, but also as the reducing agent for the reduction of Ag^+ into Ag_{NPs}. The size of Ag_{NPs} prepared by this method is around 20 nm. After being drop-coated onto SPCE and dried, the MWCNTs/POE-imide/Ag_{NPs}-modified SPCE was applied to sensing H_2O_2. Our preliminary results show that there is no observable response from MWCNTs/POE-imide modified SPCE to H_2O_2. However, a reduction peak resulted from the reduction of H_2O_2, at around -0.12 V (vs. Ag/AgCl) was observed. This low overpotential could be attributed to the synergetic effect of MWCNTs and Ag_{NPs}. Further studies on the improvement of the sensor response by controlling the size and loading of Ag_{NPs} are in progress.

Keywords: H_2O_2, MWCNTs, Silver nanoparticles

D-6
CHALLENGES IN THE PREPARATION OF COLLOIDAL GOLD PARTICLES AS A LABEL FOR ANTIBODIES IN LATERAL FLOW DEVICES

Barbara Cvak[1*], Alexandra Molinelli[2], Rudolf Krska[3]

[1 3] Christian Doppler Laboratory for Mycotoxin Research, Center for Analytical Chemistry, IFA Tulln, University of Natural Resources and Applied Life Sciences Vienna, Tulln, Austria
[2] Romer Labs Division Holding GmbH, Technopark 1, 3430 Tulln, Austria
[*] Corresponding author–E-mail: barbara.cvak@boku.ac.at; Phone: +43 / 2272 / 66280–419; Fax: +43 / 2272 / 66280–403

Due to their unique chemical and physical properties colloidal gold particles have been studied for many years. They provide many properties for their use as color reagent in biochemical test systems like lateral flow devices (LFDs). A lot of different methods have described synthesis of gold particles of defined particle size and shape. In most studies chemical reduction of the metal salt by sodium citrate or other reducing agents is still widely used. With a defined amount of reducing agent and reaction conditions during synthesis, the size of emerging particles can be controlled easily. In this study new findings about the synthesis of gold colloids and their application as label for antibodies in lateral flow devices are presented. Colloidal gold particles have been synthesized in a range of 15 to 50 nm in diameter. The absorption maxima of the solutions are determined by UV/VIS measurement to deduce particle size approximately. To confirm these results and to determine particle size exactly, the colloids have been aligned with transmission electron microscope (TEM). The colloidal gold particles with 40 nm in diameter have been used for labeling monoclonal anti-mycotoxin antibodies. To determine the amount of antibodies coupled to these particles, smaller colloids with 15 nm have been labeled with anti-species specific antibodies. Both solutions have been added together and can give information about the coupling ratio of antibodies to colloidal gold particles by TEM measurements. Furthermore, the anti-mycotoxin antibody/colloidal gold conjugate has been used in strip test format. Due to the red colored gold particles antibodies have been labeled with, a well visible colored line in the test zone of the strip test can be formed. The intensity of this test line can be measured with a photometric reader which enables semi-quantitative analysis of the target analyte. The synthesis is reproducible, allows a good control of particle size and delivers homogenous particles for labeling in immunochemical test systems.

Keywords: colloidal gold, synthesis, immunoassay

D-7
DEVELOPMENT OF MICROSCOPIC TECHNIQUES FOR DIFFERENTIAL DETECTION OF TECHNOGENIC NANOPARTICLES IN FOODSTUFFS

Anatoly Zherdev[1*], Irina Safenkova[2], Mikhail Savvateev [3], Boris Dzantiev[4]

[1 2 4] Institute of Biochemistry Russian Acad. Sci., Moscow, Russia
[3] Co Ltd "AIST-NT", Moscow (Zelenograd), Russia
[*] Corresponding author–E-mail: zherdev@inbi.ras.ru; Phone: +7 495 954-28-04; Fax: +7 495 954-28-04

Application of different techniques isolation, identification and quantitative analysis of technogenic nanoparticles in foodstuffs was comparatively studied. Dimensional characteristics of various industrial products of silver and titanium oxide nanoparticles were measured using electron microscopy, atomic force microscopy and dynamic light scattering. Correspondence between the results obtained by different methods was analyzed. To avoid problem of differentiating images of technogenic nanoparticles and nanobiopolymers, possibilities of electropower microscopy for this purpose were studied. Titanium oxide nanoparticles detection by this technique in liquid samples and extracts was characterized by atomic force microscope SmartSPM. The use of not-spending micafor for the electropower microscopy became possible due to spending surface of the SmartSPM sample holder. The electropower microscopy assumes two passes of a cantilever on the same line: 1) determination of a surface relief and 2) determination of electric properties of the sample by its scanning with input of constant voltage in the course of cantilever's movement on fixed height over the measured relief. The most informative detecting parameter for the second pass is the phase of cantilever's fluctuations. Efficiency of different methods for isolation of nanoparticles from foodstuffs and packaging was characterized as well as reproducibility of measurement results.

Keywords: technogenic nanoparicles, atomic force misroscopy

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Residues – Pesticides

(E-1 – E-45)

E-1

HEAD-SPACE SOLID-PHASE MICROEXTRACTION FOR THE DETERMINATION OF SELECTED PESTICIDES IN WATER AND APPLE SAMPLES USING GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTION

Manuela Correia[1*], Sandrine Eap[2], Artur Dias[3], Cristina Delerue-Matos[4]

[1 4] REQUIMTE/ISEP, Porto, Portugal
[2] IUT d'Orsay, France
[3] ISEP, Porto, Portugal
[*] Corresponding author–E-mail: mmb@isep.ipp.pt; Phone: +351228340500; Fax: +351228321159

In the last decades, the use of pesticides in agriculture has allowed for a substantial increase in food production. However, environmental contamination, resistance phenomena, impacts on natural ecosystems and food contamination are a few examples of the risks associated to this use that cannot be ignored.

Food safety and security are key issues in modern society. More than ever, scientists, regulators and consumers are engaged in guaranteeing that food products are exempt from contaminant residues, or are contaminated at a "secure level". Nevertheless, the determination of pesticide residues in complex matrices, such as food samples, is a complex and laborious task. It is usually accomplished by chromatographic techniques and involves several preliminary steps including sampling, extraction, and clean-up. The modern "greener" vision of Chemistry also demands for the use of new analytical approaches, including faster, simpler and cleaner methodologies.

Solid phase microextraction (SPME) is a convenient extraction method because integrates sampling, extraction, concentration and sample introduction into a single step without the use of solvents. In the recent years, SPME has gained widespread acceptance in the analysis of (semi)volatile food components, including contaminants [1-3].

The purpose of this study was to develop an analytical methodology for the analysis of two organophosphorus (chlorpyrifos, chlorfenvinphos) and two chloroacetanilides (alachlor and metolachlor) in water and apple samples using HS-SPME and gas chromatography–electron capture detection (GC-ECD). The HS-SPME process was optimized by testing parameters such as extraction time and temperature, pH, and ionic strength, using a 100 μm PDMS fiber. An increase in the extraction efficiency was obtained on increasing the temperature up to 75°C, for all analytes except for metolachlor, for which the maximum extraction efficiency was obtained at 55°C. Salt addition also led to an increase in the extraction efficiency. Regarding the value of pH, no significant effect on the extraction efficiency was observed for both isomers of chlorfenvinphos, while for alachlor and metolachlor, the efficiency decreased in the pH range 2–7, above which, it increased. The optimised methodology was applied to natural water samples and to commercial samples of different apple varieties.

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Keywords: pesticides, solid-phase microextraction, gas chromatography

E-2
LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Angela Santilio[1*], Graziella Amendola[2], Tiziana Generali[3], Roberto Dommarco[4]

[1 2 3 4] National Institute of Health (ISS), Rome, Italy
[*] Corresponding author–E-mail: angela.santilio@iss.it; Phone: +39 06 4990 2746; Fax: + 39 06 4990 2360

Chlormequat and Mepiquat are quaternary ammonium salts. They are used as plant growth regulator for growth control in cereals (wheat, rye, oats and triticale), pear and grapes.

The European Union (EU) has established maximum residue levels (MRLs) for Chlormequat and Mepiquat on cereals (barley, oats, rye, wheat, millet, maize), thereby emphasising the need for a routine analytical methodology to be used in monitoring programmes.

The aim of this work is to study the performance of and easy and fast method to determine these compounds on oatmeal.

The method consist of an extraction of the compounds of interest by methanol follow to direct injection in LC/MS/MS.

Chlormequat and Mepiquat were determined on an Aquasil C18 column (150 $\times$ 2.1 mm; 5 µm) by gradient elution with a mobile phase consisting of acetonitrile/5 mmol/L NH_4 acetate + 0.1% acetic acid at flow rate of 0.4 ml/min. Electrospray ionisation mass spectrometry in positive mode was employed. For quantification the transition 122 $\rightarrow$ 58 m/z and 114 $\rightarrow$ 98 m/z were used for Chlormequat and Mepiquat, respectively.

The performance of the method were studied in terms of recoveries at two spiked levels (2.5 mg/kg and 5.0 mg/kg for Chlormequat; 2 mg/kg and 1 mg/kg for Mepiquat), accuracy and linearity. For both compounds, the mean recoveries were ranged between 70–79% with relative standard deviation (RSD%) less than 20%. Limit of quantification (LOQ) was calculated as 0.05 mg/kg for both Chlormequat and Mepiquat.

The method can be considered useful for the analysis of the compounds studied on oats flour and further investigations are in progress to test the performance of the method on oat grain and flake oat.

Keywords: Chlormequat, Mepiquat, residue, Oat, LC/MS/MS

E-3
ORGANOCHLORINE PESTICIDE RESIDUES IN MILK IN TEHRAN, IRAN

Mahnoosh Parsaeimehr[1], Jamileh Salaramoli[2*], Beizaee Azadeh[3], Hejazi Marzieh[4], Tahereh Aliesfahani[5]

[1] Department of Veterinary Medicine, Islamic Azad University-Shoshtar branch, Iran
[2 4 5] Department of Toxicology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[3] Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[*] Corresponding author–E-mail: mparsamehr99@yahoo.com; Phone: +98 021-66438141; Fax: +98 021-66438141

Objective: Persistent Organic Pollutants (POPs) are bioaccumulative pesticides which persist in the environment over extended time periods and biomagnify as they move up through the food chain. They cause adverse human health or environment.

Due to their lipophilic properties, OCPs are firstly stored in fat rich tissues and subsequently are excreted through milk. They get accumulated in fat rich products such as milk and butter and consumers of dairy products are exposed to these residues, thus, milk samples have shown to be good indicator samples for the contamination of persistent organic pollutants (POPs) in the food chain.

On the other hand, OCPs pose a serious risk to health, especially for infants, since they are more sensitive than adults.

Method: In this study, the residues of five organochlorine pesticides, lindane, aldrin, dieldrin, endosulfan, dichlorodiphenyltrichloroethane (DDT) in 30 cow milk samples marketed in city of Tehran, Iran during 2007-2008 measured. Detection and measurement of the toxins were achieved by using GC-ECD.

Result & discussion: The results obtained had shown that organochlorine compounds were discerned in some examined cattle milk samples. The highest residual level of these pesticides were Aldrin (9.2 ng/g), Dieldrin (5.8 ng/ g), DDT (3.8 ng/g), Lindane, also known as gamma-hexachlorocyclohexane, (2.6 ng/g) and Endosulfan (1.4 ng/g). In milk samples, Aldrine was detected at higher average (2.07 ng/g) followed by it's metabolite Dieldrin (1.17 ng/g), DDT (1.43 ng /g), Lindane (1.1 ng/g) and Endosulfan (1.01 ng/g). Only Aldrin residual level was higher than permitted levels.

Our this investigation showed the average of these OCs residue were lower than accepted MRLs (Reg. (EC) N°149/2008) for cattle milk except for Aldrin. It also reflected the reduction use of organochlorine pesticide in recent years. However, these may imply recent or continuous use of OCs on the cattle and/or their feedstuff as well as the former use of organochlorine pesticides.

Keywords: Organochlorine pesticide residue, milk, Lindane

E-4

PERSISTANT ORGANOCHLORINE PESTISIDE RESIDUES IN COW'S BUTTER IN TEHRAN, IRAN

Jamileh Salaramoli[1*], Azadeh Beizaee[2], Mahnoosh Parsaeimehr[3], Hejazi Marzieh[4], Tahereh Aliesfahani[5]

[1 4 5] Department of Toxicology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2] Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[3] Department of Veterinary Medicine, Islamic Azad University-Shoshtar branch, Iran
[*] Corresponding author–E-mail: a79_beizaee@yahoo.com; Phone: +98 021-66438141; Fax: +98 021-66438141

Obejective: Organochlorine insecticides are among the most important organotoxins and make a large group of PBTs (Persistent, Bioaccumulative, Toxic substances) pesticides. Physicochemical properties of these pesticides, especially their high lipophilicity, facilitate the absorption and storage of these toxins in human and animal bodies and consequently, they can translocate and excrete through milk and milk fat. They get accumulated in fat rich products such as butter and consumers of dairy products are exposed to these residues, thus, butter (milk basis) have shown to be good indicator samples for the contamination of persistent organic pollutants (POPs) in the food chain and good indicator of cattle exposure to there pesticides.

Method: An analysis of 30 samples of butter (milk basis) in Tehran, capital of Iran, during 2006–2007. Detection and measurement of the toxins were achieved by using GC-ECD.

Result & Discussion: Butter samples analysis revealed the presence of Lindane, Aldin, Dieldrin, Endosulfan, DDT in 60%, 88.8%, 100%, 67% and 55.5% of samples, respectively.

All levels of organochlorine pesticide residues (Lindane, Endusulfan, DDT) in butter samples were well below the maximum permissible limits given by the FAO/ WHO and the detected levels of Aldrin and dieldrin are lower than other countries. only in a few samples, Aldrin and Dieldrin residual levels were a little higher than applied MRLs for Aldrin and Dieldrin according Reg. (EC) No 839/2008, 0.006 mg/kg.

Present levels of the contaminants are substantially lower, which indicate the gradual phase out of these compounds.

Keywords: Organochlorine pesticide residue, butter, Lindane

E-5
MULTIRESIDUE METHOD FOR THE DETERMINATION OF ORGANOPHOSPHORUS, ORGANOCHLORINE, AND PYRETHROID PESTICIDE RESIDUES IN BUTTER

Graziella Amendola[1*], Tiziana Generali[2], Patrizia Pelosi[3], Roberto Dommarco[4]

[1 2 3 4] Istituto Superiore di Sanità (ISS – National Institute of Health) Roma, Italy
[*] Corresponding author–E-mail: graziella.amendola@iss.it; Phone: +39 06 49902615; Fax: +39 06 49902360

The presence of pesticide residues in animal origin products could rise from the treatments of pesticides on feeds, from environmental contamination and from the use of pesticides as veterinary drugs.

Recently our laboratory has developed a method for the determination of Organochlorine (OC), Organophosphorus (OP) and Pyrethroid (PYR) pesticide residues in butter, in order to check the compliance

with Maximum Residues Levels (MRLs) established in Regulation (EC) n. 396/2005, to protect human and animal health.

In general the selective extraction of pesticide residues from fatty matrices presents some difficulties and butter has an 80–84% of fat. We have obtained good results by simple and fast extraction and clean up steps.

Butter (10 g) has been extracted by acetonitrile at room temperature and easy and fast steps of cleanup have been followed by freezing the sample at -20°C and using a C_{18} cartridge which allow a removal of fat about 99%. The sample was divided for the analysis of OP and OC/PYR pesticides separately.

The OP compounds were directly determined by gas chromatography with Flame Photometric Detector (GC-FPD) by matrix matched standards and using Thriphenylphosphate (TPP) as internal standard.

The OC/PYR residues were analysed after a further clean up in order to reduce much more the fat residue. Sample was loaded into a Florisil ready to use cartridge eluted by mixtures of hexane-toluene at different percentage. The quantitative determination was performed by gas chromatography coupled with a mass spectrometry detector (GC-MSD), using matrix matched standards and PCB 209 as internal standard.

The method was tested for 49 compounds at the spiking level range of 0.01–0.1 mg/kg. The recoveries obtained were between 70–120% for all the pesticides studied except for pirymiphos methyl, profenofos and HCB while azinphos methyl was not recovered at all.

This method was used to participate at the 4th European Proficiency Test in butter fat (EUPT AO-04) and good performance has been obtained.

Linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), accuracy and precision data, obtained according to SANCO/2007/3131 guide line, will be presented.

Keywords: butter, pesticide, residues, gascromatography determination

E-6

OPTIMIZING RECOVERIES OF PLANAR PESTICIDES IN SPINACH USING TOLUENE AND QUECHERS KITS WITH GRAPHITIZED CARBON

Ulrik Wittek[1], Thierry Faye[2], Limian Zhao[3], Belén de la Iglesia[2], Joan Stevens[4*]

[1] Agilent, Waldbronn, Germany
[2] Agilent, Massy, France
[3 4] Agilent, Wilmington, DE
[*] Corresponding author–E-mail: joan_stevens@agilent.com; Phone: +1 (302)-633-8839; Fax: +1 (302)-633-8955

This application note describes the impact of toluene addition in the dispersive SPE step to the analysis of pesticides in spinach using Agilent SampliQ QuEChERS AOAC kits for highly pigmented fruits and vegetables. Graphitized carbon black (GCB) is required in the dispersive SPE kits in order to remove high levels of pigments from the matrix. However, it also retains pesticides with planar structures resulting in poor recovery and precision. The eight problematic pesticides found in the original AOAC method, by either LC/MS/MS or GC/MS, generated poor results with about 20–60% recovery with >15% RSD. In the modified AOAC method, an aliquot of toluene was added to the dispersive SPE clean-up tube, ratio of 8:3 (ACN extracts/toluene). It significantly improved the extraction efficiency of those problematic planar pesticides. With the modified AOAC method, the eight problematic pesticides generated substantially improved recoveries, 50–100%, and < 10% RSD. However, the addition of toluene also introduced more matrix impurities into final sample, and caused problems for some pesticides which gave good results originally. Therefore, the modified AOAC method cannot be considered to be a “drop in” replacement for the original AOAC method; but it can be a very useful alternative for the problematic pesticides affected by GCB in the pesticides analysis of highly pigmented matrix.

Keywords: QuEChERS, food safety, pesticides, GC/MS

E-7
DETERMINATION OF PESTICIDES IN FOOD EXTRACT SAMPLES AT LOW PPB LEVELS USING A NEW BENCH TOP GC-MS/MS SYSTEM

Chris Sandy[1*]

[1] Agilent Technologies, Wokingham, UK
[*] Corresponding author–E-mail: chris_sandy@agilent.com; Phone: + 44 7836 236597

The GC-MS detection, confirmation and quantitation of trace level agrochemicals in food-stuffs such as fruit, vegetables, herbs, spices and meat products can be impaired by co-extracted matrix components. Matrix components that elute during the chromatographic run can mask the presence of contaminants and interfere with their confirmation / measurement due to co-eluting ions. Furthermore, higher-boiling matrix components can remain on the GC capillary column between sample analyses. These components can subsequently cause loss of chromatographic peak shape, retention time shifts of target analytes and contamination of the mass spectrometer ion source requiring more frequent maintenance of both the gas chromatograph and the mass spectrometer.

This poster shows the performance of a new bench-top GC-MS/MS system where Capillary Flow Technology is used to facilitate capillary column back flush between every sample analysis. Data is shown from both vegetable extracts and a meat fat extract analysed in MS-MS mode that demonstrates both quantitative accuracy and reproducibility for more than 60 agrochemicals spiked into the extract samples at concentration levels ranging from 0.2–50 ppb.

Keywords: Food, Pesticides, GC-MS/MS, Back flush

Dr Jim Garvey, Department of Agriculture, Fisheries and Food, Dublin, Republic of Ireland

E-8

FAST ANALYSIS OF MULTIPLE PESTICIDE RESIDUES IN APPLE JUICE USING DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AND MULTIDIMENSIONAL GAS CHROMATOGRAPHY- MASS SPECTROMETRY

S. C. Cunha[1]*, J. O. Fernandes[2], M. B. P. P. Oliveira[3]

[1 2 3] REQUI*M*TE, Department of Bromatology, Faculty of Pharmacy, University of Porto, Rua Aníbal Cunha 164 4099-030 Porto, Portugal
* Corresponding author–E-mail: sara.cunha@ff.up.pt; Phone: 00351222078910

Dispersive liquid-liquid microextraction (DLLME) recently proposed by Berijani *et al*. (2006) proved to be a fast, inexpensive and effective sample preparation technique for the analyses of many compounds including some class of pesticides. In this work, a new method based in DLLME-MDGC/MS is presented for the determination of multi pesticide residues in fruit juices. Several parameters of the extraction procedure such as type and volume of extraction solvent, type and volume of dispersive solvent and addition of salt were evaluated to achieve the highest yield and the lowest detection limits. The DLLME procedure optimized consists in the formation of a cloudy solution promoted by the fast addition to the sample (5 ml) of 500 µl of a mixture of carbon tetrachloride (extraction solvent, 100 µl) and acetone (disperser solvent, 400 µl). The micro-droplets formed and dispersed among the aqueous sample solution are further joined and sedimented (85 µl) in the bottom of the conical test by centrifugation.

Despite a slightly decreasing in the enrichment factor acquired, the use of a larger volume of the solvent than proposed by previous works, allows an increased the repeatability and extraction efficiency thus better instrumental signals. Once extracted, all the 24 pesticide were directly injected and separated by a dual GC column system, comprising a short wide-bore capillary column with low film thickness (5 m ×x 0.32 mm i.d., 0.1 µm) connected by a pressure-adjusted continual flow type switching device (Deans switch) to a second chromatographic column (15 m × 0.25mm i.d., 0.25 µm), with identical stationary phase (DB-5). The instrumental setting used, in combination with carefully optimized operational fast GC and mass spectrometry (MS) parameters, markedly decreased the retention times of the targeted analytes, the total chromatographic run being 8 min. Mean recoveries for apple juice spiked at three of concentrations ranged from 60 to 105% and the repeatability from 1 to 21%. The limit of detections of the 24 pesticides ranged from 0.06 to 1.30 µg/l.

Reference:
S. Berijani, Y. Assadi, M. Anbia, M.-R.M. Hosseini, E. Aghaee, J. Chromatogr. A, 1123 (2006) 1.

Keywords: DLLME, Pesticide multiresidues,MDGC/MS, juice

E-9

DEVELOPMENT AND APPLICATION OF A PESTICIDE LIBRARY FOR THE IDENTIFICATION AND CONFIRMATION ANALYSIS IN VARIOUS SAMPLE MATRICES BY LC/MS/MS

André Schreiber[1*], Lutz Alder[2]

[1] Applied Biosystems, Concord, ON, Canada
[2] Federal Institute for Risk Assessment, Berlin, Germany
[*] Corresponding author–E-mail: Andre.Schreiber@lifetech.com; Phone: +1 905 6609006

A new LC/MS/MS library containing more than 500 pesticides has been developed. Spectra were acquired using fast reversed phase LC coupled to a hybrid triple quadruple linear ion trap mass spectrometer. The MS/MS system was operated in Enhanced Product Ion scanning mode with standardized Collision Energy settings of 20, 35 and 50V and Collision Energy Spread of 35±15V. The acquisition and library search parameters have been optimized and then validated by library searching after injection of different dilutions and re-injection over a three months time period. Furthermore inter instrument repeatability was investigated.

Finally, the developed library was successfully used the screen food and drinking water samples for pesticides. A QuEChERS procedure was used to extract fruit and vegetable samples. Water was injected directly into the LC/MS/MS system. Multiple Reaction Monitoring (MRM) was used to screen for and quantify hundreds of targeted compounds. Traditionally the ratio of two MRM transitions is used for compound identification. However, the combination of selective and sensitive MRM detection and Enhanced Product Ion scanning with library searching allows screening for a larger panel of analytes and was able to reduce the number of false negative and false positive results. A similar experimental setup combining Enhanced MS with Enhanced Product Ion scanning can be used for General Unknown Screening. Further compound confirmation can be necessary using a different LC setup or GC analysis after extracting a second aliquot of the sample.

Keywords: LC/MS/MS, screening, spectra library

E-10
THE POSSIBILITY OF SOLID ALIQUOTS FOR QUECHERS EXTRACTION SALTS

Jack Cochran[1*], Neil Mosesman[2], Bill Grove[3], Brian Jones[4]

[1 2 3 4] Restek, Bellefonte, PA, USA
[*] Corresponding author–E-mail: jack.cochran@restek.com; Phone: 01-814-353-1300

The QuEChERS sample preparation approach is by now well used and several commercial vendors provide pre-packaged extraction salts and dispersive solid phase extraction sorbents. In some cases the extraction salts (magnesium sulfate, etc.) come in the 50 mL centrifuge tube used for extraction, which is inconvenient because the method specifies addition of homogenized sample and acetonitrile extraction solvent to that tube *prior* to salt addition. This necessitates a transfer of salts, from one tube to another, increasing the risk of leakage due to salts in cap threads, and possibly increasing plastic waste.

We have been exploring the use of "solid aliquots" for QuEChERS extraction salts to make the delivery of these salts even easier. Preliminary tests have been conducted using encapsulation, and by forming the salts (EN Method 15662 initially) into a tablet. Either method allows an easy delivery of extraction salts to sample/acetonitrile in a centrifuge tube, and decreases the risk of spillage and leakage. This presentation will focus on initial results achieved for QuEChERS extractions with solid aliquots, discussing features, benefits, and manufacturing difficulties.

Keywords: QuEChERS, solid aliquots, extraction, pesticides

E-11

LC-MS/MS ANALYSIS OF TRIAZINE PESTICIDES IN DRINKING WATER USING A NEW SOFTWARE FOR STREAMLINED METHOD DEVELOPMENT

Charles Yang[1], Jonathan Beck[2*], Jamie Humphries[3]

[1 2] Thermo Fisher Scientific, San Jose, USA
[3] Thermo Fisher Scientific, Austin, USA
[*] Corresponding author–E-mail: charles.yang@thermofisher.com; Phone: 9256280929

Introduction: A software program has been developed with built-in workflows for streamlining routine analysis in environmental and food safety labs. Incorporating a LCMS methods database that can customized by the user to include unique compounds, the analyst is able to access commonly encountered contaminants found in the environment. To demonstrate the software's capabilities, we analyzed a mixture of triazine compounds spiked into drinking water samples. 20mL direct injections (with online preconcentration) of the triazine samples were analyzed, allowing for the detection of low pg/mL (ppt) level concentrations.

Method: Water with 0.1% Formic Acid was spiked with a mixture of pesticides ranging from 1 to 100 pg/mL. 20 mL of the spiked water and blank samples of water were injected directly onto a loading column (Hypersil GOLD 20 × 2.1mm 12µ). After an appropriate time, depending on the volume injected, a multi port valve is switched to enable the load column to be back flushed onto the analytical column (Hypersil GOLD 50 × 2.1mm 3µ), where the compounds are separated prior to introduction into a triple quadrupole mass spectrometer. After all of the compounds are eluted, the valve is switched back to the starting position, the loading column is cleaned with a high organic phase and equilibrated, as is the analytical column.

Preliminary Data: The compounds analyzed include: Ametryn, Atraton Atrazine, Prometon, Prometryn, Secbumeton, Simetryn, Simazine, Terbutryn.. The response for all of the analytes in the mixture was linear over the range 1–100 pg/mL. In addition to the advantage of having an online sample preparation setup, the large injection volume allows for the pre-concentration of samples on the loading column, and the samples are then eluted onto the analytical column. This allows for excellent peak shapes for the analytes, which would not be attainable if the same injection volume was injected directly onto the analytical column. Furthermore, significant time savings are realized by this experimental setup versus off line SPE and concentration

Keywords: Pesticides, Online, SPE, Water, Environmental

E-12

INTRALAB VALIDATION OF UNI EN 15662 METHOD (DETERMINATION OF PESTICIDE RESIDUES IN FOODS OF PLANT ORIGIN) USING ASCENTIS EXPRESS RP AMIDE HPLC COLUMN ON LC-MS/MS AND CLEAN-UP BY DISPERSIVE SPE–QUECHERS-METHOD

Roberto Ferrari[1*], Enio Belotti[2], Luca Meni[3], Marco Ruggeri[4]

[1] Sigma aldrich srl, Milan, Italy
[2 3 4] Water&Life, Entratico (BG), Italy
[*] Corresponding author–E-mail: roberto.ferrari@sial.com; Phone: +39 3482314156; Fax: +39 0233417213

Recently, LC/MS/MS methods are proving to be very effective for the analysis of pesticides in food; several methods currently exist for the extraction and analyses of multi-residue pesticides from a variety of food matrices. A new method, known as the "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, has recently been introduced and subsequently improved. This method employs dispersive solid phase extraction (SPE) and chromatography with mass spectrometric detection (GC-MS or LC/MS/MS) techniques. This method recently became European Norm (EN 15662).

Most separation methods utilise C_{18} reverse phase HPLC columns. In this poster we describe an inter-lab validation procedure and its results using a new Fused Core™ Ascentis Express RP Amide HPLC column.

Ascentis Express RP-Amide columns provide a host of useful benefits that comes from both the phase technology and the particle technology. They comprise particles that have a solid core and a porous outer layer bonded with an embedded polar group (EPG) stationary phase on the surface. This results in a highly ordered packed column bed that has very significantly less diffusion, resulting in twice the efficiency compared to 3 µm columns. They also exhibit the same high speed and high efficiency of sub-2 µm particles but at a much lower backpressure, making these advances realisable for conventional HPLC instrumentation and UHPLC alike.

A total number of 190 pesticides divided into 7 mixes have been tested to develop and optimise the separation. For the validation of the method, a different representative fruit and plant origin matrix spiked with a mix of 29 pesticides has been chosen and follow the SANCO/3131/2007 document indication. "Method Validation and quality control procedures for pesticides residues analysis in Food and Feed.

Keywords: Pesticides residues, QuEChERS

E-13

MULTIRESIDUE ANALYSIS OF PESTICIDES IN VEGETABLES AND CITRUS FRUITS BY LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY

Pilar Flores[1*], Pilar Hellín[2], José Fenoll[3]

[1 2 3] Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario, IMIDA, C/ Mayor s/n, La Alberca 30150 Murcia, Spain
[*] Corresponding author–E-mail: mpilar.flores@carm.es; Phone: 34 968366804; Fax: 34 968366792

In this work, an analytical multiresidue method using liquid chromatography tandem-mass spectrometry (LC-MS-MS) with triple quadrupole in multiple reaction monitoring (MRM) mode for the simultaneous determination of 54 pesticides in vegetables (pepper and tomato) and citrus fruits (orange and lemon) has been developed. The procedure involves initial single phase extraction of sample with acetonitrile by agitation, followed by liquid-liquid partitioning formed by addition of NaCl. The average recovery by the LC-MS-MS method obtained for these compounds varied from 65.5 to 114.5% with a relative standard deviation between 2.3 and 7.4%. The method presents good linearity over the range assayed 10–500 µg/L (except famoxadone 50–1000 ↕ µg/L) and the detection and quantification limits for the pesticides studied varied from 0.03 to 14.9 µg/kg and from 0.1 to 49.7 µg/kg, respectively. The proposed method was used to determine pesticides levels in vegetables and citrus fruits samples from two experimental orchards and two greenhouses.

Keywords: Residues, pepper, tomato, orange, lemon

E-14

MONITORING OF PESTICIDE RESIDUES IN NORTH HUNGARIEN REGION BY SCREENING OF MORE THAN 250 COMPOUNDS IN FRUIT AND VEGETABLE SAMPLES USING VARIAN INSTRUMENTS

Kadenczki Lajos[1*]

[1] Agricultural Office of B.A.Z. County, Plant Protection and Soil Conservation Directorate
Pesticide Residue Analytical laboratory
[*] Corresponding author: Henriette Dobrik Szemáné; E-mail: kadenczki.lajos@borsod.ontsz.hu;
Phone: 00/36/46/503-419; Fax: 00/36/46/503-419

QuEChERs method (MSZ EN15662:2008) was introduced to our laboratory in 2006 in order to determine pesticide residues in fruit and vegetable samples.

In Hungary almost 250 componds are registered. In the past 2–3 years about 100 pesticides were drawn back by the registration authority. Due to free trade of products (after Hungary had become an EU member state) about another 50–60 compounds are entered to the country by import fruits and vegetables. Their screening is also necessary, which means, that about 400–410 compounds should be analyzed in order to completely cover those pesticides, which can be found in the samples either they are import agricultural products or due to the Hungarian agricultural practice.

QuEChERs is an efficient, cheap and quick method for the determination of pesticide residues. Using LC/MS/MS triple-quadrupol and GC/MS/MS triple-quadrupol it is particularly effective, fortunately our laboratory achieved both in 2007. With these Varian instruments we are able to screen more than 250 pesticides. About another 100 compounds are determined by dual GC/ECD, and GC/PFPD. So about 100 compounds are analysed more than one system.

A mixture of representative compounds is used in each sample set in order to control the method. This mixture contains 31 different pesticides which covers all types of pesticides to be analyzed. PF-38 and Triphenyl-phosphat are used as ISTD, its grate advantage is that it can be analyzed by all kinds of detection techniques.

Our poster presents the method in details as well as the results of the national monitoring program and import sample analyses during 2008.

Keywords: pesticides, residues, determination

E-15

HIGH SENSITIVITY MULTI-RESIDUE PESTICIDE ANALYSES IN FOODS USING THE TSQ QUANTUM GC-MS/MS

Richard Fussell[1], Mike Hetmanski[2], Michal Godula[3*]

[1 2] Food and Environmental Research Agency, Sand Hutton, York, YO41 1LZ, UK
[3] Thermo Fisher Scientific Praha, Slunecna 27, 100 00 Praha 10, Czech Republic
[*] Corresponding author–E-mail: michal.godula@thermofisher.com; Phone: +420777114430

GC-MS/MS was evaluated for the multi-residue analysis of approximately 100 pesticides in various matrices. Samples were extracted and cleaned-up using the QuEChERS procedure[1]. The samples were extracted with acetonitrile in the presence of magnesium sulfate, sodium chloride, disodium hydrogen citrate and trisodium citrate. Extracts were then cleaned-up by dispersive SPE using magnesium sulfate, and PSA.

A TSQ Quantum GC-MS/MS system was used for this study. The system comprised a Trace GC Ultra gas chromatograph equipped with a TriPlus autosampler interfaced with a TSQ Quantum triple quadrupole MS/MS detector operated in EI mode.

The pesticides evaluated included captafol, captan, chlorothalonil, dichlofluanid, dicofol folpet and tolylfluanid. These compounds have caused analytical problems, especially with GC-MS analyses of sample extracts produced by the QuEChERS procedure. The use of PTV, backflush and H-SRM (Highly-Selective Reaction Monitoring) techniques were also evaluated. The data from the optimized GC-MS/MS methodology was also directly compared to data derived from analysis of the same samples by “conventional” methodology (e.g. acetone or ethyl acetate-based extraction and single quadrupole GC-MS); to assess detection and quantitation limits, reproducibility and robustness for both analytical approaches.

[1] http://www.quechers.com

Keywords: pesticides, TSQ QUantum GC-MS/MS, QuEChERS

E-16

VALIDATION OF AN OFF LINE SPE LC-MS/MS METHOD FOR THE DETERMINATION OF SYSTEMIC INSECTICIDE RESIDUES IN HONEYBEE AND POLLEN SAMPLES COLLECTED IN APIARIES FROM NW SPAIN

María García-Chao[1], María Jesús Agruña[2], María Llompart[3], Thierry Dagnac[4*]

[1 2 4] Galician Institute for Food Quality (INGACAL), Agronomic and Agrarian Research Centre (CIAM), A Coruña, Spain
[3] University of Santiago de Compostela, Department of Analytical Chemistry, Santiago de Compostela, Spain
[*] Corresponding author–E-mail: thierry.dagnac@xunta.es; Phone: +34 981 64 79 02

The use of pesticides to protect crops against plagues and insects is one of the most important ways to assure agricultural quality and productivity. However, bad application practices may cause the contamination of different environmental compartments and animal species, as a consequence of migration or accumulation of those compounds. Fipronil, imidacloprid and thiametoxam are systemic or systemic-like insecticides widely used in maize crops. Their heavy action in the nervous system of target insects also means a high toxicity to non target pollinator insects such as honey bees which can get in touch with them through pollen and nectar during foraging activities. These insecticides have even been suspected to cause a significant decrease of honeybee colonies that has been observed in many countries since the past decade. Since September 1st 2008, the European Commission set new MRLs in food and feed of plant and animal origin. The pesticides included in this study have MRLs in honey and pollen between 10 and 50 ng/g. In the present work, an analytical method was developed with the aim of determining residues of fipronil and some of its metabolites (fipronil sulfone, fipronil sulfide, fipronil desulfinil and fipronil carboxamide), thiamethoxam and imidacloprid in honey and pollen samples. The extraction optimization was performed using a Doehlert experimental design by studying two factors, the mixture and the ratio of solvents used. Prior to the extraction procedure, raw hive samples containing honey, pollen and wax were centrifuged at 4000 rpm. The upper solid material was removed, and 1 g of the lower phase was mixed with 3 mL of the optimized mixture of methanol/water (20/80). The extract was passed through a florisil cartridge and the target compounds were eluted with methanol and analyzed by LC-MS/MS in selective reaction monitoring (SRM) mode. The method was validated according to the Commission decision 2002/657 and the ISO 11843 standard for the following parameters: recovery, repeatability and reproducibility at 0.5, 1 and 1.5 the MRLs, decision limit (CCalpha) and detection capability (CCbeta). Ion suppression/enhancement effects into the ion source were also assessed. The limits of detection were included between 1 and 5 ng/g. The validated method was applied to the determination of the target pesticides in 120 samples collected in colonies from 90 apiaries of NW Spain (two sampling campaigns during 2008). None of the target insecticides were detected in any of the samples. This study was included in a global project aiming at assessing the colony collapse disorder of honeybees in Galicia.

Keywords: insecticides, honeybees, LC-MS/MS, method validation

This study was funded by the Agricultural Ministry of the Autonomic Government of Galicia.

E-17
FAST GC-TOF FOR MULTI-RESIDUE ANALYSIS OF PESTICIDES: TAKING A SECOND LOOK

Andrew Wyeth[1], Karen Inwood[2], Sujot Babbra[3], John Points[4*]

[1 2 3 4] LGC, Teddington, UK
[*] Corresponding author–E-mail: john.points@lgc.co.uk; Phone: +44 208 943 8429

The regulatory analysis of pesticide residues requires generic multi-analyte methods that are fully quantitative, provide unequivocal confirmation of identity, achieve 0.01 mg/kg detection limits in a wide variety of different test matrices, and comply with the requirements of SANCO/3131/2007 for all analytes within the quantitative scope. A number of methods are in use in different laboratories, using different mass spectrometry techniques.

A method is described using fast gas-chromatography with time-of-flight mass spectrometry. This technique has a number of benefits; it is generic (250+ residues included); sufficiently sensitive; rugged; does not rely on complicated pre-set parameters or acquisition "windows"; rapid; uses relatively inexpensive equipment in comparison to other advanced MS techniques; and collects full MS spectra thus allowing the use of deconvolution and library matching software. The method has been in routine use in our laboratory for UK regulatory testing for two years, and our experiences are described.

An additional advantage of GC-TOF is the ability to retrospectively mine data for evidence of novel analytes. A real example is described. Following a RASFF notification, historical chromatographic data was re-examined for evidence of a previously unsought residue, and clear evidence of its presence was retrospectively found.

Keywords: time-of-flight, multi-residue, pesticides

E-18

USING Q-TRAP INSTRUMENT FOR FINDING, IDENTIFYING AND STRUCTURAL ELUCIDATION OF PESTICIDE METABOLITES IN PESTICIDE TREATED PLANT PRODUCTS

Martin Dušek[1*], Miloslav Šanda[2], Petr Cuhra[3], Sona Baršová[4]

[1 3 4] Czech Agriculture and Food Inspection Authority (CAFIA), Prague, Czech Republic
[2] Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic
[*] Corresponding author–E-mail: martin.dusek@szpi.gov.cz; Phone: +420257199549

Pesticides which are applied as a pre and post harvest treatment of fruit and vegetables are usually biochemically modified or degraded through specialized enzymatic systems. These biotransformation reactions are known as Phase I and Phase II reactions. A common Phase I oxidation involves conversion of a C-H bond to a C-OH, while Phase II reactions — usually known as conjugation reactions — involve an interaction of the polar functional groups of Phase I metabolites with other components of the plant, e.g. typically with sugars accompanied by creation of O-glycosides.

A common strategy for metabolite analysis consists of using two mass spectrometry (MS) systems, a triple quadrupole MS system to identify metabolites, and a separate ion trap MS system to characterize the metabolites. In this scenario the hybrid triple quadrupole linear ion trap (Q-TRAP) has been used for metabolite analysis instead of two separated systems.

The combination of the MS/MS neutral loss scanning mode and collection of EPI spectra has been used for finding of O-glycosides in pesticide treated plants. The EPI spectra were triggered by the neutral lost scan mode searching for the mass lost corresponding to the lost of glucose molecule. Collected EPI spectrum shows fragmentation pattern of parent O-glycoside (Phase II metabolite) which involves oxidized molecule of pesticide (Phase I metabolite). Thus O-glucoside metabolites can be positively identified via EPI spectra. In some cases, introducing of a hydroxyl group into the pesticide molecule can be observed in different positions. The mass weight of such Phase I metabolites is therefore identical and thus, in some cases, it is possible to find more than one compound with the same or almost the same EPI spectrum in the pesticide treated samples. Characterizing the position of the substitution of C-H bond at the molecule with C-OH bond — the structural elucidation these Phase I metabolites — has been realized by combination of MS/MS/MS scan mode with the collection of IDA-triggered EPI spectra.

Keywords: pesticide metabolites, glucose conjugates, Q-trap

E-19
GCMS ANALYSIS OF PESTICIDES IN GRAPES USING QUECHERS SAMPLE EXTRACTION

Anila Khan[1], Rob Bunn[2], Ruth Lewis[3], Luisa Pereira[4], Paul Humphrey[5*]

[1 2 3 4] Thermo Fisher Scientific, Runcorn, UK
[5] Thermo Fisher Scientific, Hemel Hempstead, UK
[*] Corresponding author–E-mail: paul.humphrey@thermofisher.com; Phone: +44-1442-233-555; Fax: +44-1442-233-667

Introduction: The method uses QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) which is a dispersive SPE method for extracting multi-residue pesticides from fruits and vegetables. The advantages of this methodology are speed, easy execution, minimal amount of solvents required and importantly its economical when compared to conventional solid phase extraction.

The aim of the work presented in this poster is to demonstrate that QuEChERS sample preparation gives high recoveries and is reproducible. Additionally, GC column selection is studied by comparing the method performance of a generic 5% phenylsiloxane phase column with a pesticide dedicated column.

Methodology: The pesticides are a mixture of organophosphate, organochlorine, pyretheroid, benzenoid, triazole and dicarboximide compounds.

The QuEChERS procedure usually involves two simple stages: sample extraction followed by dispersive SPE. An extra clean up step, which includes solvent exchange, is introduced to enhance the chromatographic separation.

The analysis is performed on a 5% phenyl polysilphenylene-siloxane phase column 30 m × 0.25 mm × 0.25 µm connected to a 5 m × 0.25 mm guard column via a glass fitting which is leak-free, and also on a pesticide column 30 m × 0.25 mm × 0.25 µm with integral 5 m guard.

Calibration curves are constructed to determine method linearity on each separation column. The analysis was performed on a GC fitted with a single quadrupole mass spectrometer, and detection was in positive Selected Ion Monitoring (SIM) mode.

Preliminary data: The quantitation of pesticides was based on triphenylphosphine serving as the internal standard. The correlation coefficients for all eight pesticide residues were higher than 0.99. Pesticide recoveries were in the range of 76–110%, with an average RSD of 11.0%. The application specific column showed better performance for the organochlorine pesticides than the generic column.

Keywords: SPE, GCMS, pesticides, QuEChERS

E-20
DETERMINATION OF CARBAMATE RESIDUES IN MANGOSTEEN USING MODIFIED QUECHERS ANALYSIS BY LC-MS/MS

Wanisa Meecharoen[1], Nuansri Tayaputch[2], Natchanun Leepipatpiboon[3*]

[1 3] Chromatography and Separation Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.
[2] Central Laboratory (Thailand) Co. Ltd., 50 Phaholyothin Rd., Ladyao, Jatujak, Bangkok 10900, Thailand
[*] Corresponding author–E-mail: natchanun.l@chula.ac.th; Phone: +662 2187608; Fax: +662 2541309

The mangosteen (*Garcinia mangostana* L.) is a tropical fruit in South East Asia, it is known as the "Queen of fruit". Chemical residue analysis of mangosteen followed the exporting standard is difficult due to interfere by dark colored and phenolic components in the fruit peel. A dispersive solid phase extraction method was successfully optimized for the removal of these interferences for carbamate residues analysis by LC-MS/MS. Extraction parameters such as organic solvent, buffer, and sorbent types were evaluated. 10 g of mangosteen sample were extracted with acetonitrile (10 mL) in combination of sodium chloride, anhydrous magnesium sulfate and sodium acetate (1:4:1 g) clean up with mixed sorbent of PSA and alumina (1:1) provided optimum clean up. Method performance for the analysis of carbamate pesticides are: limit of detection 0.005 mg/Kg, limit of quantification 0.01 mg/kg, recoveries 66–116%, RSD 9–19%. The repeatabilities typically $<$ 20% have been achieved for range 0.01–0.10 mg/Kg fortified level, which exceeded the benchmark parameters of Directive EC 396/2005.

Keywords: Pesticide residues, Carbamate, Mangosteen, QuEChERS

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E-21

APPLICATION OF GRAPHITIZED CARBON BLACK CLEANUP FOR GC/MS/MS AND UPLC/MS/MS MULTIRESIDUE ANALYSIS OF PESTICIDES IN GREEN LEAFY VEGETABLES

Stanislaw Walorczyk[1*], Dariusz Drozdzynski[2]

[1 2] Institute of Plant Protection–National Research Institute, Poznan, Poland
[*] Corresponding author–E-mail: s_walorczyk@tlen.pl; Phone: +48 61 864 91 81; Fax: +48 61 864 91 80

A multi-residue method has been developed for the trace determination of 162 pesticides in green leafy vegetables and others high in natural pigments by using a modified quick, easy, cheap, rugged, effective and safe (QuEChERS) method. The effectiveness of different amounts of graphitized carbon black (GCB) on the clean-up was evaluated applying increasing amounts of GCB (5–50 mg) to purify spiked extracts. A compromise between effective cleanup (less coloured extract) and satisfactory recoveries was achieved for the content of GCB between 10 to 15 mg per 1 mL of acetonitrile extract. For higher GCB contents, adsorption of susceptible pesticides such as chlorothalonil, cyprodinil, fenazaquin, mepanipyrim, pirymethanil, quinoxifen, quintozene, thiabendazole was observed. The final extracts were analyzed by gas chromatography/tandem mass spectrometry (GC/MS/MS) and ultra performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS). A high degree of confidence in the identification of pesticide residues was achieved by entering two multiple reactions monitoring (MRM) transitions per compound, following recent EU guidelines (SANCO/2007/3131). Validation studies were carried out on lettuce and recovery data were also generated for other matrices during routine use of the method. The recovery and RSD results satisfied the EU criteria (i.e. average recoveries were in the range 70–120% with RSDs $\leq$ 20%) for 114 and 153 of the target pesticides at the 0.005 and 0.01 mg/kg spiking level, respectively, and for all the pesticides but captan, clomazone and methiocarb-sulphon at higher spiking levels. The developed methodology was applied to the determination of pesticide residues in a variety of samples comprising lettuce, cabbage, leek, green beans, spinach, fennel, rucola, parsley leaves, broccoli, chicory and chives, of which approximately 30% contained pesticide residues. A total of 34 different pesticides were determined at concentration levels ranging from 0.006 mg/kg (boscalid) to 2.3 mg/kg (chlorotalonil). Most frequently found pesticides were procymidone, cypermethrin, chlorpyrifos, cyhalothrin-lambda, azoxystrobin and cyprodinil.

Keywords: Pesticide, Chromatography, Mass spectrometry, Vegetables

E-22

VALIDATION AND UNCERTAINTY ANALYSIS OF 186 PESTICIDE RESIDUES IN HAZELNUT BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

Muammer Kaplan[1*], Elmas Oktem Olgun[2]

[1 2] TUBITAK Marmara Research Center Food Institute, Gebze, Kocaeli, Turkey
[*] Corresponding author–E-mail: muammer.kaplan@mam.gov.tr; Phone: +90-262 6773241; Fax: +90-262 6412309

Hazelnut (Corylus avellana L.) is an important industrial commodity. It is high in oil content (60-70%) and represents high oil commodities. A multi residue method based on the modified Quick Easy Cheap Effective Rugged and Safe (QuEChERS) sample extraction was developed for determination of pesticides in hazelnut samples using LC-MS/MS separation technique. The electron spray ionization mass spectrometric detection in the selected ion monitoring mode using one target and one qualifier ions for the routine analysis of 186 pesticides in hazelnut has been developed. Chromatographic conditions were optimized to achieve quantitation and confirmation of 186 pesticides, isomers or degradation products, belonging more than 40 different chemical classes, by LC-MS/MS. The validation study was carried out on hazelnut matrix following DG SANCO/2007/3131. Linearity was studied in the 0.5–2 MRL concentration range. The calibration curve was established by the analysis of each pesticide at seven calibration levels that is 0.1, 0.2, 0.4, 0.6, 0.8, 1 and 2 MRL. The calibration curves were best fitted to a linear curve. The majority of the correlation coefficients were higher or equal to 0.98. The recoveries for all the pesticides studied were within the range of 60–125% at all the fortification levels. The calculated limits of detection for each pesticide ranged from 0.3 to 67 µg/kg. In this work, a “bottom-up” approach was used for the estimation of measurement uncertainty. Individual contributions of every step and input to the analytical method are estimated and the overall uncertainties were calculated following the recommendations of the EURACHEM/CITAC 2000 guide. The overall method performance was found to be satisfactory. The validated method is currently being used in routine analysis in our laboratory.

Keywords: pesticide, hazelnut, validation, uncertainty analysis,

E-23
MONITORING OF BIFENAZATE IN COMMERCIAL AGRICULTURAL PRODUCTS BY HPLC

Eun Heui Park[1*], Jin Ha Lee[2], Myong Shik Cho[3], Myoung Jin Go[4]

[1 2 3 4] Food & Drug Administration, Gwangju, Korea
[*] Corresponding author–E-mail: chemo815@kfda.go.kr; Phone: 82-62-602-1507; Fax: 82-62-602-1440

This study was carried out to develop the analytic method for bifenazate and monitoring in commercial agricultural products. The analytical method for bifenazate was established using a HPLC. The percentage recovery of bifenazate spiked in sample was found to be 70.1~96.9% and limit of quantitation (LOQ) for analysis method of bifenazate was 0.05 mg/kg. We validated the method for the linearity, the precision and the reproducibility. We investigated the residues of bifenazate using the developed method. We monitered in 260 commercial agricultural products(seventeen kinds of cereal grains, vegetables, beans, nuts, fruits and mushrooms) from seven metropolitan cities and nine provinces. Bifenazate was not detected at all. Also, analytical condition of LC/MS/MS were set up for bifenazate.

Keywords: bifenazate, HPLC, residues

E-24

CHEMICAL SCREENING OF CONTAMINANTS IN THE FOOD CHAIN USING LC- HIGH RESOLUTION ORBITRAP-MASS SPECTROMETRY

Paul Zomer[1*], Frans Schoutsen[2], Hans Mol[3]

[1 3] RIKILT – Institute of Food Safety, Wageningen, The Netherlands
[2] Thermo Fisher Scientific, Breda, The Netherlands
[*] Corresponding author–E-mail: paul.zomer@wur.nl; Phone: +31 317 487257; Fax: +31 317 417717

There are many different groups of toxicants which might be present in the food chain. Examples are pesticides, mycotoxins, plant toxins, and veterinary drugs. Until recently analysis methods only dealt with compounds within one of these groups but the last few years a trend towards methods covering different classes of residues and contaminants can be observed. Most of these wide-scope methods use LC coupled to full scan mass spectrometry as the separation and detection method. Because of the different properties of the many compounds to analyze for, a generic extraction method with little or no clean-up is necessary [1]. The resulting complex extracts are a challenge for the mass spectrometer to detect the compounds of interest at low (10–100 µg/kg) levels. In this work we explore the potential of a new, single stage, ultra-high resolution mass spectrometer (Exactive Orbitrap). It is demonstrated that the use of very high resolving power, up to 100,000 [FWHM], is required to obtain reliable and correct mass assignment (within 2 ppm) in the most complex extracts [2].

In chemical screening assays involving many hundreds of compounds data evaluation is typically done by automated library-based detection. Here the number of false negatives should be low (typically <5%). At the same time the number of false positive hits needs to be limited to the minimum because this means unnecessary confirmation work. In the poster, factors which have an impact on the number of (false positive) hits (e.g. retention time window, mass window, size of the database, use of fragmentation) will be discussed and screening results for a wide range of matrices with and without spiking with 150 analytes will be presented.

[1] Mol *et al*, Anal Chem, 80(24) 9450 (2008)
[2] Kellmann *et al*, J Am Soc Mass Spectrom. 20(8) 1464-76 (2009)

Keywords: Pestcides, Natural Toxins, Veterinary Drugs, full-scan, high-resolution

E-25
DETERMINATION OF PESTICIDES RESIDUE IN FRESH FRUITS AND VEGETABLES BY GC/MS

Biljana Marošanović[1*], Jelena Banić Simičić[2]

[1 2] SP Laboratorija, Bečej, Serbia
[*] Corresponding author–E-mail: splaboratorija@soyaprotein.com; Phone: +381 21 68 11 757; Fax: +381 21 69 12 545

SP Laboratory was formed at 1980. and acredited from Acreditation Body of Serbia (ISO 17025) at 2001. It is a commercial laboratory for food, feed, stuffs for general use analysis. SP Laboratory has acreditation for determination of pesticides residue in fresh fruits and vegetables.

During summer of 2009. more than 100 commercial samples of fresh fruits and vegetables (apple, raspberry, blackberry, plum, grape, peach, tomato, pepper, cucumber, lettuce, onion) made in Serbia, were analyzed on pesticides residue.

Determination of pesticides residue in fresh samples was analyzed by GC/MS. Pesticide screening of samples was made with RTL PEST 3 Library (Agilent) and quantitative analysis with reference standard supstances. Instrument for analysis was GC 6890N / MS 5975 by Agilent and capillary column HP5-MS (30 m $\times$ 0.250 mm $\times$ 0.25 µm).

Samples were preparated by liquid/liquid and solid phase extraction and reconstituted in acetone.

Validation of method was made for 49 organochlorine, 68 organophosphorus, 26 pyretroide, 6 synergist and 80 organonitrogen pesticide residues in fresh samples.

In samples 14 different pesticide residues were determinated, 60% of samples had more than one pesticide residue. About 70% pesticides residue in samples were organonitrogen fungicides, 20% were pyrethroid insecticides and 10% other contaminents.

Therefore, about 75% of analyzed samples from Serbian markets were without any pesticides residue.

Keywords: pesticides residue, fruits, vegetables, GC/MS

E-26

MULTI-RESIDUE METHOD FOR DETERMINATION OF 350 PESTICIDES IN FOOD EXTRACTS BY UTILIZING NEW GC-MS/MS TECHNOLOGY

Thierry Faye[1*], Joerg Riener[2]

[1 2] Agilent Technologies
[*] Corresponding author–E-mail: thierry_faye@agilent.com; Phone: +33643477118

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 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 food analysts. Their work is made even more difficult by the fact that traces of pesticides have to be determined in complex matrices. In this study a single method for detection, quantification, and confirmation of 350 pesticide residues in food extracts by the Agilent 7000 GC/MS/MS Triple Quadrupole coupled to a 7890A GC has been developed. For the majority (98%) of the pesticides at least two multiple reaction monitoring (MRM) transitions were set 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. A thoroughly optimization was performed for each analyte (Retention Time Locking, RTL) to achieve individual optimum collision energy voltages. A HP-5MS UI MS column (30 m × 250 µm × 0.25 µm) was used for the chromatographic separation with helium as carrier gas allowing an excellent separation of the analytes in a 41 minutes period. The time for sample preparation could be reduced significantly by usage of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) multi residue method for sample preparation. However, this saved time is nowadays spent in the evaluation of the huge amount of analytical data. Utilizing the features of the MassHunter software all the data necessary for performance and plausibility check are displayed in a simple way that gives the operator all information of the batch “at a glance”.

The developed method exhibited its versatility by its good linearity was linear in the range of 1–200 µg/kg, with correlation coefficients larger than 0.99. We found that pesticides could be identified using this high-throughput procedure with a limit of detection (LOD) in vegetable matrices of 0.01 mg/kg (ppm) or lower, which is the regulatory level for baby food and banned substances and is typically the most sensitive MRLs used by the European Union, on average the LOD was 0.5 µg/kg. This robust method enables to determine pesticides at low µg/kg (ppb) in food extracts with a simple clean up procedure.

Keywords: GC/MS/MS, triple quadrupole, pesticides, food

E-27

PESTICIDE MULTIRESIDUE METHOD IN FRUITS USING LC-MS/MS: ANALYTICAL AND REGULATORY ASPECTS

AN Cunha[1], MCC Silva[2], M Silva[3], HCMP Silva[4], EC Albuquerque Junior[5], DL Telles[6], ACP Araújo[7*]

[1 2 3 4 5 6 7] ITEP, Recife/PE, Brazil
[*] Corresponding author–E-mail: adelia@itep.br; Phone: 5581 32724314; Fax: 5581 32724313

Introduction: The LC-MS/MS allows the development of multiresidue methods, that is, the identification and quantification of a large number of compounds with a single chromatographic run. For analysis confirmation the following parameters have to be considered: retention time, parent ion, two fragmentation transitions and the ion ratio. Considering the number of pesticides and a large variety of matrices, monitoring and routine laboratories sometimes have to face analytical difficulties such as:

1) exotic tropical natural fruit interferences and few references available in the literature;
2) a need for monitoring metabolites which increases the list of pesticides in the method; ¨
3) pH interference in the integrity of the compound;
4) interference of a compound in the MRM of another compound leading to ion ratio values not acceptable; in this case, a single method with more than 2 transitions is needed for proper confirmation;
5) intrinsic properties of some molecules leading to poor reproducibility of the ion ratio,
6) need of improvement of National pesticide MRLs database for some pesticides.

Objective: In this work difficulties related to detection/confirmation of around 200 pesticides analysis in tropical fruits taking into account the actual analytical strategy in use in the laboratory were evaluated.

Methodology: The extraction was performed using acetone (Luke modified) followed by LC-MS/MS. Routine samples received in the laboratory such as mango, papaya, apple, grape and cashew were used for the development of this work.

Results and Discussion: The analysis of propiconazole in cashew, compound not authorized for this culture, could not be fully analyzed due to a natural interference of the matrix; various metabolites were introduced to follow legislation, some of them difficult to be purchased (piraclostrobin,dicofol,carbofuran, and others), leading to a considerable increase in the list of compounds analyzed; etrinphos rapidly degrades in the acid pH apples making it impossible the confirmation analysis; the presence of prochloraz and thiabendazole in the same sample, both authorized as post-harvest fungicides in mango and papaya demanded single methods for confirmation with more than two transitions; famoxadone, frequently detected in apples, papayas and grapes, showed lack of reproducibility during fragmentation. Moreover, taking the actual Brazilian Regulations some aspects of this analysis are of difficult interpretation; take the example of carbofuran detected in mangoes, papayas and grapes but not allowed for these cultures, nevertheless, carbofuran is the main metabolite of carbosulfan, which has established MRLs for these same cultures.

Keywords: LC-MS/MS, pesticide residue, tropical fruits

FINEP, CNPq

E-28
PROFICIENCY TESTING FOR DETERMINATION OF PESTICIDE IN PAPAYA PULP – 4TH ROUND

A. Nóbrega[1], I. H. P. Bastos[2*], m. H. W. M. Cardoso[3], a. V. Gouvêa[4], m. H. De la cruz[5], m. H. P. Moraes[6], p. Santos[7], j. M. R. Caixeiro[8], v. S. Cunha[9], f. Violante[10]

[1 2 3 4 5 6] FIOCRUZ/INCQS – Department of Chemistry/Pesticide Residue Laboratory, Rio de Janeiro, Brazil
[7 8 9 10] INMETRO, Rio de Janeiro, Brazil
[*] Corresponding author–E-mail: lucia.bastos@incqs.fiocruz.br; Phone: 55-21-3865.5187

The results of a proficiency test patronized jointly by INMETRO and FIOCRUZ/INCQS is presented. The aim of the study was the determination of pesticide residues in papaya. The papaya was bought at local Rio de Janeiro market and after evaluation to absence of the pesticides studied the pulp was considered adequate for the purpose. Known amounts of endosulfan-sulfate, procymidone, fenitrothion, trifluralin and tetradifon as ethyl acetate solutions, were added to a puree prepared. Once fully homogenized, aliquots of the sample material were transferred to glass jars and they were sent to twenty-seven participating laboratories. Reference values for the concentrations of pesticides in the sample were assigned after analyzing statistically the analytical results for the reference material in agreement with ISO 13528. The results of the homogeneity and stability tests were evaluated according to ISO GUIDE 35. Samples were considered homogeneous and stable for the period studied. The evaluation of the results submitted by participating laboratories was performed in accordance with the guidance ISO / IEC Guide 43-1. Only twenty-five participating laboratories sent the results, twenty Brazilian laboratories and five Latin-American laboratories. Variation coefficients and z scores were calculated to measure the relative distance of laboratory results to the design value as well as to evaluate the analytical capacity of each participant. It was found that 75% of the reported results were satisfactory, 9% were questionable and 15% were unsatisfactory. Four laboratories did not detected, at least one of the analyzed pesticide. The results of this study indicated that efforts are needed to increase the quality of the measurements of pesticide residues, in papaya, performed by some of the participating laboratories. Problems were found particularly in which it concerns the qualitative identification of the spiked pesticides.

Keywords: pesticide residues, papaya, profficiency testing

FINEP

E-29
USE OF GC-QTOFMS TO IDENTIFY PESTICIDE RESIDUES IN COMPLEX MATRICES

Viorica Lopez-Avila[1*], George Yefchak[2]

[1 2] Agilent Technologies, Santa Clara, USA
* Corresponding author–E-mail: viorica_lopez-avila@agilent.com; Phone: 408-553-2709; Fax: 408-553-3677

This paper describes the use of a research GC-QTOFMS to identify pesticide residues using a combination of accurate mass measurements that have errors less than a few parts per million (ppm), isotope abundance ratios, and high resolution (>10,000). The accurate mass measurements were collected with a GC-QTOFMS comprising a GC, an electron ionization (EI) source from a low resolution MS, and a commercial Agilent QTOFMS that was upgraded with a 4 GHz data acquisition system. To implement EI, we removed the electrospray source and the first octapole assembly from the QTOFMS and replaced them with the EI source. The mass axis was calibrated using seven peaks from perfluorotributylamine (PFTBA) introduced via the calibration gas inlet) and a background phthalate ion at m/z 149.02332 was used as real time reference mass ("lock mass"). Octafluoronaphthalene (OFN, peak at *m/z* 271.9867) was used to establish mass accuracy, resolution and instrument sensitivity. The signal-to-noise for the OFN peak at 10 pg was 245 and a calibration curve covering a range of 1 to 1000 pg had a correlation coefficient of 0.995 and %RSDs of 19%, 18%, and 12% at 1, 10, and 100 pg per injection, respectively. An example will be presented for GC-QTOFMS analysis of 20 pesticides that were spiked into a citrus oil extract.

Keywords: GC-QTOFMS, accurate mass, pesticides

E-30

THE USE OF THE SCHEDULED MRM™ ALGORITHM TO EXTEND THE SCOPE AND INCREASE THE THROUGHPUT OF PESTICIDE SCREENING BY LC/MS/MS

Andre Schreiber[1*], Nadia Pace[2], Henri Snijders[3]

[1 3] Applied Biosystems
[2] MDS Analytical Technologies
[*] Corresponding author–E-mail: andre.schreiber@lifetech.com; Phone: +1-905-660-9006

Pesticide application on our food is a typical process used to prevent the destruction of crops by pests in order to increase crop yield. Unfortunately, however, after application of pesticides on food, pesticide residues can remain on food, soil and water which can, in turn, have harmful effects on the consumer. As a result, maximum residue levels have been established as a means to determine foods allowable for import and export. Testing these food products has become an important necessity. Hundreds and hundreds of pesticides are used around the world on crops. For this reason, there is a need to develop methods to accommodate for the analysis of such high numbers of compounds. For such methods, a targeted approach is ideal which includes all known pesticides in a single run. Here we present a high-throughput LC/MS/MS method that combines the high sensitivity MRM screening with fast EPI identification and allows detection of low concentration pesticides, with LOD below 0.1 µg/L. The developed LC/MS/MS method was used to monitor pesticides in various fruit and vegetable samples after simple QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction. Additionally, water samples were injected directly into LC/MS/MS. A reversed phase LC system using a polar modified column (Restek Ultra Aqueous C_{18}), a gradient of water/methanol with 10 mM ammonium acetate buffer, and a QTRAP® mass spectrometer with Electrospray Ionization (ESI), Multiple Reaction Monitoring (MRM) and Enhanced Product Ion (EPI) scan were used to detect pesticides. This set-up allows the simultaneous screening, quantitation and identification of hundreds of pesticides in food and water samples. In one single experiment more than 600 MRM transitions were monitored without cross talk using traditional MRM and Scheduled MRM™ functionality. The use of the Scheduled MRM™ algorithm greatly enhances accuracy and reproducibility of LC/MS/MS detection of a large set of targeted analytes at low concentration levels. In the same experiment fast confirmatory EPI scans were triggered by intelligent software using Collision Energy Spread (CES) settings. In comparison to fixed Collision Energies CES was found to give more reproducible and richer MS/MS spectra and thus greatly enhancing the quality of library searching. Library searchable EPI spectra were generated at concentrations as low as 0.1 µg/L.

Keywords: pesticides, Scheduled MRM, LC/MS/MS

E-31

OPTIMISATION OF ANALYSIS OF ACIDIC HERBICIDES IN CEREALS, EVALUATION OF VARIOUS MS DETECTION TECHNIQUES FOR QUANTIFICATION

Jana Urbanova[1*], Ondrej Lacina[2], Tomas Cajka[3], Vladimir Kocourek[4], Jana Hajslova[5]

[1 2 3 4 5] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: jana.urbanova@vscht.cz; Phone: +420220443272

Pesticides represent an important group of agrochemicals used for food crop protection, on this account, unavoidably, their residues can often be detected in treated crops at the harvest time. Regarding the high amount of registered active ingredients possessing different physico-chemical properties, not all of them can be included in multiresidue methods employed for their control. Acidic herbicides represent an example of such problematic class of pesticides. The definition of maximum residue limit often involves not only the free form but also its esters or conjugates. For this the reason extraction step has to be modified to release those bounded compounds.

This study deals with optimization of analysis of widely used acidic herbicides like 2,4-D, dicamba, dichlorprop, fluazifop, fluroxypyr, haloxyfop, MCPA, mecoprop etc. in cereals by high performance liquid chromatography coupled with mass spectrometry. Alkaline hydrolysis was involved to determinate sum of free and conjugated form, and then the QuEChERS [1] based extraction procedure followed. With respect to the carboxylic group in the molecular structure of tested herbicides, pH value of matrices before extraction had to be carefully decreased because it significantly influences recoveries. Performance characteristics were evaluated in various mass analyzers, (*i*) triple quadrupole Quattro Premier XE (Waters, USA), (*ii*) quadrupole-linear ion trap QTRAP 5500 (Applied Biosystems, USA), (*iii*) time-of-flight LCT Premier XE (Waters, USA) and (*iv*) orbitrap Exactive (Thermo Scientific, USA). Since the group of examined pesticides ionises both in positive and negative mode of electrospray thus possibility of polarity switching was also tested and the impact on detection limits within a single run was assessed.

[1] Anastassiades M, Lehotay S, Stajnbaher D, Schenck F: Journal of AOAC 2, 86 (2003)

Keywords: mass spectrometry, acidic pesticides, cereals

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E-32

ROUTINE MULTIRESIDUE ANALYSIS OF PESTICIDES IN CEREALS AND FEEDINGSTUFFS USING ACETONITRILE EXTRACTION AND GC/MS/MS – INTERPRETATION OF THE RESULTS

Stanislaw Walorczyk[1*], Dariusz Drozdzynski[2], Boguslaw Gnusowski[3]

[1 2 3] Institute of Plant Protection–National Research Institute, Poznan, Poland
[*] Corresponding author–E-mail: s_walorczyk@tlen.pl; Phone: +4861 8649181; Fax: +4861 8649180

The presentation will show results of the routine application of a previously developed multiresidue method for determining pesticides in cereals and dry animal feeds [1–2]. Recently, the scope of the method was extended to 154 pesticides in a single injection gas chromatographic-tandem quadrupole mass spectrometric acquisition method (GC-MS/MS). The pesticides were extracted by using a modified buffered QuEChERS method then cleaned-up by dispersive solid phase extraction (dispersive-SPE) with PSA and C18 sorbents and optionally by a freezing-out clean-up step. The method was originally validated on wheat grain but during the routine use the recovery data for other matrices have been generated on an on-going basis. Currently, the work is underway to add some new pesticides to be analyzed by ultra performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) as a complementary technique. Up to now, the developed methodology was applied for the determination of pesticide residues in 464 samples encompassing cereals grains, bran, whole ears, straw, soya beans and some mixtures and compound feeds. Of the samples analyzed, approximately 20% contained pesticide residues at concentration levels ranging from 0.01 mg/kg (pirimiphos-methyl, tebuconazole, malathion and bifenthrin) to 6.5 mg/kg (pirimiphos-methyl). A total of 27 different pesticides were determined, of which most frequent were: pirimiphos-methyl (30 cases), tebuconazole (17 cases), deltamethrin (8 cases) and malathion (8 cases). Persistent environmental pollutants such as DDT and lindane were determined in 3 and 1 sample, respectively. In this poster, validation data, quality control data and the summary of results along with exposure assessment will be presented.

[1] S. Walorczyk J. Chromatogr. A, 1165 (2007) 200-212.
[2] S. Walorczyk J. Chromatogr. A 1208 (2008) 202-214.

Keywords: pesticide, cereals, feed, GC/MS/MS

E-33

FAST PTV–GC–HRTOFMS METHOD FOR THE CONTROL OF MULTIPLE PESTICIDE RESIDUES WITH THE FOCUS ON THOSE NOT AMENABLE TO LC–MS ANALYSIS

Marketa Hakenova[1*], Tomas Cajka[2], Petr Dohnal[3], Jana Hajslova[4], Vladimir Kocourek[5]

[1 2 3 4 5] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: marketa.hakenova@vscht.cz; Phone: +420 220 444 372

Hundreds of pesticides have been registered for the protection of food crops during their growth and storage. Multiresidue methods are used to control maximum residue limits established for the particular pesticide/commodity combination. The aim of this study was to optimise the GC sample introduction procedure involving injection of the QuEChERS extracts without a solvent exchange. Programmed temperature vaporizing (PTV) injection was employed for this purpose in a solvent vent mode. A high-resolution time-of-flight spectrometer (HRTOFMS) was used for detection of particular analytes.

Although LC–MS based methods are currently used for determination of most of pesticides, there are several important pesticides that are not, or only poorly, ionisable in LC–MS (e.g. aldrin, dieldrin, α-endosulfan, β-endosulfan, endrin, HCB, hexachlorbenzen, α-HCH, β-HCH, γ-HCH, δ -HCH). Thus, GC procedure is the only viable option for their analysis.

The validation study of selected analytes was performed for several matrices with different water content (baby food, lettuce and flour) and was investigated at two different concentration levels (0.01 and 0.1 mg/kg). Performance characteristics such as recovery, repeatability of the measurement, linearity of calibration, limit of detection and quantification were evaluated.

Keywords: pesticides, PTV, GC–HRTOFMS, multiresidue method

This study was carried out within the project NAZV 1G46073 supported by the Ministry of Education, Youth and Sports of the Czech Republic.

E-34

AN ALTERNATIVE APPROACH FOR THE DETERMINATION OF CAPTAN AND ITS DEGRADATION PRODUCT THPI (1,2,3,6-TETRAHYDROPHTHALIMIDE)

Sarka Prinosilova[1*], Marketa Hakenova[2], Tomas Cajka[3], Petr Dohnal[4], Jana Hajslova[5], Vladimir Kocourek[6]

[1 2 3 4 5 6] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: sarka.prinosilova@vscht.cz; Phone: + 420 220 444 347

Captan, a non-systematic fungicide is widely used to protect a variety of crops. Alike similar fungicide, folpet, this *N*-trihalogenmethylthio compound is relatively easily degraded yielding tetrahydrophthalimide (THPI) as a major breakdown product [1].

Currently, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) represents the most common approach in the analysis of pesticide residues, nevertheless the LC–MS/MS cannot be used for the control of crops treated with fungicide captan because of its poor ionization in an atmospheric pressure ionization (API) interface and, consequently, high detection limit. On this account, GC–MS remains the only viable option in captan residue analysis. However, captan is somewhat thermally unstable and its decomposition may easily occur under the conditions of hot splitless injection [2]. The extent of this phenomenon largely depends on the type of sample matrix and also on injection port contamination.

The possibility to improve accuracy and decrease detection limit of captan by the implementation of programmable temperature vaporization (PTV) injection was tested. The possibility to determine THPI (breakdown product) together with captan in treated fruits was demonstrated, too. Since fairly better performance characteristics were observed for THPI the possibility to convert the parent compound into this marker compound prior to the analysis by means of pH value changes was investigated. This proposed approach should be applied in the routine GC analysis of captan/THPI. In the case of positive samples containing higher concentration of THPI, an additional analysis employing ethyl acetate extraction followed by gel permation chromatography is required to determine the parent compound concentration.

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[2] The e-Pesticide Manual (Twelfth Edition) Version 2.2, CDS Tomlin

Keywords: captan, degradation, GC/MS, PTV, THPI

This study was carried out within the project NAZV 1G46073 supported by the Ministry of Education, Youth and Sports of the Czech Republic.

E-35

LC-MS/MS METHOD FOR SIMULTANEOUS DETERMINATION OF MULTIPLE PESTICIDE RESIDUES AND MAJOR GROUPS OF MYCOTOXINS IN CEREALS

Ondrej Lacina[1*], Jana Urbanova[2], Marta Kostelanska[3], Alexandra Malachova[4], Jana Hajslova[5]

[1 2 3 4 5] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: ondrej.lacina@vscht.cz; Phone: +420-220 443 272

In the recent decade, liquid chromatography–tandem mass spectrometry (LC-MS/MS) operated in a multiple reaction monitoring (MRM) mode, has become the main tool in the analysis of food and environmental contaminants represents various structure classes. This approach allowed the introduction of multiresidue methods with up hundreds target analytes determined in a single run. However, most of these methods are focused only on one group of food contaminants such as pesticides, veterinary drugs, mycotoxins, plant toxins, etc. Considering the fact, that sample preparation prior LC-MS/MS detection are in principle similar for most of these methods, we attempted to determine pesticide residues and mycotoxins – analytes that although of fairly different origin, possess similar range of physico-chemical properties.

To detect all analytes in single run, several optimizations and compromises have to be done. No clean-up step was employed to cover analytes with wide range of physico-chemical properties, so in a few cases extensive matrix effects were observed. Some problems were evaluated with ionization of analytes, because majority of pesticides as well as aflatoxins and fumonisins, ionize in a positive electrospray mode (ESI+) only. On the other hand, B-trichothecenes are usually analyzed under negative ionization in a form of acetate or formate adducts. Although far from best conditions, the B-trichothecenes provide ions also in ESI+. Moreover under these conditions is possible to distinguish totally co-eluted isomers 15-acetyldeoxynivalenol and 3-acetyldeoxynivalenol in one run. The final comprehensive LC-MS/MS multi-residue method was validated for 17 *Fusarium* toxins, 4 aflatoxins, ochratoxin A, sterigmatocystin, 4 ergot alkaloids, 3 *Alternaria* toxins and over 250 pesticides and their degradation products. Thanks to high sensitivity of QTRAP 5500 tandem mass spectrometer, the detection limits (LOD) of all analytes are below EU MRL limits. A new algorithm for optimization of detection analytes in multiresidue target analysis called Scheduled MRM™ was used. This automatically associates retention times with MRM transitions and creates an optimized acquisition method to maximize dwell times (time spent acquiring the specific MRM transition) and cycle time (number of acquired points per peak).

Keywords: mycotoxines, pesticide residues, cereals, LC-MS/MS

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E-36

DEVELOPMENT AND APPLICATION OF A PESTICIDE LIBRARY FOR THE IDENTIFICATION AND CONFIRMATION ANALYSIS IN VARIOUS SAMPLE MATRICES BY LC/MS/MS

André Schreiber[1*], Lutz Alder[2]

[1] Applied Biosystems, Concord, ON, Canada
[2] Federal Institute for Risk Assessment, Berlin, Germany
[*] Corresponding author–E-mail: Andre.Schreiber@lifetech.com; Phone: +1 905 6609006

A new LC/MS/MS library containing more than 500 pesticides has been developed. Spectra were acquired using fast reversed phase LC coupled to a hybrid triple quadruple linear ion trap mass spectrometer. The MS/MS system was operated in Enhanced Product Ion scanning mode with standardized Collision Energy settings of 20, 35 and 50 V and Collision Energy Spread of 35±15V. The acquisition and library search parameters have been optimized and then validated by library searching after injection of different dilutions and re-injection over a three months time period. Furthermore inter instrument repeatability was investigated.

Finally, the developed library was successfully used the screen food and drinking water samples for pesticides. A QuEChERS procedure was used to extract fruit and vegetable samples. Water was injected directly into the LC/MS/MS system. Multiple Reaction Monitoring (MRM) was used to screen for and quantify hundreds of targeted compounds. Traditionally the ratio of two MRM transitions is used for compound identification. However, the combination of selective and sensitive MRM detection and Enhanced Product Ion scanning with library searching allows screening for a larger panel of analytes and was able to reduce the number of false negative and false positive results. A similar experimental setup combining Enhanced MS with Enhanced Product Ion scanning can be used for General Unknown Screening. Further compound confirmation can be necessary using a different LC setup or GC analysis after extracting a second aliquot of the sample.

Keywords: LC/MS/MS, screening, spectra library

E-37

EVALUATION OF THE QUECHERS SAMPLE PREPARATION METHOD AND COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY FOR THE ANALYSIS OF PESTICIDES IN DIETARY SUPPLEMENTS

Jack Cochran[1*], Michelle Misselwitz[2], Julie Kowalski[3], Rick Lake[4]

[1 2 3 4] Restek, Bellefonte, PA
[*] Corresponding author–E-mail: jack.cochran@restek.com; Phone: 01-814-353-1300; Fax: 01-814-353-1309

Recently the US FDA announced that makers of dietary supplements (e.g. vitamins, herbal and botanical pills, etc.) will have to test their products for purity. Since dietary supplements contain natural products where pesticides have been employed, methods for pesticide analysis must be evaluated, including sample extraction and cleanup, and instrumental techniques such as gas chromatography. The QuEChERS approach offers a rapid way to prepare samples for analysis, but dietary supplement extracts can be so complex as to make trace-level pesticide determinations problematic.

An elegant way to solve complex separation problems is to use comprehensive two-dimensional GC (GC×GC). GC×GC is a way to increase peak capacity by applying two independent separations to a sample in one analysis. Typically GC×GC involves a serial column configuration (with different stationary phases) separated by a thermal modulator. Chromatography is performed on the first column, and then effluent from the first column is continually (and quickly) focused and injected onto the second column. By keeping the second column short, a series of high speed chromatograms are generated, and the first column separation can be maintained. Separation results are plotted as a retention plane (column 1 retention time × column 2 retention time). In this work, a combination of QuEChERS and GC×GC will be evaluated to determine spiked and incurred pesticides in dietary supplements.

Keywords: dietary supplements, pesticides, QuEChERS, GC×GC-TOFMS

E-38

AUSTRALIAN NATIONAL RESIDUE SURVEY – CASE STUDY PESTICIDE RESIDUE MANAGEMENT CONTINUUM MONITORING PROGRAM FOR GRAINS – A CASE STUDY

Ian Reichstein[1*]

[1] National Residue Survey, Australian Government Department of Agriculture, Fisheries and Forestry, PO Box 858, Canberra, ACT, 2601, Australia
[*] Corresponding author–E-mail: ian.reichstein@daff.gov.au; Phone: +61 2 62725668; Fax: +61 2 6272 4023

Australia exports a major portion of its agricultural production and is highly dependent on maintaining and developing access to, and competitiveness in, export markets.

The majority of Australian primary producers rely on pesticides to protect their crops from pests and diseases. The Australian Pesticides and Veterinary Medicines Authority (APVMA) supports Australian agriculture by registering and allowing the supply of safe and effective animal health and crop protection products.

The APVMA sets maximum residue limits (MRLs) based on good agricultural practice and with Food Standards Australia New Zealand (FSANZ) work together to ensure that the level of residues resulting from the use of chemical products are within health limits (ADI and ARfD).

A residue risk management continuum is established when the effectiveness of chemical registration and control of chemical use regulations is assessed through residue monitoring programs.

In the early 1960s, the Australian Government established a non-regulatory body, the National Residue Survey (NRS). In 2008–2009, random monitoring programs were conducted for over 50 commodities (21 grains, 5 horticultural commodities, 11 fish species, 12 animal species, honey and egg with over 20,000 samples collected for analytical testing.

The NRS grain residue monitoring program is presented as a case study of the residue risk management continuum demonstrating to overseas markets the high level of residue integrity of Australian grain. Over 4,000 grain samples are collected and analysed per annum.

The NRS grains program provides an independent verification of the residue status of exported and domestically traded Australian grain. NRS residue testing data collected over decades also provides long term trends demonstrating a decline in the frequency of residue detections and the levels of residue detected.

To be confident that residue testing results meet the requisite standards, the reliability of the Australian analyses must be assured. The NRS laboratory performance evaluation system has been developed to provide that assurance, using a range of proficiency tests and other techniques in the selection of laboratories for NRS work.

Residue testing results are reported against both Australian MRLs and international MRLs applying in the export market. NRS retains databases of overseas MRLs and compares its residue testing results of exported commodities against those standards. Grain marketers receive residue testing reports for each shipment prior to arrival at the overseas market.

Keywords: Australia, pesticide, residue, monitoring, export

E-39

COMPARISON OF MINIAUTURIZED EXTRACTION TECHNIQUES FOR THE ANALYSIS OF PESTICIDES IN AQUEOUS SAMPLES BY GAS CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

Antonia Garrido-Frenich[1], Jose Luis Martinez-Vidal[2], Rosa Martinez-Ocaña[3], P. Baquero-Feria[4], F.Javier Arrebola[5*]

[1 2 3 4 5] Research Group Analytical Chemistry of Contaminants, Department of Analytical Chemistry, University of Almeria, 04120 Almeria, Spain
[*] Corresponding author–E-mail: arrebola@ual.es; Phone: +34 950 015823; Fax: +34 950 015483

Determination of pesticides in food and water is relevant for consumer protection. The extraction procedure used in the analytical method requires special attention in order to reach properly the low limits of pesticides defined by the European legislation [1]. Miniaturised extraction techniques like solid-phase microextraction (SPME) and hollow fiber liquid phase microextraction (HF-LPME) provide a precise isolation of a wide range of pesticides in drinking water with simplicity and suitability, using a small amount of sample, without or with a minimal amount of organic solvents and reasonable enrichment factor [2,3].

Two new miniaturised methods have been developed, validated and compared using SPME and HF-LPME in combination with gas chromatography-triple quadrupole tandem mass spectrometry (GC-QqQ-MS/MS) for determining a total of 77 pesticides in drinking water.

In the case of SPME, extraction temperature and time were optimized by experimental design, although other parameters, as desorption time, pH and ionic strength were set up too. For the HF-LPME, the extraction and desorption solvents nature (octanol: dihexyl ether (75:25, *v/v*) and cyclohexane respectively), as well as the extraction and desorption time, ionic strength and pH, were studied. The proposed methods were totally validated (recovery, precision, lower limits, range of work, potential matrix effect, etc) and applied to the analysis of 41 different water samples (10 natural spring, 25 tap, and 6 commercial mineral water samples). No pesticides were detected above the allowed limits but some of the analyzed samples contained pesticide traces over the concentration of the first calibration level. In all the cases, similar results were achieved using the two compared methods.

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[2] F.J. Arrebola, S. Cortes Aguado, N. Sánchez-Morito, A. Garrido Frenich, J.L. Martínez Vidal, Anal. Lett. 37 (2004) 99.
[3] P. Plaza Bolaños, R. Romero-González, A. Garrido Frenich, J. L. Martínez Vidal, J. Chromatogr. A 1208 (2008) 16.

Keywords: water analysis, pesticides, SPME, HF-LPME

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E-40

MULTI-TARGET SCREENING OF UP TO 650 PESTICIDES IN A SINGLE LC/MS RUN BY EXACT ION TRACES

Arnd Ingendoh[1*], Petra Decker[2], Marcus Macht[3], Michal Bohac[4]

[1 2 3] Bruker Daltonik GmbH, Bremen, Germany
[4] Bruker Daltonics CZ
[*] Corresponding author–E-mail: ai@bdal.de; Phone: +49 421 2205 100; Fax: +49 421 2205 101

Novel aspect: Using high mass accuracy as selective parameter for the screening and quantitation of hundreds of pesticides in food.

Introduction: The usefulness of LC/MS/MS methods for the unambiguous identification and quantification of pesticides in complex matrix samples are well known. Triple quadrupole systems have proven to be useful for this task due to their high specificity in MS/MS mode and their low detection limits. However, working in MS/MS mode makes any MS system blind for all other compounds than the current MS/MS transition is designed for. Therefore, it is difficult to develop methods for simultaneous analysis of high numbers of pesticides. Thus, other ways of achieving specificity are of interest, such as the high mass accuracy and mass resolution of an ESI-TOF system. It can generate high specificity without limiting the number of simultaneously observed target compounds.

Methods: The QTOF approach enables the screening for several hundred of possible pesticides within one run. The selectivity is based on the accurate mass, with mass traces defined within < 0.002 Da over a dynamic range of > 4 orders of magnitude. By using a database of several hundred pesticides, spiked samples can be easily detected. Sensitivity in the range of low ppb range or even below can be achieved.

Preliminary results: Results for various matrices will be presented and discussed for potential need of sample preparation. An excellent linear range of 4 orders of magnitude is achieved, allowing the quantitation of the pesticides. In contrast to classical screening approaches by triple quadrupole instruments there are several benefits:

1) A high number of targets can be screened at the same time without loss of sensitivity
2) Unknown peaks can be identified based on accurate mass and true isotopic pattern
3) Data can be reprocessed later for additional compounds (archiving)
4) Profiling of the data allow for further statistical data evaluation.

Benefits and requirements of the method will be discussed in detail.

Keywords: multitarget screening, pesticides, hrEIC, quantitation

E-41
MONITORING OF PESTICIDE RESIDUES IN VARIOUS FOOD COMMODITIES FOLLOWING STORE TREATMENT

Petr Dohnal[1*], Marketa Hakenova[2], Jana Hajslova[3], Vladimir Kocourek[4], Radek Aulicky[5], Vaclav Stejskal[6]

[1 2 3 4] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[5 6] Crop Research Institute, Drnovska 507, 161 06 Prague 6 – Ruzyne
[*] Corresponding author–E-mail: petr.dohnal@vscht.cz; Phone: 220444372

Post harvest protection of crops is an important task because pests can cause significant losses of stored product. There are several ways how to prevent the excessive damage of raw commodities, especially cereals, nuts and seeds. Fumigation using gases is often used for protection of stores and processing facilities, where air-tightness of treated space is achievable. Despite this technique is considered as “non-residual”, it is rather time consuming, fumigants are highly toxic and only skilled certified personnel can perform it.

As there is renewed emphasis on chemicals with little mammalian toxicity, techniques based on application of modern insecticidal aerosols are commonly preferred for pest control of stored food. Synthetic pyrethroids and organophosphates are commonly used in stored food protection.

The aim of this study was to assess the dynamics of insecticide residues applied in experimental rooms by either smoke generator or ultra-low-volume (ULV) aerosol applicator.

Different approaches to the aerosol application techniques (smoke generators, ultra low volume applicators) and three types of active components (pirimiphos-methyl, cypermethrin, bendiocarb) were used in this study. Samples of eight commodities (flour, pea, oat flakes, rice, natural rice, wheat, sunflower seed and rape seed) were placed in the experimental area and the closed space was treated by aerosol. Concentrations of insecticide in the air were monitored few hours after treatment and then its residues were determined in all samples of food commodities. Both spatial and time distribution of pesticide residues was observed in rooms of different size.

New method of sampling and determination of tested pesticides in the ambient air using ultrasonic extraction of membrane filters and SOXTEC extraction of polyurethane foams followed by GC/MS detection were developed and validated. Samples of food commodities were extracted with ethyl acetate followed by GPC clean up and GC/MS detection.

Nine experiments in food storage facility and three in model room were carried out during last two years and the validity of results is discussed also in connection with analytical methods used. Significant differences in pesticide dynamics were also noticed when the same amount of active compound was applied using different application technique. It can be also concluded that the geometry of treated space play important rule and it is difficult to simply extrapolate result from model experiments to real conditions.

Keywords: pesticides, storage treatment, GC/MS

This study was carried out with support from project CHEMKONTAM – project 2B06099 of The Ministry of Education, Youth and Sports, Czech Republic, National program for research II

E-42

LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY FOR THE DETERMINATION OF PESTICIDE RESIDUES IN RIVER WATERS OF SERBIA

Zoran Sojanovic[1*], Gorica Vukovic[2], Marinela Tadic[3]

[1] Republic Hydrometeological Service of Serbia, Belgrade, Serbia
[2 3] Institute of Public Health, Belgrade, Serbia
[*] Corresponding author–E-mail: sofronije2000@hotmail.com; Phone: +381600271013

Liquid chromatography tandem mass spectrometry was applied for the trace level determination of seventeen pesticides listed in EN/ ISO 11369: 1997 in river water samples. The samples were collected from January to Mart 2009. The volumes of the sample and the final extract used in the method were 250 ml and 0, 25 ml, respectively. The water samples were cleaned up and concentrated by OASIS HLB cartridges. Extracts were analized by liquid chromatography/ESI/tandem mass spectrometry. The obtained method detection limits were less than 0,010 µg/L, and the mean recoveries were 86.0–104.8% with the relative standard deviation of 2, 8–11.5% for all compounds. The accuracy and precision were determined via recovery experiments, spiking reagent water at 50, 100 and 500 ng/L, at six replicates per level. The concentration detected of the different compounds were: 0.010–0.493 µg/L for atrazine, 0.010–0.060 µg/L for chlorotoluron, 0.010–0.047 µg/L for desethylatrazine, 0.010–0.112 µg/L for isoproturon, 0.010–0.053 µg/L for metazachlor, 0.010–0.038 µg/L for metolachlor, 0.010–0.050 µg/L for sebuthylazin, 0.010–0.039 µg/L for simazine and 0.010–0.048 µg/L for terbuthylazine.

Keywords: Water samples, Pesticides, LC–MS/MS

E-43
ORGANOCHLORINE AND ORGANOPHOSPHORUS PESTICIDE RESIDUES IN BABY FOOD PRODUCTS ON ROMANIA IN 2008

Carmen Hura[1*], Bogdan Andrei Hura[2], Cristina Perju[3]

[1 2 3] Institute of Public Health, Iasi, Romania
[*] Corresponding author–E-mail: chura@iasi.mednet.ro; Phone: 40 232 410399; Fax: 40 232 210399

In the present of agricultural practice, pesticides provide an unquestionable benefit for crop protection; however, the presence of pesticide residues in food can negatively affect human health. Special attention is paid to the safety of children and infants, as they represent a vulnerable risk group of population. The toxicity of pesticides are in infants and children may differ quantitatively and qualitatively from in adults. Considering the multitude of risks associated with pesticide intake by infants, the European Union has set a very low limit for pesticide in infant food. According to this regulation infant formulae must not contain residues of individual pesticides at levels exceeding 0.01 mg/kg (MRL).

The authors present the results obtained in 2008 in the research of some chemical pollutants with cancer risk (organochlorine and organophosphorus pesticides residues) in baby food products from Romania.

We analyzed 466 different baby food products samples (processed cereal-based foods) from Romania. The analysis of the organochlorine pesticides was performed by gas chromatography with an electron capture detector (EC) and the organophosphorus pesticides with a phosphorous nitrogen detector (NP) (Shimadzu A 2010 model), after the acetonitrile extraction of the pesticide residues from samples and the clean-up with SPE (we tested 48 pesticides).

The GC/ECD and GC/NPD analysis of pesticides in all researched samples showed that most of the detected and quantified residues were below 0.01 mg/kg which corresponds to the maximum residual limit for pesticide residues in baby food.

Keywords: pesticide, baby food analysis, GC

E-44

THIOURACIL: THE CHALLENGE OF DEVELOPPING A QUANTITATIVE ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD IN URINE WITHOUT DERIVATISATION

Julie Vanden Bussche[1*], Lynn Vanhaecke[2], Yoann Deceuninck[3], Karolien Verheyden[4], Klaas Wille [5], Karen Bekaert[6], Bruno Le Bizec[7], Hubert De Brabander[8]

[1 2 4 5 6 8] Ghent University, Faculty of Veterinary Medicine, Research group of Veterinary Public Health and Zoonoses, Lab Chemical Analysis, Salisburylaan 133, B-9820 Merelbeke, Belgium
[3 7] LABERCA-Ecole Nationale Vétérinaire de Nantes, Route de Gachet, BP 50707, F-44307 Nantes Cedex 3, France
[*] Corresponding author–E-mail: julie.vandenbussche@ugent.be; Phone: 0032 9 264 73 40

Thiouracil (TU) belongs to the xenobiotic thyreostats, a group of growth-promoting agents that are banned in Europe since 1981. Thyreostats negatively affect the meat quality of previously treated animals and are listed as compounds with teratogenic and carcinogenic properties (International Agency for Research on Cancer), which in turn represents a possible human health risk.

To monitor the illegal use of thyreostats in livestock, sensitive and specific analysis methods have been developed. The most sensitive method available today exploits a 3-iodobenzyl bromide derivatisation. This method has been used to establish a correlation between the presence of thiouracil in bovine urine and the administration of a Brasscicaceae diet, known to contain precursors of natural-occurring thyreostats. Thiouracil itself was not detected in the diet, which in turn provokes the question: Does thiouracil have a semi-endogenous status or does it concern false-positive results due to the derivatisation? In the work of the national control plans it is of uttermost importance to discard false results, therefore, the goal of this study was to develop an analytical method for the quantification of TU from urine without derivatisation.

A difficult, yet critical step in the determination of TU from a urine matrix is the sample extraction itself. TU, as a result of its polar nature and multiple tautomeric forms, undergoes interferences from matrix constituents, resulting in the disability of widely accepted techniques such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE) to extract TU. Various techniques such as desalting, denaturation, hydrolysis, reducing agents and mechanical disruption were tested during this study to evaluate the most optimal extraction conditions. Best results were obtained by using mechanical disruption at elevated temperatures, followed by LLE with ethyl acetate. Analysis was conducted on an Accela UHPLC system (Thermo Fisher Scientific, San Jose, USA) coupled to a TSQ Vantage triple quadrupole mass analyzer (Thermo Fisher Scientific, San Jose, USA), and detection was performed by tandem MS in the positive ion mode.

An effective UHPLC-MS/MS method was devised for the detection of TU in urine. This method should provide the answer to the alleged semi-endogenous status of thiouracil. The outcome of this study has a significant impact on the monitoring behavior of thyreostats, therefore future research will include all xenobiotic thyreostats and focus on the validation of this procedure in accordance with the EU criteria as described in Commission decision 2002/657/EC.

Keywords: Thiouracil, Thyreostats, UHPLC, mass spectrometry

E-45

NEW WAYS OF SAMPLE ANALYSIS: APPLICATIONS OF MINIATURIZAION AND AUTOMATION TOWARDS TRACE LEVEL PESTICIDE RESIDUE ANALYSIS

Manasi Saha[1*], Robert Gooding[2], John Jones[3]

[1 2 3] BASF Crop protection, Agricultural Research Center, Research Triangle Park, NC 27709
[*] Corresponding author–E-mail: manasi.saha@basf.com; Phone: (919) 547-2232

There are tremendous demands for increasing sample throughput today governed by the regulatory trends and demands for increased food monitoring all over the globe. Crop protection and food industries as well as monitoring and enforcement laboratories are continually searching for new ways to improve the efficiency and speed of residue analysis while simultaneously reducing the cost. It is a challenge to conduct analyses of thousands of samples collected for field dissipation and field residue studies or for monitoring purposes at the sourcing or retail level, using fast, short and rugged methods that quantitate multiple analytes, with simple shake, dilute and injection. Towards these goals, analytical methods with limits of quantitation as low as 0.01 ppm level were developed for numerous active ingredients and metabolites, in soil, water and plant matrices. Sample analyses utilize automation (automated solvent delivery system) and miniaturization (0.1 g sample size; 96-well plate setting) in conjunction with LC-MS/MS determination.

The methodology, and data proving linearity, accuracy and precision of the method will be presented and will be discussed in detail.

Keywords: Trace level Pesticide Residue Analysis

RESIDUES – DRUGS ET AL.

(F-1 – F-41)

F-1

ESTIMATING THE DECISION LIMIT AND THE DETECTION CAPABILITY USING MATRIX-MATCHED CALIBRATION DATA

Panagiotis Steliopoulos[1*]

[1] CVUA Karlsruhe, Karlsruhe, Germany
[*] Corresponding author–E-mail: panagiotis.steliopoulos@cvuaka.bwl.de; Phone: +49 721 926 5451

In the European Union, analytical methods that are used in official residue control of pharmacologically active substances have to be validated in accordance with Commission Decision 2002/657/EC. This Decision makes high demands on the analytical proficiency and lays down a set of rules and recommendations for the determination of performance characteristics. Chapter 3 of the CD 2002/657 offers examples and suggestions for validation procedures but explicitly allows alternative approaches. Two essential performance characteristics that shall be ascertained are the decision limit CCα and the detection capability CCβ. CCα is the measured concentration from which on it can be concluded with an error probability α that a sample is non-compliant. Put another way, CCα is the critical limit to decide whether a particular threshold value is exceeded significantly. For substances with an established maximum residue limit (MRL), the threshold value equates to this MRL. For banned substances the threshold is zero. CCβ is the true concentration lying above CCα with a statistical certainty of 1 – β. A crucial point is that these performance characteristics have to be determined on the basis of experimental results that were obtained under within-laboratory reproducibility conditions.

In this work, an approach to estimate CCα and CCβ is presented that is intended for cases in which quantification is accomplished via matrix-matched calibration. This type of calibration enables to quantify accurately without the necessity of correcting results for the bias error caused by matrix effects. The approach utilizes calibration data series obtained at different times and under varying conditions. It is based on the idea to consider the set of the calibration submodels to be an ensemble of equally probable events. Briefly speaking, the approach suggests, first, generating for each run the cumulative distribution function of future results x* given the threshold value (MRL or 0) as true concentration, and then, calculating a function which assigns to every result x* the mean over all run-specific probabilities at x*. The concentration at which this function equals 1 – α is CCα. The concentration at which this function equals 1 – β, minus the threshold plus CCα is CCβ. By way of illustration, the methodology is applied to a data set acquired within a validation study of an LC-MS assay for the determination of the macrolide antibiotic tilmicosin in muscle.

Keywords: decision limit, detection capability, CD/2002/657

F-2

INTERLABORATORY METHOD PERFORMANCE STUDY OF A SURFACE PLASMON RESONANCE BIOSENSOR SCREENING ASSAY FOR FLUOROQUINOLONE ANTIBIOTICS IN POULTRY MUSCLE, TROUT AND EGG

Stefan Weigel[1*], Bjorn Berendsen[2], Mariel Pikkemaat[3], Alexander Elferink[4], Anne-Catherine Huet[5]

[1 2 3 4] RIKILT–Institute of Food Safety, Wageningen, Netherlands
[5] CER, Marloie, Belgium
[*] Corresponding author–E-mail: stefan.weigel@wur.nl; Phone: 0031 317 485518

An interlaboratory method performance study was carried out to compare the performance of a previously developed immunochemical biosensor screening method for fluoroquinolone antibiotics in poultry muscle, fish and egg in different laboratories. The assay is capable of detecting the presence of at least 13 different (fluoro)quinolones in the target matrices at low levels (0.5–50 µg/kg). For each matrix blank and incurred test samples with proven homogeneity were prepared blinded and in duplicate. They contained fluoroquinolones typically applied to the respective species at levels of 0.5, 1 and 1.5 times the maximum residue levels. The samples were distributed to the nine participating laboratories in Europe and North America. The valid results returned from eight laboratories demonstrated that the assay is suitable for the intended purpose (qualitative screening). For chicken and fish all incurred samples were identified as suspect positives, while in the case of egg one sample (1%) was falsely declared as compliant. In both the blank chicken and egg samples one false positive result (6%) was reported. The evaluation of the numerical result values showed that the assay is not suited for the generation of semi-quantitative results due to unacceptable high variations (11–72%).

Keywords: Screening, biosensor, antibiotics, fluoroquinolones

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F-3

NATURAL TRACES OF THIOURACILE IN MOST SPECIES OF MEAT PRODUCING ANIMALS. TOWARD A THRESHOLD IN URINE?

Bruno Le Bizec[1*], Emmanuelle Bichon[2], Gaud Pinel[3], Yohann Deceuninck[4], Stéphanie Prévost[5], Fabrice Monteau[6], Jean-Philippe Antignac[7]

[1 2 3 4 5 6 7] LABERCA, ENVN, Nantes, France
[*] Corresponding author–E-mail: lebizec@vet-nantes.fr; Phone: 33 2 40 68 78 80; Fax: 33 2 40 68 78 78

Thyreostatic drugs (TS), illegally administrated to livestock for fattening purposes, are banned in the European Union since 1981 (Council Directive 81/602/EC). Since 2006, thiouracil has been formally identified in urine of non treated animals, but in the thyroid gland as well. Various animal species are concerned, e.g. bovine, ovine, caprine, equine, or porcine. Findings were independent from age or gender, but appeared associated with animal diet. A positive correlation has been demonstrated when animal are fed with cruciferous. Urine concentrations in the low $\mu g.L^{-1}$ have been reported in a couple of member states. Thiouracil can not be anymore systematically interpreted as the consequence of an illegal administration. Thus, we investigated on a large scale (n >2000 animals) – covering various species, ages, genders, seasons, and feed – the distribution of the so-called endogenous levels of thiouracile residues in urine. Threshold values may be proposed to guarantee – at a 95% or 99% confidence level – the compliance (or not) of suspected urine sample where thiouracil residues have been identified. The robustness of the method regarding quantitation of thiouracil in urine (including stability) will be obviously discussed in this presentation.

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Keywords: Thiouracil, threshold determination, thyrostats

F-4

ANALYSIS OF RESIDUES OF FLUOROQUINOLONES IN ANIMAL TISSUES. ASSESSMENT OF EXTRACTION METHODS

Ramon Companyó[1*], Susana Salvador[2], Fernando Gómez-Granados[3], Maria Dolors Prat[4]

[1 2 3 4] Universitat de Barcelona. Barcelona. Spain
[*] Corresponding author–E-mail: compano@ub.edu; Phone: 0034934039119

Advanced analytical instrumentation, such as that based on liquid chromatography coupled to mass spectroscopy, is now capable of detecting veterinary drug residues at low concentration levels in food samples. However, accurate analysis of these compounds in animal tissues also requires an extraction method that provides suitable recoveries for the analytes and a clean-up step to remove some of the co extracted compounds. These sample treatment steps are crucial in the whole procedure to ensure the validity of the measurements.

In quantitative analysis, a good estimation of recovery rates after extraction and purification should be done during the validation process. Recoveries are a good measure of the efficiency of the sample treatment, and they must be used to correct the results to ensure accuracy. Because of the scarcity of suitable certified reference materials in the field of veterinary drug analysis, recoveries are usually estimated from spiking experiments. Alternatively, calibration methods based on blank samples spiked prior to the extraction that provide recovery-corrected data can also be used. In both cases, the assumption is made that added and incurred analytes behave in the same way, but this is questionable. Therefore, there is a risk that spiked samples may lead to an overestimation of the extraction efficiency.

In this study we compare three sample treatment methods, which show different extraction efficiencies, for the analysis of incurred residues of fluoroquinolones in animal tissue samples. First, spiked beef samples consisting of muscle, kidney and liver tissues were used to estimate extraction recoveries of seven fluoroquinolones (marbofloxacin, norfloxacin, ciprofloxacin, enrofloxacin, danofloxacin, sarafloxacin and difloxacin). Then, the methods were applied to the analysis of incurred residues of enrofloxacin and ciprofloxacin in tissue samples from animals treated with enrofloxacin.

The selected methods, which are based on extraction with dichloromethane, acetonitrile and water, resulted in noticeable differences in recovery rates (from 60–90% of dichloromethane method to 16–30% when extracting with water). However, after recovery correction, similar mean values were obtained when the three methods were used to analyse incurred residues of ciprofloxacin and enrofloxacin.

Keywords: fluoroquinolones, sample treatment

F-5

ASSESSMENT OF GEL PERMEATION CHROMATOGRAPHY FOR SAMPLE CLEAN-UP IN THE ANALYSIS OF RESIDUES OF COCCIDIOSTATS IN EGGS

Joaquim Chico[1], Antoni Rubies[2], Francesc Centrich[3], Ramon Companyó[4], Maria Dolors Prat[5], Mercè Granados[6*]

[1 4 5 6] Universitat de Barcelona. Barcelona, Spain
[2 3] Agència de Salut Pública de Barcelona. Barcelona, Spain
[*] Corresponding author–E-mail: mgranados@ub.edu; Phone: 34 93 403 48 75

Poultry is especially prone to coccidiosis, a disease that causes intestinal lesions. In modern poultry production anticoccidial drugs are widely used to prevent or treat coccidiosis in broilers and pullets. However, according to UE legislation, anticoccidials can not be administered to laying hens, and consequently, there are no maximum residue levels established for eggs, which means that these compounds cannot be present in eggs.

The effective control of anticoccidials requires very sensitive analytical methods, mostly based on liquid chromatography coupled to mass spectrometry (LC-MS). Methods include the extraction of the analytes from the egg sample with a proper solvent, and because the complexity of the egg matrix, *i.e.* high level of lipids and proteins, the extracts are also complex and a clean-up step is mandatory before the LC analysis.

In this contribution we present a method for the analysis of residues of several anticoccidials (clopidol, ronidazole, ethopabate, diclazuril, monensin, salinomycin, naransin, maduramicin, lasalocid) in eggs by LC-ESI-MS/MS, which includes a clean-up step by gel permeation chromatography (GPC).

The analytes are extracted with ethyl acetate, and the extracts are injected in a GPC system equipped with an Envirogel column. The mobile phase consists of ethyl acetate–cyclohexane (1:1). The fraction containing the analytes is collected, evaporated, reconstituted with the LC mobile phase and injected into the LC-ESI-MS/MS system. Separation is performed in the gradient elution mode using a C_{18} column and with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phases. Ronidazole-d_3 and nigericin are used as internal standards. The analytes are detected in the positive ionization mode, except diclazuril, which is detected in the negative mode. The method permits the detection of anticoccidials below the 1 μg kg^{-1} level and has been validated according to Decision 2002/657/EC. Absolute recoveries are in the range 55–85%, except for clopidol, which is 30%.

Keywords: clean-up, GPC, coccidiostats, egg

F-6
CONFIRMATORY METHOD FOR THE DETERMINATION OF STREPTOMYCIN IN APPLES BY LC-MS/MS

Detlef A. Bohm[1*], Carolin S. Stachel[2], Petra Gowik[3]

[1 2 3] Federal Office of Consumer Protection and Food Safety (BVL), National Reference Laboratories for Contaminants and Residues, P.O. Box 11 02 60, D-10832 Berlin, Germany,
[*] Corresponding author–E-mail: detlef.bohm@bvl.bund.de; Phone: +49-(0)30-84122343

Streptomycin is an aminoglycoside antibiotic that is particularly active against aerobic gram-negative bacteria. As a pesticide this substance can be used for the treatment of fire blight, which is amongst common diseases in apples. With Regulation (EC) No 396/2005 a default maximum residue limit of 10 µg/kg came into force for streptomycin in apples [1] in September 2008. As a veterinary drug strepto-mycin is authorised for use in cattle and pigs. Maximum residue limits were laid down in Annex I of Council Regulation (EEC) No 2377/90 [2]. Residues of streptomycin have to be controlled in various food products such as meat, milk, honey and also in apples. A method with SPE clean-up step and LC-MS/MS measurement was developed, which is able to con-firm and quantify streptomycin in apples. All steps and conditions of the analytical procedure are de-scribed. The method was validated for streptomycin in apples in accordance with SANCO/2007/3131 [3]. The relevant parameters of the method are calculated and discussed so that the fitness for pur-pose of the method in question can be demonstrated comprehensively.

[1] Regulation (EC) 396/2005, Off. J. Eur. Commun. L70 (2005) 1
[2] Council Regulation (EEC) 2377/90, Off. J. Eur. Commun. L224 (1990) 1
[3] Document No SANCO/2007/3131

Keywords: streptomycin, apples, LC-MS/MS, validation,

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F-7

VALIDATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTING SULFONAMIDE ANTIBIOTICS IN PIG FEED RESOURCES

Ramon Companyó[1*], Vanesa Jiménez[2], Javier Adrian[3], Jacinto Guiteras[4], María Pilar Marco[5]

[1 2 4] Universitat de Barcelona. Barcelona. Spain
[3 5] IQAC-CSIC. Barcelona. Spain
[*] Corresponding author–E-mail: compano@ub.edu; Phone: 0034934039119

The widespread use of antibiotics in veterinary medicine is the main cause for the alarming emergence of bacteria with resistance to antimicrobials. The addition of antibiotics to animal feed as growth promoters is now forbidden, but they can still be added for therapeutic or prophylactic purposes. The problem, however, is that these medicated feeds, containing relatively high antibiotic concentrations (100 -1000 μg g^{-1}), are usually prepared in the same production lines as regular feeds, and this leads to a high risk of cross-contamination, because full removal of all antibiotic residues before switching back to normal production is difficult; as a consequence, the routine control of regular feeds is necessary. Sulfonamides are among the most used veterinary drugs in Europe but only two of them, sulfadiazine (SDZ) and sulfamethazine (SMZ), are authorized in Spain for use in medicated pre-mixtures and are mainly used for pig-rearing; fraudulent use of other sulfonamides cannot be discarded, however.

A new analytical screening methodology based on an Enzime-Linked Immusorbent Assay (ELISA), for the control of SDZ and SMZ and also of other unauthorized sulfonamides in pig feeds has been developed. Analytes are first extracted from the feed by manually shaking for 1 minute with a 95:5 mixture of acetonitrile and water, with recoveries of 80 -100%; the extract is then diluted to avoid the effect of organic solvent and matrix, and the sulfonamides are finally determined by an ELISA, making use of previously developed and optimized immunoreagents (As155/SA2-OVA). The accuracy, precision, LOD and Detection Capability (CCβ) were assessed. LOD were 0.2 μg g^{-1} for SDZ and 0.04 μg g^{-1} for SMZ. Besides SDZ and SMZ, other sulfonamides can also be detected with this immunoassay, and the LOD and cross-reactivities for six of them were determined. A novel strategy for an efficient determination of CCβ in an ELISA screening method is also proposed; with this approach, estimated CCβ values of 0.8 μg g^{-1} for SDZ and 0.1 μg g^{-1} for SMZ were obtained.

Finally, real pig feed samples were analyzed by this new ELISA methodology and by a previously developed LC method, and results confirm the adequacy of the immunoassay for screening purposes.

Keywords: sulfonamide antibiotics, ELISA, feed

F-8

A DECANOIC ACID REVERSE MICELLE-BASED SOLVENT FOR THE MICROEXTRACTION OF QUINOLONE ANTIBIOTIC RESIDUES FROM AQUACULTURE FISHES AND SEAFOOD

Soledad Rubio[1*], María Dolores Sicilia[2], Esther María Costi[3]

[1 2 3] University of Córdoba, Córdoba, Spain
[*] Corresponding author–E-mail: qa1rubrs@uco.es; Phone: 34-957-218644; Fax: 34-957-218644

Decanoic acid (DeA) reverse micelle-based solvents have emerged in the last few years as a valuable alternative for the extraction of organic compounds prior to their chromatographic determination [1]. The most noticeable characteristics of these supramolecular solvents include: capability to solubilise compounds through different interaction mechanisms, high number of solubilisation sites, low cost and experimental convenience.

This article deals with the evaluation of DeA reverse micelle-based solvents for the extraction of oxolinic acid (OXO) and flumequine (FLU) from aquaculture products. Residues of these acidic quinolone antibiotics (AQAs) are frequently found in farming fishes and seafood because of their wide use as effective and low-costly antimicrobial agents. The current maximum residue levels (MRLs) of OXO and FLU permitted by the European Union in edible animal tissues are 100 μg Kg^{-1} and 600 μg Kg^{-1}, respectively. Reported methods for the determination of AQAs in edible animal tissues usually involve laborious and time-consuming sample treatments including extractions in organic solvents and one or more clean-up steps.

The supramolecular solvent used for extractions in this work was synthesized by dissolving DeA in THF and adding water as coacervating agent (95% *v/v*). DeA form reverse micelles in THF, which grow in the presence of water owing to their partial desolvation. The larger aggregates formed are insoluble in the water:THF solution and separate from it as an immiscible liquid (the supramolecular solvent). The procedure used for the extraction of AQAs from aquaculture products was as follows: 400 μL of the supramolecular solvent synthesized were added to about 200 mg of chopped sample. Then, the mixture was vortex-shaken and centrifuged. Analytes were determined in the supramolecular extract by liquid chromatography/fluorescence detection.

The proposed supramolecular solvent-based microextraction (SUSME) approach surpasses to those using organic solvent in: 1) Efficiency; recoveries achieved are quantitative and independent on the composition of sample matrices. They ranged between 99–102% with relative standard deviations in the interval 0.2–5% for salmon, sea trout, sea bass, gilt-head bream and prawn samples fortified at analyte concentrations equal and below the corresponding EU MRLs. 2) Simplicity; neither clean-up or solvent evaporation is required. 3) Rapidity; the sample treatment spend about 30 min. 4) environmental friendliness; the consumption of organic solvents is significantly reduced and their emission to the atmosphere avoided.

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Keywords: microextraction, supramolecular solvent, quinolones, fishes

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F-9

SUPRAMOLECULAR SOLVENT-BASED MICROEXTRACTION OF SULPHONAMIDE ANTIBIOTIC RESIDUES FROM MEAT PRIOR TO THEIR DETERMINATION BY LIQUID CHROMATOGRAPHY/FLUORESCENCE DETECTION

Soledad Rubio[1*], María Dolores Sicilia[2], Esther María Costi[3]

[1 2 3] University of Córdoba, Córdoba, Spain
[*] Corresponding author–E-mail: qa1rubrs@uco.es; Phone: 34-957-218644; Fax: 34-957-218644

Quantifying sulphonamide antibiotic (SA) residues in meet is a difficult task owing to the complexity of the samples, the polarity of the analytes and the low concentrations at which they should be determined (below 0.1 mg kg^{-1}, the maximum residue limit permitted for SAs in Europe and the USA). Reported procedures for the extraction of SA residues from edible animal tissues are lengthy, involve numerous reextraction and cleanup steps, consume large amounts of toxic solvents and/or need the use of equipments no usually available in laboratories. Beside, recoveries obtained frequently are low and/or dependent on the food matrix composition. The quantification of SAs in the extracts is frequently performed by liquid chromatography (LC) with fluorescence (FL) detection after analyte derivatization. Reported LC/mass spectrometry-based methods also provide the sensitivity required for the determination of SA residues in meet but matrix-matched external calibration is required in order to avoid interferences due to matrix components.

This research deals with the evaluation of a supramolecular solvent made up of reverse micelles of decanoic acid (DeA) dispersed in a continuous water:tetrahydrofuran (THF) phase for the extraction of eight SAs (sulfadiazine, sulfamerazine, sulfamethoxypyridazine, sulfachloropyridazine, sulfadoxine, sulfamethoxazole, sulfadimethoxine and sulfaquinoxaline) from meat samples prior to their determination by LC/FLU. SAs were extracted from 200 mg-samples using 1 mL of DeA reverse micelle-based solvent and directly determined in the extract after derivatization with fluorescamine. SAs were effectively extract from meat using the optimized supramolecular solvent-based microextraction (SUSME) approach independent on the analyte polarity and the composition of sample matrices. The capability of the DeA reverse micelle-based solvent to extract highly polar analytes [octanol-water partition coefficients (log K_{ow}) of SAs ranged between -0.09 and 1.68] can be explained on the basis of the different mechanisms that it provides for analyte solubilisation, i.e. hydrophobic interactions and hydrogen bonds formation, and the high number of solubilisation sites that in it exists. Quantitation limits for the determination of SAs using the proposed SUSME/LC/FLU method were in the interval 0.02–0.06 mg kg^{-1} and the precision, expressed as relative standard deviation ([SAs]= 0.1 mg kg^{-1}, n=11) ranged between 2.7 and 3.9%. Recoveries obtained by applying this approach to the analysis of pork, beef, chicken, turkey and lamb samples fortified at the μg Kg^{-1} were in the interval 98–109%.

Keywords: microextraction, supramolecular solvent, sulphonamides, meat

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HPLC-DAD METHOD FOR SIMULTANEOUS DETERMINATION OF NINE β-LACTAM ANTIBIOTICS IN SHEEP MILK

Alejandrina Gallego[1*], Maria Cámara[2], Rosa Mª Garcinuño[3], Pilar Fernandez[4], J.Senen Durand[5], Pedro. J Sanchez[6]

[1 3 4 5] National University of Distance Education (UNED). Analytical Sciences Faculty. 28040 Madrid,Spain
[2 6] Regional Center of Animal Selection and Reproduction (CERSYRA), 13300 Valdepeñas, Spain
[*] Corresponding author–E-mail: agallego@ccia.uned.es; Phone: 34 913987364; Fax: 34 913988379

β-lactam antibiotics are being extensively used both in human and veterinary medicine practices. These antibiotics are frequently used in veterinary practice to treat and prevent some diseases such as ovine mastitis and microbial infections. In addition, β-lactams are usually administered at sub-therapeutic levels as feed additives or by the drinking water in order to enhance animal growth and feed efficiency. β-lactam residues in raw milk may interfere and adversely affect the manufacture of fermented dairy products such as cheese, buttermilk and yogurt due to the inhibition of the fermentation processes Drug residues in milk supplies may have public health implications and can interfere in the manufacture of dairy products, such as cheese. This study is focussed on the development of a simple and practical method capable of simultaneously extracting and analysing the selected β-lactam antibiotics ampicillin (AMP), bencylpenicillin (PEG), cephalexin (CFX), cefazolin (CFL), cefoperazone (CFP), cloxacillin (CLO), dicloxacillin (DCL), oxacillin (OXA), and phenoximethyl penicillin (PEV), in sheep milk from La Mancha, Spanish region, where sheep milk production is mainly used for processing a prestigious cheese with its own Denomination of Origin, Manchego cheese. The column, mobile phase, temperature and flow rate were optimised to provide the best resolution of these analytes. The extraction method of the antibiotic residues involves the deproteinisation of the sheep milk sample using acetonitrile and centrifugation followed by a solid-phase extraction (SPE) clean-up. Recoveries for β-lactams ranged from 43 to 93% with relative standard deviations between 0.5 and 3.9%. The limits of quantification (LOQs) for the studied compounds were lower than the MRLs established by the EU for the studied β-lactams in sheep milk, making the method suitable for performing routine analyses. The proposed multi-residue LC-DAD (UV diode-array detection) method is the first one capable of determining nine β-lactams antibiotics in samples of sheep milk.

Keywords: β-lactam antibiotics, sheep milk, LC-DAD

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F-11

EVIDENCE BIOCHIP ARRAY TECHNOLOGY AS A MULTI-ANALYTICAL TOOL FOR THE SCREENING OF RESIDUES IN DIFFERENT FOOD MATRICES.

Aaron Tohill[1], Damien McAleer[2*], R. Ivan McConnell[3], S. Peter Fitzgerald[4]

[1 2 3 4] Randox Laboratories Ltd., Crumlin, United Kingdom
[*] Corresponding author–E-mail: scientific.publications@randox.com; Phone: 44 (0) 28 9442 2413

Biochip array technology provides a platform that enables the simultaneous determination of multiple analytes with a single sample. It uses miniaturized assay procedures with implications in the reduction of sample/reagent consumption and cost-effectiveness of the tests. The core of the system is the biochip (9mm x 9mm) and represents not only the platform in which the capture ligands are immobilized and stabilised defining microarrays of discrete test sites, but is also the vessel were the simultaneous immunoreactions are performed. The use of this technology is advantageous for the rapid and simultaneous screening of multiple residues, that could be present in different foods and that could be incorporated in the food chain and the environment, as only positive results need to be confirmed. We report examples of applications of biochip arrays to different food matrices.

The immunoassays for multi-analyte determination of residues in food are competitive and are applicable to the semi-automated bench top analyser Evidence Investigator. The system incorporates dedicated software to process and archive the multiple data generated.

The biochip array for the screening of growth promoters simultaneously determines beta-agonists, boldenone, corticosteroids, nandrolone, ractopamine, stanozolol, stilbenes, trenbolone and zeranol with limits of detection (LOD) in tissue from 0.1ppb (trenbolone) to 0.9 ppb (nandrolone) and recovery >70% for different concentration levels.

The antimicrobial biochip array I enables the simultaneous determination of twelve sulphonamides (sulphadimethoxine, sulphadiazine, sulphadoxine, sulphamethizole, sulphachlorpyridazine, sulphamethoxypyridazine, sulphamerazine, sulphisoxazole, sulphathiazole, sulphamethazine, sulphaquinoxaline, sulphapyridine), with LODs ranging from 0.3 ppb (sulphadoxine) to 7.5 ppb (sulphamethazine) in honey, 0.05 ppb (sulphamethoxypyridazine) to 6.21 ppb (sulphapyridine) in tissue, 1.04 ppb (sulphamerazine) to 5.74 ppb (sulphadimethoxine) in milk and 1.92 ppb (sulphamethazine) to 66.64ppb(sulphadimethoxine) in animal feed. The sample recovery values are >70% for different concentration levels in all the sample matrices analysed.

The antimicrobial biochip array II allows multiplexed measurement of quinolones, ceftiofur, thiamphenicol, streptomycin, tylosin, tetracyclines with LODs ranging from 0.6 ppb (tylosin) to 9.7 ppb (tetracyclines) in honey, 0.61ppb (thiamphenicol) to 5.4 ppb (tetracyclines) in milk with sample recovery values >83% for different concentration levels in both matrices.

Nitrofurans (AHD, AMOZ, AOZ, SEM), chloramphenicol and triphenylmethane dyes are simultaneously determined with the antimicrobial biochip array III which presents LODs ranging from 0.2 ppb (AHD, AMOZ, chloramphenicol) to 1.0 ppb (SEM) for honey with sample recovery values >72% for different concentration levels.

In conclusion, this multi-analytical approach is applicable to different food matrices and consolidates many tests using a single platform, thereby enhancing the scope of tests and minimising the environmental impact as the consumption of solvents, plastic consumables, paper, electricity is significantly reduced.

Keywords: biochips, arrays, residues, food, multiplex

F-12

DETERMINATION OF β2-AGONISTS IN PORK USING OPT SOLID-PHASE EXTRACTION AND LC-ESI-MS/MS

Ulrik Wittek[1], Thierry Faye[2], Chenhao Zhai[3], Jianzhong Li[4], Yue Song[5], Joan Stevens[6*]

[1] Agilent, Waldbronn, Germany
[2] Agilent, Massy, France
[3 4 5] Agilent, Shanghai, China
[6] Agilent, Wilmington, DE
[*] Corresponding author–E-mail: joan_stevens@agilent.com; Phone: +1 (302)-633-8839; Fax: +1 (302)-633-8955

The β2-agonists have been used worldwide as illegal growth promoters in pork production. Recently incidences of poisoning occurred due to high levels of the β2-agonist (clenbuterol) in pork. This application employed a polymeric SPE product to extract and enrich four β2-agonists from pork with analysis by LC-MS/MS. A method for simultaneous determination of four β2-agonist residues of terbutaline, salbutamol, clenbuterol and formoterol in pork has been developed and validated. The analytes are purified by liquid-liquid extraction (LLE) and solid-phase extraction (SPE) and quantified by liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in positive ion multiple reaction monitoring (MRM) mode. The method provides a sub-ng/g level of limit of detection (LOD) for all four β2-agonists in pork. The dynamic calibration ranges for these compounds are obtained from 0.25 to 5 ng/g. The overall recoveries range from 78 to 101% with RSD values between 1.8 and 7.2%.

Keywords: SPE, food safety, residues, LC-MS/MS

F-13

CONFIRMATORY ANALYSIS OF 15 ANABOLIC STEROIDS IN MUSCLE FROM BOVINE AND PORCINE USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Mikael Pedersen[1*], Lis Abildgaard Andersen[2], Jens Hinge Andersen[3]

[1 2 3] National Food Institute, Søborg, Denmark
[*] Corresponding author–E-mail: mikp@food.dtu.dk; Phone: +45-72347429

A confirmatory method has been developed for the determination of chlormadinone acetate, megestrol acetate, melengestrol acetate, medroxyprogesterone acetate, methyltestosterone, α/β-boldenone, methylboldenone, α/β -trenbolone, α/β -nortestosterone, testosterone, epitestosterone and androstenedione in muscle from bovine and porcine. The method is used for residue import control in Denmark since the normally choosen matrices, like urine, plasma and faeces, for steroid analysis are not available. In 2005 Denmark imported 6.000 tons of beef from third countries (primarily Brasil and Australia) and the production in Denmark of beef was 130.000 tons meaning that appr. 5% of beef consumed in Denmark came directly from third countries. Each member state has to analyse samples imported from third countries for residues and with the suspected misuse of growth promoting agents in Brasil in 2007 it was decided to include analysis for steroids in meat from third countries in the Danish residue plan. Since the levels of steroids accumulated in muscle tissue are in the sub µg/kg amounts and the European Commission has set 1 µg/kg as the reference point for action (0.5 µg/kg for chlormadinone acetate) a very sensitive method has to be used and for confirmation mass spectrometry is the detector to choose.

The procedure involved hydrolysis, defattening with heptane and final clean up with SPE using C_{18}- and amino cartridges. Analytes were analysed using liquid chromatography (LC) coupled with tandem mass spectrometry (MS^2), and quantification was performed using matrix-matched calibration standards in combination with deuterated internal standards. In accordance with Commission Decision 2002/657/EC, two ion transitions were monitored for each analyte. Decision limits (CCα) were estimated by analysing 20 blank muscle samples and varied from 0.04 to 0.47 µg/mL. Detection capabilities (CCβ) were estimated using 16 muscle samples fortified at estimated CCα and were < 0.7 µg/mL. The mean within-laboratory reproducibility ranged from 5–22% (% RSD_{IR}). The method is sensitive and reliable and has been used for import control during the last two years. The three endogene hormones testosterone, epitestosterone and androstenedione, has been included in the method to get knowledge about the levels of these hormones and findings has been made several times.

Keywords: Androgens, Muscle, Methodvalidation, LC-MSMS, Analysis

F-14

DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR THE ANALYSIS OF B-AGONISTS IN ANIMAL FEED AND DRINKING WATER

Cristina Juan[1*], Francisco Moragues[2], Nuria León[3], Carmen Igualada[4]

[1 2 3 4] Laboratorio de Salud Pública de Valencia, Centro Superior de Investigaciones en Salud Pública, Valencia, Spain
[*] Corresponding author–E-mail: juan_cri@gva.es; Phone: 0034 96 192 58 22

β-adrenergic agonists are used in stock farming to promote growth and productivity in livestock or for therapeutic purposes. However in the Council Directive 96/22/EC, the European Union has forbidden its use as repartitioning agent for growth promotion due to its adverse effects on human health by carrying over from β-adrenergic agonists treated animals to the human diet. Meanwhile in the Council Regulation (EEC) Nº 2377/90 maximum residue limits (MRLs) are fixed only for one β–agonist, clenbuterol. Clenbuterol is permitted solely for tocolysis in parturient cows and the treatment of respiratory ailment of equidae. MRLs have been fixed in bovine and equidae muscle, liver, kidney and milk at values ranged 0.05-0.5 µg/kg. Nevertheless, the Community Reference Laboratories (CRLs) have suggested a minimum required performance limit (MRPL) for those substances in which MRLs have not been established to improve and to harmonize the performance of analytical methods in residue control. In this guidance paper MRPLs for all the β-agonists in feed are fixed at 50 µg/kg, and for clenbuterol, clenpenterol, ractopamine, brombuterol, mabuterol, mapenterol and hydroxymethylclenbuterol in drinking water are ranged at values of 0.2-10 ppb (µg/L).

Eight β-agonists (clenbuterol, clenpenterol, ractopamine, brombuterol, mabuterol, mapenterol and hydroxymethylclenbuterol) were simultaneously detected in feed and drinking water by a reproducible, sensitive and selective multi-residue analytical method. The developed method was based on an acidify extraction, with acid clorhidric 0.1 N, and centrifugation. The supernant was passed throught a solid phase extraction on mixed mode ion exchange polymer cartridge for clean up. The eluted residue was analyzed by liquid chromatography tandem mass spectrometry using electrospray source in positive ion mode (LC-MS/MS). Method validation was achieved in feed and drinking water according to the criteria laid down in the Commission Decision 2002/657/EC and decision limit (CCα) and detection capabilities (CCβ), accuracy and precision (repeatability and within lab reproducibility) were calculated. The CCα were below the MRPL for all β-agonists studied, 1 and 0.1 µg/kg for feed and drinking water, respectively. In conclusion, the proposed analytical method brings high quality and it was successfully applied for analysis residue control of veterinary β-agonists.

Keywords: feed, drinking water, β-agonists

F-15

TOXICITY OF CHLORTETRACYCLINE ON MAIZE SEED GERMINATION, SEEDLING GROWTH AND THE ANTIOXIDANT RESPONSE

Bei Wen[1*], Peng Wang[2], Shuzhen Zhang[3], Xiaoquan Shan[4]

[1 2 3 4] Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[*] Corresponding author–E-mail: bwen@rcees.ac.cn; Phone: 86-10-62849329; Fax: 86-10-62923563

The effects of chlortetracycline (CTC) on maize (*Zea mays L. cv. TY2*) seed germination, root and shoot lengths and their biomass, CTC accumulation, amylolytic activity, soluble protein and malondialdehyde (MDA) concentrations, and antioxidant enzyme activities in the seedlings were investigated. Germination percentages of maize had no responses to different CTC concentrations. The length and fresh weight of root and shoot decreased with CTC concentration increasing, which correlated significantly with the logarithm of CTC concentrations. Seedling contents of soluble protein, MDA, and the activities of glutathione S-transferase (GST), peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) changed significantly as a result of CTC treatment. Both the soluble protein contents and activities of SOD positively correlated with logarithm of CTC concentrations in exposure solutions. The results demonstrate the harmfulness of CTC to maize in the early developmental stage, and indicated that the inducement of free radicals is one of the possible mechanisms of CTC toxicity.

Keywords: Maize, Chlortetracycline, Phytotoxicity

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F-18
SCREENING VETERINARY DRUGS IN PRODUCTS OF ANIMAL ORIGIN

Peter Hancock[1*], James Morphet[2], Emmanuelle Cognard[3], Didier Ortelli[4]

[1 2] Waters, Manchester, UK
[3 4] Food Authority Control of Geneva, Geneva, Switzerland
[*] Corresponding author–E-mail: peter_hancock@waters.com; Phone: +44 7788 411812

Veterinary drugs are widely used to treat or prevent disease in animals which can result in trace levels of drug residues in products of animal origin such as meat, fish, milk, eggs or honey. The presence of drug residues in the food chain is of concern due to their potential detrimental effect on human health. To protect consumer health and to ensure the high quality of animal products, maximum residue limits (MRLs) to set allowed maximum levels for drugs residues in animal products have been established worldwide[1-3].

As regulations became more stringent with respect to MRLs, the need to develop qualitative methods as well as confirmation and identification techniques becomes important in order to minimize false positives. Time of flight mass spectrometry (TOF MS) screening has gained popularity due to benefits such as historical data interrogation, simplified instrumental method set-up and reduced compromise in method performance when increasing the number of compounds contained in the method. However, processing and reviewing TOF screening data is often a complex workflow where positive peaks are first identified then quantified to assess the risk posed to the consumer. Frequently the transfer from qualitative to quantitative processes is performed manually, which places a significant drain on data review resource and introduces a high probability for errors.

The use of ACQUITY UPLC coupled to quadrupole time of flight (Xevo QTOF MS) for the semi-targeted screening of more than 150 multiclass veterinary drug residues and metabolites in milk, liver, blood, fish and meat will be discussed. The data was processed using POSI±IVE software, enabling exact mass data to be qualitatively and quantitatively reviewed in a single pass, by going straight to the important quantitative results for positively detected components. The use of product ions from MS^E acquisition to add extra confidence when assigning the identity of a peak of interest will also be investigated.

[1] http://ec.europa.eu/food/food/chemicalsafety/residues/index_en.htm
[2] http://www.foodsafety.gov/%7Efsg/animal.html
[3] http://www.mhlw.go.jp/english/topics/foodsafety/positivelist/index.html

Keywords: Screening, Veterinary drugs, TOF

F-20

OMIC STRATEGIES TO SCREEN FOR THE ILLEGAL -AGONISTS IN CATTLE

Gaud Pinel[1*], Frédérique Courant[2], Yannick Blanchard[3], Yoann Deceuninck[4], Emmanuelle Bichon[5], Emmanuelle Com[6], Charles Pineau[7], Fabrice Monteau[8], Jean-Philippe Antignac[9], Bruno Le Bizec[10]

[1 2 4 5 8 9 10] LABERCA, Nantes, France
[3] AFSSA, Ploufragan, France
[6 7] Biogenouest, Rennes, France
[*] Corresponding author–E-mail: pinel@vet-nantes.fr; Phone: 33 2 40 68 78 80; Fax: 33 2 40 68 78 78

β-agonist compounds can be misused in food-producing animals for growth promoting purposes. Efficient methods based on mass spectrometry detection have been developed to ensure the control of such veterinary drug residues. Nevertheless, the use of “cocktails” composed of mixtures of low amounts of several substances as well as the synthesis of new compounds of unknown structure prevent efficient prevention. To circumvent those problems, new analytical tools able to detect such abuse are today mandatory. In this context, ‘omic technologies may represent new emerging strategies for investigating the global effects associated to a family of substances and therefore, to suspect the administration of β-agonists (either “cocktails” or unknown compounds). As a first demonstration of feasibility, tranomic, proteomic and metabolomic, have been assessed to screen for the administration of clenbuterol to calves. On the one hand, the metabolomic and proteomic approaches which were both based on high resolution mass spectrometry measurements, using either a LC-ESI(+)-Orbital trap or a SELDI-TOF system, made it possible to highlight metabolic and proteomic modifications in urine and serum, respectively, as a consequence of a clenbuterol administration. By the means of chemometrics (Student and Mann-Whitney tests, OPLS), those metabolic and proteomic differences were used to build predictive models able to suspect clenbuterol administration in calves. One the other hand, the tranomic approach allowed highlighting some genes of which the expression levels were modified upon clenbuterol administration. These new approaches may be considered of valuable interest to overcome current limitations in the control of growth promoters’ abuse, with promising perspectives in terms of screening.

Keywords: metabolomic, transcriptomic, proteomic, β-agonists, calves

F-21

HOLLOW-FIBER LIQUID-PHASE MICROEXTRACTION FOR THE DETERMINATION OF MACROLIDES AND TETRACYCLINES RESIDUE IN WATER SAMPLE

Soparat Yudthavorasit[1], Chayada Chiaochan[2], Natchanun Leepipatpiboon[3*]

[1 2 3] Chromatography and Separation Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
* Corresponding author–E-mail: natchanun.l@chula.ac.th; Phone: +662 218 7608

Hollow-fiber liquid-phase microextraction (HF-LPME), the alternative microextraction method based on the use of hollow fiber membrane, is developed for preconcentration and determination of three macrolides and two tetracyclines residue in water sample with Liquid Chromatography-Electrospray-Tandem Mass Spectrometry (LC-ESI-MS/MS) detection in positive mode. In HF-LPME, organic solvent immobilized in fiber pores creates interface between sample and acceptor solution. Macrolides and Tetracyclines in sample were extracted and enriched through organic phase into acceptor solution inside fiber lumen. The parameters affecting enrichment factor such as organic solvent composition, pH of donor and acceptor solution and extraction time were investigated. Within extraction time of 60 min, Aliquat 336 in dihexyl ether was employed as organic phase, sample and acceptor solution was adjusted pH to 8.0 and 4.0 for providing highest enrichment ability. Under optimized condition, the limit of detection of 0.02–0.18 μg L^{-1} and the limit of quantification of 0.07–0.85 μg L^{-1} were performed in the working range 0.50–50.00 μg L^{-1} and the high enrichment factor was achieved from 13 to 156 times. The proposed method offered simple, low-cost, sensitive, high enrichment potential, efficient clean-up and environmental friendly procedure with consumption of organic solvent in micro-liters and also successfully applied in real water sample at spiked level 2 μg L^{-1} with the recovery ranged from 80 to 110% and the relative standard deviation (RSD) for intra-day and inter-day precision were 3.27–10.95% and 6.50–11.44%, respectively.

Keywords: Liquid-phase microextraction, Macrolide, Tetracycline

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F-22

DETECTION OF ANABOLICS USED AS GROWTH PROMOTERS IN URINE SAMPLES DERIVED FROM YOUNG FATTENING CALVES

Vasilios Vozikis[1], Vassiliki Kotsaki- Kovatsi[2], Alexandros Spais[3], Chrissanthy Papadopoulou[4*]

[1] Institute of Food Hygiene, Center of Veterinary Institutes, Thessaloniki-Greece
[2] Department of Biochemistry and Toxicology, Veterinary Faculty, Aristotle's University of Thessaloniki, Thessaloniki –Greece
3 Department of Animal Nutrition, Veterinary Faculty, Aristotle's University of Thessaloniki, Thessaloniki – Greece
[4] Food Microbiology and Hygiene Group, Medical School, University of Ioannina, Ioannina-Greece
[*] Corresponding author–E-mail: cpapadop@cc.uoi.gr; Phone: 00306944690511; Fax: 00302651007886

Drug residues in foods of animal origin constitute a significant public health risk. Among the residual substances which can be detected in bovine meat, anabolics are considered to be a severe human health threat and tolerance limits have been imposed by two Council Directives 96/23/EC and 2003/74/EC. In the present study a survey on certain anabolics used as growth promoters in young calves has been contacted. A total of 150 urine samples from young fattening calves from intensive breeding plants of Northern Greece were examined for the presence of diethylstilboestrol (DES), metyltestosterone (MET), 19-nortestosterone (NORT), zeranol (ZE) and trembolone (TRE). For the detection of the anabolics the EIA method was employed using commercially available competitive enzyme immunoassays for the quantitative analysis of DES, MET, NORT, ZE and TRE (RIDASCREEN®). The concentration of DES measured in all urine samples was lower than 0.2 μg/kg, while the concentration of MET was found to be higher than 0.2 μg/kg in 95% of the tested urine samples. The concentration of NORT was higher than 0.2 μg/kg in 15% of the tested samples and less than 1 μg/kg in 63% of the samples. The concentration of ZE was above 0.2 μg/kg in 20% of the urine samples and below 1 μg/kg in 45% of the samples. The concentration of TRE measured in all urine samples was lower than 0.9 μg/kg. The results indicate the need of rotating tests of urine and meat samples performed constantly by the Food Inspection Authorities in order to ensure the safety of meat products intended for human consumption.

Keywords: anabolics, urine, fattening calves

F-23
SCREENING DETERMINATION OF SEMICARBAZIDE IN BABY FOOD USING ELISA

Iva Diblikova[1], Maria Vass[2], Karel Hruška[3], Milan Franek[4*]

[1 2 3 4] Veterinary Research Institute, Hudcova 70, 62100 Brno, Czech Republic
[*] Corresponding author–E-mail: franek@vri.cz; Phone: +420 533 331 901; Fax: +420 541 211 229

Monitoring of illegal use of the nitrofurazone in livestock production is carried out by detection of the marker residue, semicarbazide (SEM), which is the metabolite derived from the parent nitrofuran antibiotic. However, in baby food, azodicarbonamide (ADC), a blowing agent and flour treatment additive, was proved to be the main source of SEM contamination. Recently, ELISA for SEM in meat and egg has been developed in this laboratory [1,2]. The procedure involves acid hydrolysis for the release of protein bound residues in a sample homogenate, derivatisation with o-nitrobenzaldehyde, extraction, and ELISA detection. A modified version of this method was introduced for baby food and subsequently validated according to the criteria set out in Commision Decision 2002/657/EC. Baby food samples confirmed free of SEM by LC-MS/MS analysis were used as blanks for validation experiments. Detection capability of the assay calculated from 20 blank samples spiked with SEM was determined to be 1.4–2.3 µg/kg. For samples spiked with 5,10, 20 µg/kg of SEM, the analytical recovery was 87.6–107.2% and intra-assay and inter-assay variation coefficients were below 14.1%. Practical applicability of the ELISA was demonstrated by screening of SEM in 98 baby food samples obtained from retail outlets (from five different producers) within Brno area (Czech Republic). The collected samples were typical commercial products of a different composition packed in glass jars (100–200 g) with gasket seals. All tested samples did not contain any SEM amount detectable by the established ELISA technique. Although the use of ADC in food industry has been banned in the EU since 2005, this relatively inexpensive screening ELISA test for SEM in baby food matrices can be utilized by residues laboratories for regulatory purposes.

[1] M. Vass, I. Diblikova, I. Cernoch, M. Franek: Anal. Chem. Acta, 608 (2008) 86-94.
[2] M. Vass, I. Diblikova, E. Kok, et al.: Food Addit. Contam. 25 (2008) 930-936.

Keywords: Semicarbazide, baby food, screening

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F-24

VALIDATION OF AN ANALYTICAL METHOD FOR SCREENING OF BENZIMIDAZOLES IN MEAT AND MILK MATRICES BY UPLC-QTOF METHOD

Jean-Christophe Yorke[1*]

[1] EVIRA, Helsinki, Finland
[*] Corresponding author–E-mail: jean-christophe.yorke@evira.fi; Phone: +358 207724421; Fax: +358207724359

Benzimidazoles are veterinary anthelmintic drugs widely used for prevention and treatment of parasitic infections and are therefore monitored in national residue control plans (NRCP). The current work of the residue control system recommends applying a two stage analysis. A screening analysis followed by a confirmatory and quantitative method if positive results have been found during the first stage. Decision 2002/657/EC lays down performance criteria for screening and confirmatory methods. Screening methods has to asses a false compliant result rate of less than 5% making the validation less demanding for this type of methods. For confirmatory methods extraction procedures are more intensive limiting the throughput and rendering these methods more costly.

A high throughput qualitative method using a clean-up step followed by an UPLC-QTOF MS for screening 20 benzimidazoles in meat and milk matrices has been developed and validated.

The procedure includes a homogenization with a basic ethyl acetate solution followed by a defatting step using hexane. The chromatographic separation was achieved in 11 minutes using an Acquity BEH C_{18} 1.7 µm, 50 $\times$ 2.1 mm column with a gradient of formic acid/acetonitrile. The mass spectrometric detection was performed with QTOF MS in positive ESI full scan acquisition (100–1000 Mw range). High selectivity was achieved by extracting an ion chromatogram with a mass window of 20 mDa for each benzimidazole

Validations for milk and muscle matrices were performed analyzing 20 blank samples and 20 samples spiked at the maximum residue level (MRL) repeating these on four non consecutive days. The decision rules Cut-off factors Fm_s and the threshold values T_s have been determined with the signal intensity of the ion chromatograms. Comparison of blank samples with samples spiked at MRL allowed to verify that the rate of false negative was < 5% (β error) and also to verified that that $CC\beta <$ MRL for each benzimidazole. The values of false positives were calculated with the Student's test for independent samples in those cases where the blanks would be above the Cut off factors. Because the rate of false positives was far less than 1% we can expect to use a confirmatory method only for real positive samples.

A daily determination of T_s and Fm_s factors by injection of blanks and Qc_s was noticed to be important to control the day to day variation of the response that was dependent on the cleanness of the source.

Keywords: UPLC-QTOF, qualitative method; benzimidazoles, validation, milk and muscle matrices

F-25

DEVELOPMENT AND VALIDATION OF A MULTI-RESIDUE METHOD FOR THE DETERMINATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) IN MEAT OF MEAT-PRODUCING ANIMALS BY LC-MS/MS

Maria Strouthou[1*], Spyroula Constantinou[2], Christoforos Papachrysostomou[3], Militsa Hadjigeorgiou[4], Popi Kanari[5], Nikolaos Thomaidis[6]

[1] Laboratory of Analytical Chemistry, University of Athens, Greece, [2 3 4 5] Veterinary Drug Residues Laboratory, State General Laboratory, Cyprus
[6] Laboratory of Analytical Chemistry, University of Athens, Greece
[*] Corresponding author–E-mail: maria_strth@yahoo.com; Phone: 0035799896927; Fax: 0035722316434

Non-steroidal anti-inflammatory drugs (NSAIDs) are used extensively in veterinary medicine for the treatment of inflammation disorders, but at the same time are strongly associated with gastrointestinal problems. In order to protect consumer health, the European Union decided to regulate their use (2377/90/EC) to prevent undesired alimentary exposure and health risks to people.

The present study, describes the development, optimization and validation of a liquid chromatographic tandem mass spectrometric (LC-MS/MS) method for the determination of eleven NSAIDs belonging to different subclasses (carprofen, vedaprofen, diclofenac, flunixin, meloxicam, mefenamic acid, tolfenamic acid, niflumic acid, phenylbutazone, naproxen and ibuprofen). The method is applied to the analysis of porcine, bovine, ovine and poultry meat samples.

During method development, a comparison of mobile phases (CH_3OH:$HCOONH_4$, CH_3OH: CH_3COONH_4, CH_3CN:$HCOONH_4$, CH_3CN:CH_3COONH_4) and columns (Waters Sunfire C_{18} 3.5 μm, Waters XTerra C_{18} 3.5 μm, Phenomenex Luna C_{18} 5 μm and a Monolithic Merck Chromolith RP-18e 100–3mm) and electrospray parameters optimization (Capillary voltage, Extractor voltage, RF Lens, Dessolvation gas and Cone gas) using a chemometric approach were performed. Different sample preparation procedures, involving both enzymatic or chemical hydrolysis and solid-phase extraction, were examined and an enzymatic hydrolysis with a protease solution, followed by a Strata-X Polymeric Reversed Phase SPE was finally chosen. Matrix effects were also studied from different sample preparation procedures and with different separation columns (Sunfire and Chromolith).

The developed method was validated according to the criteria of the European Decision 2002/657/EC. The multi-residue method was able to detect and quantify the different analytes sufficiently low for both authorized and non-authorized substances.

Keywords: NSAIDs, meat, LC-MS/MS, optimization, validation

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F-26
IDENTIFICATION OF UNKNOWNS IN FOOD, FEED, BIOLOGICAL AND FORENSIC SAMPLES

Ruud Peters[1*], Efraim Oosterink[2], Alida Stolker[3], Michel Nielen[4]

[1 2 3 4] RIKILT, Wageningen, The Netherlands
[*] Corresponding author–E-mail: ruudj.peters@wur.nl; Phone: +31 317 486 348; Fax: +31 317 417 717

Nowadays a large number of compounds are determined in food and feed samples. In most cases this concerns substances that are already known and the general concern is to confirm their absence or presence. However, many more substances for which no specific tests are performed may be present in the sample. Biological tests are used to screen samples for large groups of compounds having a particular effect, but it is often difficult to identify a specific compound when a positive effect is observed. The identification of an unknown compound is a challenge for analytical chemistry in food analysis as well as in environmental, clinical and forensic analysis.

A procedure was developed for the identification of unknowns. Because of the extremely wide range of substances, in terms of molecular weight, polarity, pKa, and chemical/thermal stability, a generic sample preparations developed for four types of matrices. Analyte separation was achieved using high resolution liquid chromatography (HRLC) with careful selection of the most suitable, i.e. generic, HRLC column. A TOFMS analyzer was used for detection and identification since it can analyze samples for a theoretically unlimited number of compounds without any a priori knowledge about the presence of certain compounds. While the use of HRLC/TOFMS generates an enormous amount of data, potentially allowing the identification of unknown compounds, the resulting “forest of peaks” in a chromatogram make it difficult to distinguish individual peaks. Data processing software as MetAlign™ was used for denoising, baseline correction and the detection of individual and unique peaks in the HRLC/TOFMS data. Identification was based on an accurate mass database coupled with Search- LC and TOFMS software to enable identification of unknowns based on accurate mass, retention time indices and isotopic pattern. Analyses of test samples spiked with over 200 veterinary drugs and pesticides show that up to 95% of the spiked components are detected and identified, depending on the search parameter settings. The procedure has been used for the identification of unknown, sometimes biologically active compounds, in food and feed samples, in sport supplements, in biological and forensic samples. Unknowns identified in such samples are antibiotics, steroids and steroid esters, pharmaceutical products and other individual compounds. Recently, the procedure was successfully tested as an alternative screening method for the detection and identification of known and unknown antibiotics in meat.

Keywords: Unknown-identification, TOFMS, Accurate-mass-database, Forensic-analysis

F-27

DETERMINATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN ANIMAL MUSCLES BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Piotr Jedziniak[1*], Teresa Szprengier-Juszkiewicz[2], Malgorzata Olejnik[3]

[1 2 3] National Veterinary Research Institute, Pulawy, Poland
[*] Corresponding author–E-mail: jedzi@piwet.pulawy.pl; Phone: +48600941197

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of animals. According to EC directive 96/23 residues of these drugs have to be monitored due to a potential risk for consumer health. Differences in chemical properties and low limits of residues (MRLs and concentrations recommended by Community Reference Laboratory) in the tissues make LC-MS/MS a preffered technique for both screening and confirmatory purposes.

Considering above, method for a wide range of NSAIDs (carprofen, diclofenac, flunixin, meloxicam, phenylbutazone, oxyphenbutazone, tolfenamic acid, mefenamic acid, naproxen, ketoprofen) was developed. Additionally, deutered internal standards (flunixin-d_3, diclofenac-d_3, carprofen-d_3, tolfenamic acid-d_4, phenylbutazone-d_{10}, meloxicam-d_3) were used in a quantitative analysis. Muscle samples were minced and acetate buffer (pH 5.0) was added. Subsequently, beta-glucuronidase was used in the 1-hour enzymatic hydrolysis. After this step, the samples were extracted twice with acetonitrile. Extracts were cleaned-up with Sep-Pak Alumina N and C_{18} cartridges and analysed by LC-(ESI)-MS/MS system. NSAIDs were separated on Inertsil ODS-3 column with 30 min gradient of acetronitrile and 0.1% formic acid. Mass spectrometer worked in the multiple reaction monitoring mode (negative ionisation), for all analytes 2 transitions were monitored.

The method was validated according to the requirements described in the Commission Decision 2002/657/EC: linearity, precision (repeatability and within-laboratory reproducibility), recovery, decision limit CCα and detection capability CCβ were calculated. Selectivity and robustness of the method was also established. Within-laboratory reproducibility was in the range of 10–25%, with the recovery above 65%. The method allowed to determine NSAIDs residues above 1.0 µg/kg.

The effectiveness of the presented method was proven in the analysis of over 200 muscle samples of bovines, pigs and horses. Presence of the residues of phenylbutazone and oxyphenbutazone was confirmed in one sample.

Keywords: NSAIDs, LC-MS/MS, 2002/657/EC, phenylbutazone

F-28

LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY ANALYSIS OF COCCIDIOSTATS RESIDUES IN EGGS

Luigi Giannetti[1], Francesca Longo[2], Francesca Buiarelli[3], Mario Vincenzo Russo[4], Bruno Neri[5*]

[1 2 5] Istituto Zooprofilattico Sperimentale delle regioni Lazio e Toscana, 1411 Rome Italy
[3] Dipatimento di Chimica Università di Roma "La Sapienza" P.zzle Aldo Moro, 5 Rome Italy
[4] Facoltà di Agraria (DISTAAM) Università del Molise Via De Sanctis Campobasso Italy
[*] Corresponding author–E-mail: bruno.neri@izslt.it; Phone: +390679099430; Fax: +390679099420

The aim of the present work is the development of a rapid and effective analytical procedure for the determination of coccidiostats in eggs. Nicarbazin, robenidine, diclazuril, clazuril, toltrazuril and its metabolite were diluted by distilled water and subjected to clean-up by SPE styrene-divinylbenzene columns. Coccidiostats were eluted with methanol and determined by using HPLC-MS/MS. Separation was carried out on ODS column in gradient mode, with methanol–water mixture acidified with 0.1% formic acid as an eluent. Tandem mass spectrometry analysis was performed with turbo-ion spray ionisation in positive and negative ion mode by using multi reaction monitoring (MRM). The procedure has been successfully applied for eggs during routine and confirmatory analysis.

Validation was performed according to Commission Decision 2002/657/EC criteria by determining linearity, precision, recovery, specificity, decision limit (CCα) and detection capability (CCβ). The recovery of coccidiostats ranged from 80% to 94%, with R.S.D. of 15% for all coccidiostats under investigation.

The decision limit (CCα) ranged between 2.01 μg/kg and 6.34 μg/kg or all investigated coccidiostat; the detection capability (CCβ) ranged between 4.79 μg/kg, and 6.31 μg/kg for all investigated coccidiostats.

Keywords: coccidiostats, food, eggs, HPLC–MS/MS

F-29

DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF FIVE MACROLIDES IN EGGS AND MUSCLE USING LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY

Giulia Segato[1], Federica Gallocchio[2], Giancarlo Biancotto[3], Giovanni Binato[4], Cristiana Benetti[5*]

[1 2 3 4 5] Istituto Zooprofilattico Sperimentale delle Venezie
[*] Corresponding author–E-mail: cbenetti@izsvenezie.it; Phone: +39 049 8084335; Fax: +39 049 8830572

Macrolide antibiotics are widely used in veterinary medicine to treat respiratory diseases, or were administered as feed additives to promote growth in the past. These antibiotics are lipophilic molecules with a central lactone ring to which several amino and/or neutral sugar are bound.

They are well absorbed after oral administration and distribute extensively to tissue. Incorrect use of these drugs may leave residues in edible tissues and this can have undesirable effects on consumer health (e.g. development of allergic reactions, appearance of resistance bacteria etc.). Consequently, maximum residue limits (MRLs) have been established for these substances in animal products.

To guarantee the respect of the MRLs of these drugs in eggs and muscle, effective measures of control have to be carried out. For this aim a specific and sensitive screening method for five macrolides (spiramycin, erythromycin, tylosin, tilmicosin and josamycin) in eggs and muscle based on a HPLC-MS analysis was developed.

Different extraction procedures for both matrices were tested (with organic solvent and/or with aqueous buffers). For further clean-up different solid phase materials were examined (Reversed Phase and Strong Cation Exchange polymeric sorbents) and various elution solvent mixtures were also investigated to evaluate recovery and purification efficiency. Best results were obtained by an extraction with methanol/NaH_2PO_4 10mM pH 6 buffer 30/70 *v/v* and SPE clean up with Strong Cation Exchange polymeric sorbent.

Chromatographic separation was performed on SunFire C_{18} column (3.0 × 100 mm, I.D. 3.0 µm) using a gradient of aqueous ammonium acetate 10 mM pH 3.5 and acetonitrile as mobile phase at a flow rate of 0.25 ml min^{-1}.

The method was validated as a screening method according to the guidelines laid down by Commission Decision 2002/657/EC. Therefore performance characteristics, such as Detection capability (CCβ, < 25 µg/kg for both matrices), specificity, ruggedness were evaluated.

Keywords: macrolides, HPLC/MS, residues, 2002/657, CCß

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F-30

THE DETECTION OF VETERINARY RESIDUES IN MEAT USING LC/MS/MS ANALYSIS

Stephen Lock [1*], Donna Potts[2], Francisco Mocholí[3]

[1 2] Applied Biosystems, Warrington, UK
[3] SAILab, Barcelona (SPAIN)
[*] Corresponding author–E-mail: stephen.lock@lifetech.com; Phone: +447720276948

Introduction: Penicillin's and Tetracycline's are veterinary medicines which are used to improve the health of live stock and as such are found as residues in Meat but also in other food ingredients e.g. Honey which are used across the European Union. New legislation for these compounds has recently come into effect in Spain for the export of meat to Russia which necessitates the use of new techniques such as LCMSMS over existing techniques such as UV-VIS detection. This poster presents data on an analysis method using an API3200™ LCMSMS System where these detection limits for these residues are below current EU legislation of 100 parts per billion.

Method: Meat extracts were prepared initially using off line using solid phase extraction. Separations of penicillins such as Penicillin, Oxacillin, Cloxacillin, Nafcillin, Dicloxacillin and tetracyclins such as Tetracycline, Chlortetracycline, Oxy Tetracycline, Doxycycline & Methacycline were performed using a Phenemonex Synergi MAX™ HPLC column and a gradient elution using methanol and water. Detection was by positive Electrospray ionisation (Tetracycline) or negative electrospray ionisation (penicillins).

Preliminary data: The initial preliminary data has shown that we can detect these veterinary residues at the required levels using an off line solid phase extraction followed by LC/MS/MS analysis. CVs are good typically less than 10–15% at the limit of quantitation. Further studies investigating the use of on-line solid phase extraction as a way to reduce sample clean up and speed up the analysis time are ongoing.

Keywords: Veterinary residues, LC/MS/MS

F-31

MULTI-RESIDUE DETERMINATION OF SEVENTEEN SULFONAMIDES AND FIVE TETRACYCLINES IN FISH TISSUE USING A MULTI-STAGE LC-ESI-MS/MS APPROACH BASED ON ADVANCED MASS SPECTOMETRIC TECHNICS.

Marilena Dasenaki[1], Nikolaos Thomaidis[2*]

[1 2] Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis Zographou, 15771 Athens, Greece
[*] Corresponding author–E-mail: ntho@chem.uoa.gr; Phone: 0030 210 7274317

The extensive use of antibiotics in veterinary medicine has led to an essential monitoring of their residues in foods. Two of the most commonly used groups of antibiotics are sulfonamides and tetracyclines and their determination in foodstuffs is of particular concern because of their potential carcinogenic character. To ensure the safety of food for consumers, Regulation 2377/90 of the EU Commission has laid down maximum residue limits of 100 ng g^{-1} of both (total) sulfonamides and tetracyclines in fish tissue.

In this work, a strategy was newly developed to rapidly screen seventeen sulfonamides (sulfadiazine, sulfathiazole, sulfamerazine, sulfadimidine, sulfamethoxypyridazine, sulfamonomethoxine, sulfachloropyridazine, suladoxine, sulfaclozine, sulfadimethoxine, sulfamethizole, sulfamethoxazole, sulfisoxazole, sulfaguanidine, sulfapyridine, sulfamoxole and sulfaquinoxaline) and five tetracyclines (oxytetracycline, tetracycline, chlorotetracycline, doxycycline and demeclocycline) in a single run from the fish tissue using ultra-high-performance liquid chromatography (UHPLC) coupled with comprehensive mass spectrometric approaches including precursor ion scan and data dependent scan.

The product ions for precursor-ion scanning were selected by studying the MS/MS fragmentation of the analytes. All sulphonamides share the same diagnostic product ion at *m/z* 156 in positive MS/MS scan while for tetracycline antibiotics the diagnostic product ion was proved to be at *m/z* 153.8. Further characterization of each compound was performed using a data dependent scan. The collision energy selected for the fragmentation of each compound to the product ion was optimised as well as the ion-abundance threshold settings for data-dependent acquisition. Separation was performed on a Zorbax Eclipse Plus C18 column (50 mm × 2.1 mm, 1.8 μm) with a gradient elution using acetonitrile – 0.1% formic acid mobile phase at a flow rate of 0.2 mL min^{-1}

This approach has proven to be a powerful, highly selective, and sensitive tool for rapid screening and detection of non targeted components in fish tissue and requires a minimum sample preparation such as one extraction step and a clean up step using dispersive SPE.

Keywords: veterinary, antibiotics, screening, mass spectrometry

F-32
DETECTION OF VIRGINIAMYCIN IN POULTRY MEAT BY IMMUNOASSAY

Chen Situ[1*], Stewart Graham[2], Jun Yang[3], Rodat Cunningham[4], Chris Elliott[5]

[1 2 4 5] Queen's University Belfast
[3] Inner Mongolia Agricultural University
[*] Corresponding author–E-mail: c.situ@qub.ac.uk; Phone: +442890976546; Fax: +442890976513

Virginiamycin is a streptogramin polypeptide antibiotic produced by *streptomyces virginiae*. As one of most extensively used antimicrobial growth promoters (AGPs) in mordent agricultural food production, virginiamycin has been incorporated into animal feedstuffs at rates ranging from 5–80 mg kg^{-1} varying in different species for the past several decades. However due to the increasing concern on the link between the overuse and misuse of AGPs in animal productions and the spread and emergence of antibiotic resistance, all AGPs have been banned in the European Union (EU) since 2006. Nevertheless, virginiamycin is still permitted in other countries that produce and export large amount of food stuff of animal origin to the EU. In order to effectively monitor the compliancy of the EU-wide ban and to assure the commodity products imported to the EU are free of virginiamycin, a reliable detection system is requisite. The current detection methods are either the traditional microbiological screening which are lack of specificity or the analytical analysis which does not allow a rapid testing of large samples. We report here is the development of immunoassays (ELISA and biosensor) for rapid determination of virginiamycin in poultry meats that can be used for screening both EU produced and import food products.

Keywords: virginiamycin, immunoassay, antibiotic growth promoter

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F-33

MULTI RESIDUE ANALYSIS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN KIDNEY USING LCMSMS.

John Points[1*], Ratharamaran Ganeshasunderam[2], Nicoletta Castiglione[3], Liam Gormley[4]

[1 2 3 4] LGC, Teddington, UK
[*] Corresponding author–E-mail: john.points@lgc.co.uk; Phone: +44 208 943 8429

Analysis for residues of veterinary medicines in animal products is required by European legislation as their use may leave residues in edible tissue and the residues may have direct toxic effects on consumers. To ensure human food safety, the European Union (EU) laid down maximum residue limits (MRLs) for numerous substances, including several non-steroidal anti-inflammatory drugs (NSAIDs).

The method presented is a multi-residue screening and confirmatory method for the analysis of 10 NSAIDs in kidney using LC-MSMS. Kidney samples are homogenised and then hydrolysed using potassium hydroxide solution. The hydrolysed extract is then acidified before purification using mixed mode polymeric anion exchange solid phase extraction cartridges. The samples are then analysed using LCMSMS. Performance criteria have been calculated in accordance with Commission Decision 20052/657/EC for Carprofen, Diclofenac, Flunixin, Ibuprofen, Meloxicam, Naproxen, Niflumic acid, Phenylbutazone, Tolfenamic acid and Vedaprofen.

Keywords: NSAIDs, Veterinary Drug Residues, LC-MSMS

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F-34

IDENTIFICATION OF METABOLITES OF BRILLIANT GREEN IN TROUTS USING LTQ-ORBITRAP

Dominique Hurtaud-Pessel[1*], Pierrick Couedor[2], Michel Laurentie[3], Eric Verdon[4]

[1 2 3 4] AFSSA, LERMVD, Fougeres, FRANCE
[*] Corresponding author–E-mail: d.pessel@afssa.fr; Phone: +33-(0)-2-99-94-78-78; Fax: +33-(0)-2-99-94-78-80

The triphenylmethane dyes, Malachite Green (MG) and Crystal Violet (CV) are sometimes used illegaly as antimicrobial, antifungal and antiparasitic agents in aquaculture. These compounds are not authorized in food animal production because of their toxic effects on humans (mutagenic, carcinogenic). In vivo, MG and CV are rapidely metabolized in treated fish in their leuco forms by reduction. Another triphenylmethane compound, the Brilliant Green (BG), also called Malachite Green G, displays a similar chemical structure as MG and CV, and therefore might hold the same toxic effects. There is no such literature data on the metabolism of BG as for MG and CV, but it is presumed to metabolize in its leuco form in vivo in treated fish as well. The present study was intended to identify possible metabolites of brilliant green in trout tissues by mass spectrometry measurement using an LTQ-Orbitrap instrumentation. Trouts (Oncorhynchus mykiss) were incurred with brilliant green and flesh tissues were collected. Extracts were analysed by liquid chromatography/high resolution mass spectrometry. The presence of the leuco brilliant green form was confirmed in fish extracts by exact mass accuracy measurement, by fragmentation and comparison with synthetic chemical standard of LBG obtained from a local chemical company. Metabolomic approach was investigated to identify other possible metabolites of BG.

Keywords: dyes, brilliant green, metabolites, LTQ-Orbitrap

F-35

TRISENSOR: RAPID TEST DETECTING β-LACTAMS, SULFAMIDES AND TETRACYCLINES FAMILIES AT THE SAME TIME

Benoit Lemmens[1], Vincent Chabottaux[2*], Céline Bonhomme[3], Marie Gourmet[4], Javier Adrian[5], M.-Pilar Marco[6], Benoit Granier[7]

[1 2 3 4 7 1]UNISENSOR S.A., Zoning industriel du Dossay, Rue du Dossay n°3, B-4020 Liège, BELGIUM
[5 6] Applied Molecular Receptors Group, CIBER-BBN, IQAC-CSIC, Jordi Girona 18-26, 08034 Barcelona, SPAIN.
[*] Corresponding author–E-mail: vincent.chabottaux@unisensor.be; Phone: +32 (0) 4 252 66 02; Fax: +32 (0) 4 252 90 55

β-lactams, sulfamides and Tetracyclines are the 3 main families of antibiotics widely used by farmers to control or prevent animal bacterial diseases. Due to their human harmfulness and in respect to EU regulations, efficient controls of these antibiotics residues in food and especially in milk are needed. We have therefore developed the first rapid and easy screening test able to detect the main compounds of the 3 families of antibiotics at the same time in milk or any other matrices.

This multiplex dipstick test is a Lateral Flow (LF) assay using specific receptors and generic monoclonal antibodies. The results are visualized at the 3 specific capture lines by the use of colloidal gold-conjugates.

For the milk, it takes 6 minutes to run and does not require any cleaning or sample preparation. It is able to detect (LoD) the 3 families of antibiotics at concentrations near their respective Maximum Residue Limit (MRL) values. Accurate results can be either read by visual observation or immediately given by an optical reader that suppresses any subjectivity from the users.

In conclusion, we are thus presenting the first rapid test based on dipstick format that is able to detect more than 30 antibiotics in one analysis.

Keywords: Dipstick, Milk, β-lactams, Sulfamides, Tetracyclines

F-36

ANALYSIS OF PHARMACEUTICAL COMPOUNDS AND FRAGRANCES IN THE AQUATIC MEDIA: PATHWAYS FROM WASTEWATERS TO DRINKING WATERS

Maria Augusta Dionísio de Sousa[1*], Carlos Gonçalves[2], Jana Hajslova[3], Maria de Fátima Alpendurada[4]

[1 2 4] Faculty of Pharmacy, University of Porto, Laboratory of Hydrology /IAREN,Porto,Portugal
[3] ICT Prague – Institute of Chemical Technology, Prague, Department of Food Chemistry and Analysis, Prague,Czech Republic
[*] Corresponding author–E-mail: asousa@iaren.pt; Phone: +351 229 364 210; Fax: +351 229 364 219

There is a vast array of anthropogenic contaminants targeted in water resources. Among these are the so-called “emerging contaminants”, including pharmaceuticals and personal care products, used in a growing scale. Due to their high environmental persistence and capacity to induce specific pharmacological and physiological effects at low doses, they have become an environmental problem, spawning a new generation of water quality issues. Despite the fact that wastewater effluents are only one potential source, they are seen as the main pathway for these compounds to reach the aquatic environment, once most wastewater treatment plants (WWTPs) are not prepared for their elimination. The impact on river water quality is highly relevant to environmental risk assessment and is often reflected by unpredictable effects in wildlife and human health. The concentrations of 24 pharmaceuticals among ansyolitics, anti-inflamatories, antibiotics, lipid regulators, diuretics, anti-depressives, anti-ulcer agents, anti-diabetics and cardiotonics, 4 fragrances and 4 bad odours were first determined in effluents from 2 WWTPs (Febros and Areínho). They are located in Gaia (Porto, Portugal), and discharge their effluents into Douro river, the source of drinking water supplied to over a million people. Pharmaceuticals were analysed by LC-MS-MS. In the case of surface waters, it was preceded by sample extraction using a recent adsorbent of polymeric nature (JTBaker H2Ophilic), along with a soft clean-up strategy using Di-NH2, whenever needed, whereas to wastewaters we used Oasis MAX cartridges, serving both purposes due to their mixed composition. Fragrances were analysed by GC-MS in full scan and MS-MS modes, providing different LODs, precision and confirmation degrees. Results showed that hydrochlorothiazide, furosemide, bisoprolol, ketoprofen, naproxen, nimesulide, diclofenac, bezafibrate, gemfibrozil, alprazolam and bromazepam were detected in some effluents. Concerning surface water, among the twelve sampling spots chosen in Douro river, we detected just a few compounds at trace levels. In one site upstream of Febros WWTP we detected hydrochlorothiazide in the concentration of 31.4 μg L^{-1}, whereas in another site downstream of both WWTPs, we found paracetamol and ketoprofen, in the concentrations of 62.6 and 10.9 μg L^{-1}, respectively. Others, such as bisoprolol, diazepam and gemfibrozil were detected upstream of both WWTPs, though their concentrations were bellow the LOQ. The same happened downstream the WWTPs with paracetamol, azithromycin and simvastatin. The analysis of drinking water revealed the absence of all compounds. The drinking water treatment plant is located upstream of the WWTPs, thus eliminating these sources of contamination.

Keywords: pharmaceuticals, drinking water, wastewater

F-37

AN LC-MS/MS HIGH THROUGHPUT SCREENING METHOD FOR THE DETERMINATION OF ANTIBIOTICS IN MILK

Ruud Peters[1], Alida Stolker[2], Joe DiBussolo[3], Richard Zuiderent[4], Cláudia Martins[5*]

[1 2] RIKILT Institute of Food Safety
[3 4 5] Thermo Fisher Scientific
[*] Corresponding author–E-mail: claudia.martins@thermofisher.com; Phone: 0034607989052; Fax: 0034932230982

Veterinary drugs are being used on a large scale and administered as feed additives or via drinking water to prevent the outbreak of diseases. As a response to this, large numbers of food samples are analyzed and new, cost-effective methods have to be developed for these compounds. The combination of high sample throughput with high sensitivity and reproducibility is the focus of many recently developed methods. We propose the combination of TurboFlow technology and Liquid Chromatography-Tandem Mass Spectrometry for an efficient screening of multi-class antibiotics in milk.

The advantage of this method is the drastic reduction of sample preparation time when comparing with the normal offline sample preparation methods. The automated on-line extraction was performed using TurboFlow technology which exploits the difference between large and small molecules and column chemistry to retain compounds of interest while matrix molecules flow to waste.

Albendazole, difloxacin, oxytetracycline, phenylbutazone, salinomycin, spiramycin, sulfamethazine and tetracycline were detected and quantified by LC-ESI-MS/MS in positive selective reaction monitoring mode (SRM). Peak areas of spiked milk samples were compared to those of neat standards to assess matrix interferences and carry over. Even without internal standards, quantitative results proved to be linear in the concentration range of 5 to 500 µg/L as well as reproducible and precise (RSD 0.4–14%). The limits of detection were between 0.1 and 5.2 µg/L. Also, the results obtained show good reproducibility even when testing different milk brands. Higher fat content seem to influence the precision of the method only at the highest level of the range studied (500 µg/L). At the 100 µg/L level, the matrix effect is minimal for salinomycine Na, spiramycin, tetracycline, oxytetracycline and sulphametazine. However, values between 70 and 85% were found for phenylbutazone, albendazole and difloxacin.

The method was tested by simulating a routine screen procedure with real milk samples. The result was the detection of all the antibiotics present in the sample.

The developed automated TurboFlow-LC-MS/MS method permits simple sample preparation while minimizing matrix interferences for the detection of various antibiotics in milk products down to ug/L levels. Accurate quantitation of those compounds subject to residual matrix interferences could be accomplished by using a suitable internal standard.

Keywords: antibiotics, milk, screening, LC-ESI-MS/MS

F-38

ADAPTATION OF A MICROBIOLOGICAL METHOD FOR SCREENING ANTIMICROBIAL RESIDUES IN SHRIMP TISSUE

Dang Pham Kim[1], Guy Degand[2], Sophie Danyi[3], Guy Maguin-Rogister[4], Marie-Louise Scippo[5*]

[1] Hanoi University of Agriculture (HUA), Hanoi, Vietnam
[2 3 4 5] University of Liège, Liège, Belgium
[*] Corresponding author–E-mail: mlscippo@ulg.ac.be; Phone: +32 4 366 40 46; Fax: +32 4 366 40 54

Vietnamese aquaculture products are exported in more than 150 countries and therefore, need to be tightly controlled all the stages of aquaculture production. To control antibiotic residues, the export aquaculture products in general and shrimp products in particular are nowadays tested directly by quantitative confirmatory methods, which are heavy and expensive techniques. For these reasons, the number of analyzed samples is rather low and products for local consumers are not well controlled. To resolve these difficulties, it could be interesting to use screening cheaper methods such as microbiological inhibition tests in the residue control strategy, like in many other countries. However, microbiological inhibition screening tests play an important role to detect residues of antimicrobials in various animal food products, but there are not yet applied to control aquaculture products in general, and shrimps in particular. We have adapted a microbiological method, very useful, cheap for Vietnamese control laboratories, to screen shrimp for residues of the most commonly used antibiotics. The method was validated according to the Decision N° 2002/657/CE. *Bacillus subtilis* was used as a sensitive strain to target antibiotics. Culture conditions (pH of medium) on Petri plates were optimized to enhance the capacity of antibiotic detection. Shrimps were extracted using acetonitrile/acetone (70:30 *v/v*). After solvent evaporation, residues were dissolved in methanol before application on Petri plates seeded with B.subtilis. The method was validated for various antibiotics using spiked blank shrimp tissues, as well as with real contaminated shrimps with enrofloxacin and tetracyclin, two antibiotics often found to be used in shrimp production. For tetracyclines and quinolones, the limit of detection was below the maximum residue limit (MRL), while for sulfonamides, the limit of detection was around the MRL. The capacity of detection was confirmed on real contaminated shrimps, analyzed in parallel with a confirmatory method (LC-MS for enrofloxacin and HPLC for tetracyclin). The results of LC-MS and HPLC also showed that this method was able to detect contaminated samples, with no false negative results.

Keywords: Antibiotics, tetracyclines; (fluoro)quinolones, sulfamides, shrimp

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F-39

PRACTICAL EXPERIENCES GAINED FROM THE ROUTINE USE OF UPLC-TOF IN ANTIMICROBIAL RESIDUES SURVEILLANCE IN ANIMAL PRODUCTS

Liam Gormley[1*], Leonardo Firpo[2], Chris Hopley[3]

[1 2 3] LGC, Teddington, UK
[*] Corresponding author–E-mail: liam.gormley@lgc.co.uk; Phone: +44 (0) 208 943 7621; Fax: +44 (0) 208 943 7599

Analysis for residues of veterinary medicines in animal products is required by European legislation. Screening methods are often used in order to detect the presence of a group or class of substances at an agreed level. Any samples that screen positive using these tests are then subjected to further confirmatory testing. These methods are usually high throughput and the designed to avoid false negatives.

A considerable proportion of veterinary residues surveillance is focussed on antimicrobial compounds. A considerable proportion of this surveillance is focussed on antimicrobial compounds. Microbiological inhibition assays are commonly used for screening analysis as they are cost effective and are sensitive to a wide range of antimicrobial compounds. The disadvantage to these tests is that may only give a broad indication of the identity of the residue and further tests are usually required to obtain more information prior to a confirmatory analysis being undertaken. Other methods such as are HPLC and LC-MS/MS also used for screening analysis however the scope of these can be more restricted.

Advances in chromatography have lead to improved resolution, reduced run time and enhanced sensitivity. When coupled to time of flight mass spectrometry equipment the possibility of high throughput screening of a wide range of compounds is a realistic option in routine surveillance programmes.

In the UK Statutory Surveillance Scheme the use of UPLC-TOF has been used in parallel to existing screening technologies including microbial inhibition assays and commercially available testing kits for over 18 months. Sample matrices including kidney, muscle, milk, fish and eggs have been tested. A generic extraction method is used, followed by targeted analysis for a range of more than 50 antimicrobial compounds including beta-lactams, cephalosporins, macrolides, quinolones, sulphonamides and tetracyclines. A total of nearly 4000 samples have been tested to date. The next steps are to expand the scope of compounds covered by the test.

Keywords: UPLC-TOF Antimicrobial Generic Screening

Veterinary Medicines Directorate, UK

F-40

THE DETECTION OF ANTIBIOTIC RESIDUES IN MEAT BY AUTOMATED SPE-LC/MS/MS ANALYSIS

Francisco Mocholí[1*], Stephen Lock[2]

[1] SAILab, Barcelona, Spain
[2] Applied Biosystems, Warrington, UK
[*] Corresponding author–E-mail: Stephen.lock@lifetech.com; Phone: +447720276948

Penicillins as well as tetracyclins and sulphonamides are antibiotic veterinary medicines which are used to improve the health of live stock. They are found as residues in meat but also in other food ingredients e.g. Honey which are used across the European Union. New legislation has recently come into effect in Spain, for the export of meat to Russia, which requires the use of techniques such as LC-MS/MS to reach detection limits for these residues below the current EU legislation of 100 parts per billion. This talk presents data collected using an API 3200™ LC-MS/MS where these required detection limits are reached. The method combines on-line solid phase analysis with LC-MS/MS detection to enable a fast and automated approach to the detection of antibiotics in meat.

Keywords: LC-MS, Antibiotic Residues, Meat

F-41

INCREASING THE SELECTIVITY OF CLENBUTEROL DETECTION IN URINE SAMPLES BY USING LC/MS/MS IN MRM3 MODE

Jan Lembcke[1], Loren Olson[2], Thomas Korba[3], Andre Schreiber[4*]

[1 2 3 4] Applied Biosystems
[*] Corresponding author–E-mail: andre.schreiber@lifetech.com; Phone: +1-905-660-9006

The selectivity of MSMS in residue analysis has enabled users to decrease significantly the analysis time. In the majority of cases, single chromatographic peaks will be observed with triple quadrupole operated a unit resolution. However, as is the case with clenbuterol urine samples, the presence of endogenous species leads to interferences which negatively affect the LOQ. To improve selectivity, several solutions have been proposed; 1) improve chromatographic separation, 2) increase resolution to improve parent mass selection, and 3) using LC-FAIMS-MSMS. Here we propose an alternative to improve specificity in quantitative applications; using MRM3 on a hybrid quadrupole-linear ion trap (QqLIT). The concept is to rely on the selectivity of the fragmentation pathway. Clenbuterol generates 3 majors fragments that can be monitored in quantitative analysis; 203, 168 and 132. However, in the majority of the urine samples analyzed in this study, interferences were observed in each one of the MRM transitions. However, fragment 168 and 132 are both formed via fragment ion 203 as an intermediate. Therefore MS^3 was used to increase the selectivity of clenbuterol detection with MS^3 without changes in the LC condition or addition of hardware to the LC-MSMS system. The detection limit was improved by 4x and single LC peaks were detected (improved selectivity). Details on the operational consideration for such analysis as well as validation results for this study will be presented.

Keywords: Clenbuterol, MRM3, LC/MS/MS

NATURAL TOXINS, MYCOTOXINS

(G-1 – G-40)

G-1

THE APPLICATION OF SURFACE PLASMON RESONANCE FOR THE DETECTION OF THE TRICHOTHECENE MYCOTOXINS T-2 AND HT-2 IN CEREALS

Julie Meneely[1*], Julie P. Meneely2, Michael Sulyok3, Sabine Baumgartner4, Rudolf Krska5, Christopher T. Elliott6, Hans P van Egmon7d

[1 2 6] Institute of Agri-food and Land Use, School of Biological Sciences, Queen's University of Belfast, David Keir Building, Stranmillis Road, Belfast BT9 5AG.
[3 4 5] IFA-Tulln, University of Natural Resources and Applied Life Sciences, Vienna, Konrad Lorenzstr. 20, 3430 Tulln, Austria.
[7] National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
* Corresponding author–E-mail: j.p.meneely@qub.ac.uk; Phone: +44 2890 976562; Fax: +44 2890 976513

The trichothecenes are a major class of mycotoxins produced by various fungal genera including *Fusarium*, *Cephalosporium*, *Myrothecium*, *Trichoderma*, *Stachybotrys* and *Verticimonosporium*. The most significant fungal genus is *Fusarium* which not only produces the greatest range of toxins but is globally distributed. T-2 and HT-2, although not the most commonly occurring trichothecenes in cereals are amongst the most toxic. The mode of action is the inhibition of protein synthesis and symptoms of infection include dermal irritation, vomiting, haemorrhagic lesions, pathological changes in haematopoietic organs and depression of immune response. Currently no European legislation exists for these mycotoxins, however it is anticipated that these will be agreed in the near future and the legislation will relate to the sum of T-2 and HT-2 simply because T-2 is rapidly de-acetylated to HT-2 in vivo and the acute toxicities are within the same range. It is also believed that the levels set, particularly for infant food will be low reflecting these toxicities, therefore highlighting the need for rapid and sensitive analytical techniques capable of supporting the regulatory demands.

A sensitive and accurate surface plasmon resonance (SPR) screening assay with minimal sample preparation has been developed for the detection of T-2 and HT-2 toxins in naturally contaminated cereals using an HT-2 toxin sensor chip and a monoclonal antibody.

The antibody displayed very high specificity for HT-2 and T-2 toxins. There was no cross-reaction with other commonly occurring trichothecenes. The assay was validated with respect to limit of detection (LOD), recovery and precision, expressed as coefficient of variation (% CV) for both within-run (intra assay) and between runs (inter assay). Samples included maize derived baby food, wheat based breakfast cereal, and unprocessed wheat samples. Limits of detection (LOD) for each matrix were determined as 25 μg kg^{-1} for baby food and breakfast cereal and 26 μg kg^{-1} for wheat. The intra-assay precision (n=6) was calculated for each matrix at two concentration levels. The results were determined as 2.8 (100 μg kg^{-1}) and 1.8% (200 μg kg^{-1}) in breakfast cereal, 4.6% (50 μg kg^{-1}) and 3.6% (100 μg kg^{-1}) in wheat and 0.97% (25 μg kg^{-1}) and 6.3% (50 μg kg^{-1}) in baby food. Inter assay precision (n=3) performed at the same concentrations were calculated as 6.67% and 3.9% for breakfast cereals, 3.3% and 1.6% for wheat and 6.8% and 0.1% for baby food respectively.

Keywords: T-2, HT-2, Surface Plasmon Resonance

G-2
DETERMINATION OF MOLDS AND MYCOTOXINS IN FEEDSTUFF FORMULA AND POULTRY PRODUCTS

Kingkeaw Charoenpornsook[1*], Pilai Kavisarasai[2], Kanjana Motina[3]

[1 3] Thammasat University,Pathumthani,Thailand
[2] Bureau of Quality Control of Livestock Products,Pathumthani,Thailand
[*] Corresponding author–E-mail: KUN99@alpha.tu.ac.th; Phone: 66-02-564-4486; Fax: 66-02-564-4486

Mycotoxins are carcinogen which is very harmful to humans and animals. So, the main purpose of this study was to investigate the possible co-incident of mycotoxins with molds, percentage of corn, percentage of soybean and percentage of fishmeal in animal feedstuff formulas. The study was divided into three parts. The first part was to determine and identify the genus of mold in each feedstuff formulas. The second part was to determine the mycotoxins contamination in feedstuff formulas including to find out the relationship among molds with mycotoxins, percentage of corn, percentage of soybean and percentage of fishmeal in animal feedstuff formulas. The last part was to determine the residues of mycotoxins in poultry products, meats, livers and eggs from the experimental chickens which were feed with the desired feedstuff formulas. For the first part of the study, the results showed that all feedstuff formulas samples were contaminated with molds. They are *Aspergillus. Spp*., *Penicilium. Spp* and non-septate fungi. Using Pearson correlation coefficient statistical analysis, the results revealed that there were no correlation among molds with percentage of corn, percentage of soybean and percentage of fishmeal in animal feedstuff formulas. For the second part of the study, the results showed that mycotoxins, Aflatoxin B1, B2, Ochratoxin A, Deoxynivalenol and T-2 toxin, were found in all animal feedstuff formulas but the concentration of mycotoxins were lower than MRL. Using Pearson correlation coefficient for statistical analysis revealed that there were correlation among mycotoxins with percentage of corn, percentage of soybean and percentage of fishmeal in animal feedstuff formulas, but no correlation between molds and mycotoxins. For the last part of the study, the results showed that there were no any residues of mycotoxins were found in meats, livers and eggs.

Keywords: Mycotoxins, Feedstuff, formulas, Poultry

The National Research Council of Thailand (NRCT)

G-3
DETERMINATION OF OCHRATOXIN A IN LOCAL ROASTED COFFEE IN THAILAND

Kingkeaw Charoenpornsook[1*], Pilai Kavisarasai[2], Kanjana Motina[3]

[1 3] Thammasat University,Pathumthani,Thailand
[2] Bureau of Quality Control of Livestock Products,Pathumthani,Thailand
[*] Corresponding author–E-mail: KUN99@alpha.tu.ac.th; Phone: 66-02-564-4486; Fax: 66-02-564-4486

Ochratoxin A (OTA) is a mycotoxin which are naturally occurring chemical compounds produced by certain fungi such as *Aspergillus ochraceus*, *Aspergillus niger,* and *Aspergillus carbonarius* which are known to produce OTA in coffee. OTA has been proved to cause a threat to humans and animals when ingested. It has been frequently detected in fresh and dried commodities, fresh grapes, dried vine fruit, wine, beer including coffee. Therefore coffee becomes the favorite drink for people around the world including Thai. So the aim of this study was to determine the possibility of contamination of OTA in local coffee in Thailand. The 52 local coffee samples were purchased from markets around in Bangkok during 2007–2008. Samples were determined for OTA by HPLC after clean-up by immunoaffinity column. The result showed that no OTA was found in all samples. The method was employed to analyze OTA in roasted coffee and resulted% recovery quite low, 53.5%. This may be due to the interferences during extraction which may affect on OTA in roasted coffee determination or the process of converting green coffee to roasted and soluble coffee can be 90% reduction in OTA level. However, it can be concluded from this study that the situation of OTA contamination of local roasted coffee in Thailand is low and safe for food or feed.

Keywords: Ochratoxin A, roasted coffee

Thammasat University and The National Research Council of Thailand (NRCT)

G-4
CHEMOPREVENTION OF AFLATOXIN PRODUCTION BY ZATARIA MULTIFLORA BOISS. ESSENTIAL OIL

Hassan Gandomi [1*], Ali Misaghi[2], Afshin Akhondzadeh Basti[3], Saeed Bokaei[4], Ali Khanjari[5]

[1 2 3 4 5] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[*] Corresponding author–E-mail: gandomih@ut.ac.ir; Phone: +98-2161117042

Background: Aflatoxin-producing moulds are capable to produce a group of secondary metabolites such as aflatoxins, which are known to be potent mutagenic, carcinogenic, teratogenic, hepatotoxic and immunosuppressive, and widely distributed in nature and frequently contaminate human food resources. The use of natural antimicrobial compounds including plant extracts, spices, and their constituents may provide an alternative for synthetic fungicides, which their safety in foods is debated, to prevent fungal growth and aflatoxin formation.

Objective: In this study the effect of Zataria multiflora Boiss. essential oil on growth of Aspergillus flavus and spore production were investigated in yeast extract sucrose broth medium.

Materials and methods: Erlenmeyer flasks containing yeast extract sucrose broth and different concentrations of EO were inoculated with 10^4 fungal spores and incubated for 10 days at 26 ± 1°C. After the incubation period, cultures were filtered through whatman No. 1 filter paper. The mycelia were dried to a constant weight at 80°C and the weight of dried mat was estimated. Determination of aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) performed by RP-HPLC.

Results: The EO significantly suppressed mycelial growth and aflatoxin synthesis in broth medium at all concentrations tested. At 50 ppm, the mycelial dry mass and aflatoxin production diminished by 22.6 and 31%, respectively. Moreover, at 150 ppm of EO, the mycelial growth and aflatoxin accumulation reduced by 90 and 99.4%, respectively.

Discussion: The results of the present work showed the inhibitory effect of EO used on aflatoxin production. The inhibitory effect of EO on aflatoxin formation was greater than that on mycelial growth. Moreover, the EO had a reducing effect on ratio of total aflatoxins per mycelial dry weight indicating whatever the concentration of the EO increases not only the mycelial growth is prevented but also the ability of aflatoxin production by remaining mycelia is decreased. In conclusion, results showed that *Z. multiflora* Boiss. essential oil could be safely used as a natural preservative on foods at low concentrations to protect them from fungal infections and aflatoxin production.

Keywords: Aflatoxin, Mycelial mass, *Zataria multiflora* Boiss. essential oil, *Aspergillus flavus*

G-5
CHEMOPREVENTION OF AFLATOXIN PRODUCTION BY ZATARIA MULTIFLORA BOISS. ESSENTIAL OIL

Ali Misaghi[1*], Afshin Akhondzadeh Basti[2], Hassan Gandomi [3], Saeed Bokaei[4], Ali Khanjari[5]

[1 2 3 4 5] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[*] Corresponding author–E-mail: misaghia@vetmed.ut.ac.ir; Phone: +98-2161117042

Background: Fungi are significant spoilage microorganisms of foodstuffs during storage, rendering them unfit for human consumption by retarding their nutritive value and sometimes by producing mycotoxins. Essential oils are 'eco-friendly' and harmless to humans, therefore at present there is an increasing attention, both in industry and academic research, to medicinal and aromatic plants for their potential antimicrobial effects.

Objective: in this study the effect of *Zataria multiflora* Boiss. essential oil against growth and spore production of *Aspergillus flavus* ATCC 15546 were investigated in PDA medium.

Materials and methods: Effect of EO on radial growth of mycelium was assayed using an agar dilution method. PDA plates containing different concentrations of EO were inoculated with spore suspension at the center and incubated for 10 days at 26 ± 1°C and the average of two perpendicular diameters of colony was daily calculated. For spore production assay, PDA plates containing different concentrations of EO were spreaded evenly with 10^5 spores and incubated for 7 days at 26 ± 1°C. The fungal spores produced in each dish were collected and conidial concentration was estimated by a haemocytometer.

Results: The radial growth reduced by 21.3% and 79.4%, at 50 and 200 ppm, respectively. The growth was completely prevented at EO ≥ 400 ppm on PDA, and minimum fungicidal concentration (MFC) of the oil was estimated at 1000 ppm. Moreover, Spore production was inhibited by 21.7% and 92.5%, at 50 and 200 ppm, respectively.

Discussion: the results of this study showed that EO effectively inhibited radial growth and spore production in a dose-dependent manner. Moreover, the effect of EO on spore production was greater than on radial growth. Reduction of spore production could limit the spread of pathogens by lowering the spore load in the storage atmosphere and on surfaces. The results showed that *Z. multiflora* Boiss essential oil has an antifungal activity and can be used as a potential mold inhibitor in foods.

Keywords: Fungal growth, Spore production, *Zataria multiflora* Boiss. essential oil, antifungal effect

G-6
OCHRATOXIN A CONTENT IN URINE FROM PORTO INHABITANTS: IS THERE A RELATIONSHIP WITH SEASON?

S. Duarte[1], J. Bento[2], A. Pena[3], C. M. Lino[4*], C. Delerue-Matos[5], T. Oliva-Teles[6], S. Morais[7], M. Correia[8], M.B.P.P. Oliveira[9], S. Casal[10]

[1 2 3 4] Group of Bromatology, Center of Pharmaceutical Studies, CEF, University of Coimbra, Health Sciences Campus, Azinhaga de Santa Comba, 3000-548, Coimbra, Portugal
[5 6 7 8] REQUIMTE, Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto
[9 10] REQUIMTE, Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, Rua Anibal Cunha, 164, 4099-030, Porto, Portugal
* Corresponding author–E-mail: clino@ff.uc.pt; Phone: +351 239488477

Ochratoxin A (OTA), that together with aflatoxins launched the distinctive and different discipline of mycotoxicology in the early 60's, remains one of the most important fungal toxins for the noteworthy toxic effects reported – carcinogenicity, nephrotoxicity and immunotoxicity. To study its occurrence and exposure risk in humans, two key approaches can be attempted: analysis of foodstuffs (the main source of contamination) or biological fluids. The latter approach proved to be accurate, and improvement on the understanding of OTA toxicokinetics allowed the estimation of the daily intake through the calculation of the OTA serum concentration. Though such inference has not yet been attained for OTA urine analysis, the latter has proven to be a better indicator of OTA consumption than plasma, besides entailing a more rapid and non-invasive procedure of collection. Data on the relationship of OTA urine content with seasonal variation is also lacking.

In an endeavor to contribute to this knowledge, a randomized urine sampling was carried out in 49 healthy Porto inhabitants (north of Portugal), in two different seasons: winter 2007 and summer 2008. The reference method was a previously developed methodology by Pena *et al*. (2006) that involved immunoaffinity clean-up and spectrofluorimetric detection.

The incidence and average level of contamination was higher in the winter than in the following summer (96.7 *versus* 57.9% and 0.021 *versus* 0.017 ng/ml). Furthermore, two of the urine samples collected during the summer presented values (0.1069 and 0.1970 ng/ml) considered outliers, and were thus removed from the casuistic study.

Climatic factors may justify this discrepancy in the values found between the two periods, namely the temperature and humidity in which the raw materials and food derivates are kept before processing, as well as dietary ones, since the kind, the nature, and amount of foodstuffs eaten during each season are different.

In conclusion, with this work it comes into view that Porto inhabitants are subjected to different risks of exposure to OTA according to the season considered, which may arise from climatic and dietary variations that need further scrutiny.

Keywords: ochratoxin A, urine, season, Porto

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G-7
OCHRATOXIN A CONTAMINATION OF BREAD–PORTUGAL NATIONWIDE SURVEY DURING THE WINTER 2007/2008

S. Duarte[1], J. Bento[2], A. Pena[3], C.M. Lino[4*], J.A. Pereira[5], C. Delerue-Matos[6], T. Oliva-Teles[7], S. Morais[8], M. Correia[9], M.B.P.P. Oliveira[10]

[1 2 3 4] Group of Bromatology, Center of Pharmaceutical Studies, CEF, University of Coimbra, Health Sciences Campus, Azinhaga de Santa Comba, 3000-548, Coimbra, Portugal
[5] CIMO/Escola Superior Agrária, Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855, Bragança, Portugal
[6 7 8 9] REQUIMTE, Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto
[10] REQUIMTE, Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, Rua Anibal Cunha, 164, 4099-030, Porto, Portugal
[*] Corresponding author–E-mail: clino@ff.uc.pt; Phone: +351 239488477

Ochratoxin A (OTA) remains one of the most important mycotoxins known, due to its ubiquitous occurrence, wide range of susceptible food commodities and observed toxic effects, in both animals and humans. The reported toxic effects include carcinogenic, nephrotoxic, teratogenic, neurotoxic and immunotoxic.

Humans can be exposed to OTA directly through consumption of contaminated plant food or indirectly through consumption of tissues of animals exposed to contaminated materials. Nevertheless, the most important exposure way remains the direct one, through the consumption of mainly cereals and their derivates. Of these, bread arises as one of the most important since it is greatly consumed by all social classes. From the many types of bread commercialized, wheat and maize bread are the most important that can contain other cereals (e.g. oat, rye), ingredients (e.g. nuts, raisins) and be prepared with the whole or the inner grain.

The purpose of this work was to undertake a nationwide survey in order to assess the contamination of bread by OTA and thus estimate the exposure risk of the Portuguese population through this staple foodstuff.

The procedure adopted was developed by Juan *et al.* (2007), and re-validated before the analysis of 274 maize and wheat bread samples. The samples were collected from random bakeries and supermarkets from north to south of the continental territory, during the winter of 2007/2008. Briefly the procedure involved the extraction of the mycotoxin with a PBS:methanol solution and a clean-up step through immunoaffinity columns. After evaporation of the eluted methanol, the sample extract was reconstituted in mobile phase and injected in a HPLC-FD equipment.

The results showed a broad contamination of maize and wheat bread, with the former presenting the highest frequency (83.3% *versus* 76.9%) and average contamination (0.40 *versus* 0.13 ng/g) values, in agreement with national and international previous studies. Furthermore, one sample of maize bread surpassed the European maximum legal limit set at 3 ng/g.

However, because maize bread is 4 times less consumed that wheat bread, its contribution to OTA human exposure is less significant that wheat bread (0.14 *versus* 0.24 ng/kg bw/day).

In sum, the bread consumed by the Portuguese population is widely contaminated with OTA, suggesting a need for additional attention to OTA contamination of this staple food and the implementation of surveillance and inspection programs to limit the exposure.

Keywords: ochratoxin A, bread, Portugal

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G-8
OCHRATOXIN A CONTENT IN URINE SAMPLES FROM BRAGANÇA AND ALENTEJO: A COMPARATIVE ANALYSIS (WINTER 2007)

J. Bento[1*], S. Duarte[2], A. Pena[3], C.M. Lino[4], J.A. Pereira[5]

[1 2 3 4] Group of Bromatology, Center of Pharmaceutical Studies, CEF, University of Coimbra, Health Sciences Campus, Azinhaga de Santa Comba, 3000-548, Coimbra, Portugal
[5] CIMO/Escola Superior Agrária, Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855, Bragança, Portugal
[*] Corresponding author–E-mail: sofiacanceladuarte@gmail.com; Phone: +351 239 488477

Ochratoxin A (OTA) is a mycotoxin which possesses a variety of toxic effects, including enzyme inhibition, immunosuppression, teratogenicity, nephrotoxicity, and carcinogenicity. It is produced by fungi for which foodstuffs such as beans, cereals, fruits, and seeds constitute an ideal growing media. It has proven itself at least partly resistant to food processing methods, meaning it is also present in derived products and thus finds its way into the human organism. Recent studies have suggested that, though OTA can be found in both plasma and urine – through which it is eliminated, though with great difficulty – the latter provides a better indication of OTA ingestion. Its collection procedure is also less invasive, and developments in analytical methodology allow an equally precise analysis.

In an effort to assess the Portuguese regional differences of exposure to this mycotoxin of the populations of Bragança and Alentejo, samples of urine from inhabitants of Bragança – eleven men and nineteen women – and Alentejo – eighteen men and twenty-two women – were tested for OTA through extraction with IACs and quantification by LC-FD.

Both regions featured similar contamination frequencies (96.7% for Bragança and 97.5% for Alentejo), with all negative samples being female. Mean values were also similar (0.022 ng/mL for Bragança *vs*. 0.021 ng/mL for Alentejo), as were maximum values (0.069 for Bragança, 0.064 ng/mL for Alentejo). In both regions, the highest contamination value was found in a female sample.

In Alentejo, mean value was found to be higher in males (0.025 *vs*. 0.018 ng/mL in females), while in Bragança the reverse was true, though the difference between genders was small (0.020 ng/mL for males, 0.023 ng/mL for females).

Females in both regions presented similar values for incidence, and mean and maximum contamination levels, while men from Alentejo featured a much higher maximum level (0.044 ng/mL *vs.* 0.027 ng/mL for men from Bragança).

Keywords: Ochratoxin A, urine, regional, Portugal

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G-9
OCHRATOXIN A IN BREAD CONSUMED IN LISBON IN SUMMER 2008: OCCURRENCE AND EXPOSURE ASSESSMENT

S. Duarte[1*], J. Bento[2], A. Pena[3], C.M. Lino[4]

[1 2 3 4] Group of Bromatology, Center of Pharmaceutical Studies, University of Coimbra
[*] Corresponding author–E-mail: sofiacanceladuarte@gmail.com; Phone: +351 239 488 477

Mycotoxins continue to grab worldwide attention and concern for the hazard and impact they present for both human and animals. Ochratoxin A (OTA), in particular, for the wide range of toxic effects that include the observed teratogenic, embriogenic, genotoxic, neurotoxic, immunosuppressive, carcinogenic (IARC group 2B), and nephrotoxic. Furthermore, its ubiquitous presence in various foods and feeds raises concern about the food safety and human exposure.

The scope of the study was the determination of the potential hazard posed by the OTA contamination of a priority foodstuff, like bread, chosen on the basis of its importance in both the total diet and the economy. So, a screening of OTA contamination in bread commercialized in the region of the Portuguese capital, Lisbon, was undertaken. Three types of bread were analysed: wheat bread (n=32), maize bread (n=8) and Mafra bread (n=11), a regional type of wheat bread. All the samples were bought in random bakeries and supermarkets during the summer of 2008. After collection, the already validated methodology of Juan *et al.* (2007) was followed in order to determine the OTA contamination in the 51 bread samples.

The results showed an important contamination of both maize and wheat bread, with slightly higher values of frequency (87.5% *versus* 81.3%) and average level of contamination (0.27±0.05 *versus* 0.23±0.18 ng/g) in the case of the former. Mafra bread presented almost half of the values of maize bread for frequency (45.5%) and average level of contamination (0.13 ng/g), thus contributing in much lesser extent to OTA estimated daily intake through bread consumption.

The average dietary exposure to OTA was calculated for adult inhabitants, combining contamination data with national values of mean weight and food consumption data. The value obtained was higher for exposures calculated from the consumption of wheat bread (0.23 ng/kg bw/day), because of the higher consumption when compared to maize bread (0.09 ng/kg bw/day). Nevertheless, neither estimation surpassed the tolerable weekly intake proposed by European Food Safety Authority (EFSA).

In conclusion, the Lisbonian inhabitants are exposed to an elevated percentage of OTA contaminated bread, with mean values that do not differ much between wheat and maize bread. The exception is the regional type of bread (Mafra bread) with low values of incidence and average concentration.

Keywords: Ochratoxin A, bread, Lisbon, Summer

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G-10

PROTEOMIC IDENTIFICATION OF AZASPIRACID TOXIN BIOMARKERS IN BLUE MUSSELS MYTILUS EDULIS

Judith A. P.E. Kouassi Nzoughet[1*], John Hamilton[2], Catherine Botting[3], Alastair Douglas[4], Lynda Devine[5], John Nelson[6], Christopher Elliott[7]

[1 7] Queen's University Belfast, Institute of Agri Food and Land Use, Belfast, United Kingdom
[2 4] Agri Food and Biosciences Institute for Northern Ireland, Belfast, UK
[3] University of St Andrews, UK
[5 6] Queen's University Belfast, Belfast, United Kingdom
[*] Corresponding author–E-mail: akouassi01@qub.ac.uk; Phone: 00442890976543; Fax: 00442890976513

Azaspiracids are a class of recently discovered algal derived shellfish toxins. Their distribution globally is on the increase, with mussels being most widely implicated in azaspiracid related food poisoning events. Evidence that these toxins were bound to proteins in contaminated mussels has been recently shown [1]. In the present study characterization of these proteins in blue mussels, *Mytilus edulis*, was achieved using a range of advanced proteomic tools. Four proteins, present only in the hepatopancreas of toxin contaminated mussels, sharing identity or homology with Cathepsin D, Superoxide dismutase, Glutathione S-tranferase π and a bacterial flagellar protein, have been characterized [2]. Several of the proteins are known to be involved in self-defence mechanisms against xenobiotics or up-regulated in the presence of carcinogenic agents. These findings would suggest that azaspiracids should now be considered and evaluated as potential tumorigenic compounds. The presence of a bacterial protein only in contaminated mussels was an unexpected finding and requires further investigation. The proteins identified in this study should assist with development of urgently required processes for the rapid depuration of azaspiracid contaminated shellfish. Moreover they may serve as early warning indicators of shellfish exposed to this family of toxins.

[1] K.J. Nzoughet et al., Azaspiracid: First evidence of protein binding in shellfish. Toxicon 51 (2008) 1255–1263
[2] Nzoughet et al., Proteomic Identification of azaspiracid toxin biomarkers in blue mussels *Mytilus edulis.* MCP published April 23, 2009, 10.1074/mcp.M800561-MCP200

Keywords: Azaspiracids, proteomics, biomarkers

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G-11

DETERMINATION OF FUMONISINS B1, B2 AND B3 IN HERBAL TEAS AND MEDICINAL PLANTS BY LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

Gorica Vukovic[1*], Snezana Pavlovic[2], Mihailo Ristic[3], Sanja Lazic[4]

[1] Institute of Public Health, Belgrade, Serbia
[2 3] Institute for Medical Plant Research, Belgrade, Serbia
[4] Faculty of Agriculture, Novi Sad, Serbia
[*] Corresponding author–E-mail: goricavukovic@yahoo.com; Phone: +381112078782

The purpose of this study was to measure the potential levels of fumonisin B1 (FB1), fumonisin B2 (FB2) and fumonisin B3 (FB3) contamination in several herbal teas and medicinal plants that are consumed traditionally in Serbia. Fumonisins were isolated by immunoaffinity column (Fumoniprep®, R-Biopharm, Rhone LTD, Scotland) and their chromatographic separation was achieved using reverse phase chromatography on XBridge column (Waters Corporation, Milford, USA). Mobile phase A was methanol containing 0.5% formic acid and mobile phase B was 0.5% formic acid (pH was adjusted at 3.8). Elution of fumonisins was performed in isocratic conditions (70% A and 30% B) at flow rate 0.3 ml/min. Fumonisins were detected by tandem mass spectrometry (LC-MS/MS) with triple quadrupole mass spectrometer (Agilent 6410 Triple Quadrupole Mass Spectrometer, USA) in positive electrospray ionisation mode using multiple reaction monitoring (MRM). For FB1 precursor ion was 722 (*m/z*), while primary product ion 334 (m/z) and the secondary product ion 352 (*m/z*) were monitored in the two MRM transitions. For FB2 and FB3 different retention time was obtained but with the same transitions, the precursor ion was 706 (*m/z*), and the product ions 336 and 318 (*m/z*). The mean recovery for fumonisin B1, at 10, 400 and 1000 μg kg^{-1} spiking levels, was 94% (% RSD 3.8). The limit of detection, calculated from those concentrations that provide a signal to noise ratio of 3:1, was 0.25 μg kg^{-1} for FB1 and FB3 and 0.5 μg kg^{-1} for FB2. Limits of quantification, estimated as those concentrations of analyte which yield a signal to noise ratio of at least 10:1, ranged from 1 μg kg^{-1} for FB1 and FB3 to 2 μg kg^{-1} for FB2. A total of 36 commercially available herbal tea and medicinal plant samples were analyzed. FB1 was detected in six samples (from 15 to 364 μg kg^{-1}), since FB2 and FB3 were not detected.

Keywords: fumonisins, LC-MS/MS, medicinal plants, teas

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G-12

EFFECT OF SOLVENT EXTRACTION OF DRIED SHIITAKE ON THE GROWTH OF MYCOTOXINS-PRODUCING FUNGI

Awanwee Petchkongkaew[1*], Hatairat Kasmanee[2], Nilobon Laowkasamsukwong[3], Sunthorn Kanchanatawee[4], Patricia Taillandier[5]

[1 2 3] Thammasat University, PrathumThani, Thailand
[4] Suranaree University of Technology, Nakhon Ratchasima, Thailand
[5] ENSIACET-INPT, Toulouse, France
[*] Corresponding author–E-mail: awanwee@tu.ac.th; Phone: +66 (0)87 254 5445; Fax: +66 (0) 564 4486

The objective of this study was to investigate the effect of solvent (Chloroform, Ethyl acetate, Ethanol and Water) extraction of dried Shiitake on the growth of mycotoxins-producing fungi which are Aspergillus parasiticus, Aspergillus ochraceus, Aspergillus flavus, Fusarium moniliforme, Fusarium verticilioides, Fusarium graminearum, Fusarium oxysporum, Penicillium expansum and Penicillium citrinum. The results showed that chloroform extract had the highest inhibition activity whereas the aqueous extract showed the lowest inhibition activity. Hence, the percentages of growth inhibition of F.monoliforme, F.graminearum, F.verticilioides, F.oxysporum and P.citrinum by chloroform extract were 91.18, 88.70, 84.83, 83.33 and 82.4%, respectively. On the contrary, 31.48, 27.62, 23.79, 23.33 and 14.26% were the percentage of growth inhibition of A. flavus, P. expansum, F. verticilioides, F. oxysporum and A. parasiticus by the aqueous extract.

Keywords: Dried Shiitake, Mycotoxin-producing fungi

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G-13
NON-INSTRUMENTAL IMMUNOCHEMICAL TESTS FOR RAPID MYCOTOXINS DETECTION

Irina Goryacheva[1], Tatiana Rusanova[2*], Sarah De Saeger[3]

[1 2] Saratov State University, Russia
[3] Ghent University, Belgium
[*] Corresponding author–E-mail: tatyanar@mail.ru; Phone: +79271565785; Fax: +78452516960

Mycotoxins are toxic secondary fungal metabolites with deleterious effects for humans and animals. Many kinds of food and feed can be contaminated with mycotoxins since they can be formed in commodities before as well as after harvest. Many countries have set regulations with maximum levels for the major mycotoxins in different commodities. Screening methods for rapid mycotoxin detection in a nonlaboratory environment have become increasingly important. Applying screening tests allows one to shorten time and to lower costs for food and feed quality control. At the first step of analysis the main problem is the confirmation of absence or presence of toxin which concentration is higher or comparable to the fixed cut-off. The main group of non-instrumental rapid tests is immunoassay based: these methods use high specificity and affinity of antibody as the main "driving force". The common formats of immunochemical tests use membranes as solid supports (e.g. dipsticks, lateral flow and flow-through tests). However membrane application has limitations for sample volume, hence restricting sensitivity. Also in the case of high-coloured extracts visual evaluation of results could be impeded.

A variant of the column device for immunochemical detection of mycotoxins in intense coloured matrices or matrices with strong matrix effect was developed combining two columns: 1) the clean-up column containing appropriate sorbent and 2) the transparent detection column, containing one or two detection gel layers with specific antibodies and control layer. The assay was combined with a simple rapid extraction procedure and validated in accordance with EU maximum levels. The duration of the procedure was about 20 min for 6 samples. Column immunochemical test-methods allow combining clean-up, pre-concentration, and detection of one or two mycotoxins in one step. In addition possibility of matching solid-phase extraction and detecting step makes this test-method applicable to use in case of complicated matrix such as feed, red wine, beer etc. For example developed set-up allowed us to detect Ohratoxin A in red wine without prior extraction with cut-off level at 2 μg L^{-1} according to EU.

In this presentation principles and perspectives of this new device and procedures for mycotoxin screening will be discussed.

Keywords: mycotoxins, rapid test, immunoassay

G-14
ANALYSIS OF OCHRATOXIN A USING ONLINE IMMUNOAFFINITYCHROMATOGRAPHY COUPLED WITH HPLC-FLD

Philipp Bischofberger[1], Martin Sibum[2], Ria Rhemrev[3], Simone Staiger[4], Scarlett Biselli[5*]

[1 4 5] Eurofins, Hamburg, Germany
[2] Spark Holland, Emmen, The Netherlands
[3] ResQ Lab, Nijeveen, The Netherlands
[*] Corresponding author–E-mail: ScarlettBiselli@eurofins.de; Phone: +494049294260

Online SPE processing is becoming increasingly popular for automated analysis, due to the improvement of laboratory efficiency, enabling fast and effective clean-up and concentration of the target compound.

Ochratoxin A is a toxic and carcinogenic fungal secondary metabolite. It is a potent nephrotoxin, carcinogen and teratogen. Hence European Union legislative bodies are imposing low Ochratoxin A limits on many affected matrices. Very sensitive methods are necessary to meet the official food control requirements. Therefore the standard reference procedures are based on immunoaffintity column clean-up, coupled to LC with fluorescence detection. Immunoaffinity column clean-up is widespread in mycotoxin analysis due to its high sensitivity and selectivity. It is, however, the most critical and time consuming step in the process. Commercial laboratories are challenged to manage an increasing amount of samples with unvarying precision and sensitivity.

A high-speed analysis based on immunoaffinity SPE cartridges coupled to LC and fluorescence detection represents an optimized solution for these competing requirements. Fully automated sample application and clean-up by immunoaffintity SPE, and subsequent LC-FLD analysis can be coupled to obtain runtimes of 10' per sample. The Clean-up steps and HPLC-runs are performed simultaneously. The application of the developed method shall meet the criteria of the European legislation and shows the same performance characteristics as obtained by the reference method.

Keywords: Mycotoxins, Online SPE, Immunoaffinity

G-15

RAPID IMMUNOASSAY-BASED STRIP TESTS FOR THE QUANTITATIVE DETERMINATION OF MYCOTOXINS IN FOOD AND FEED: CHALLENGES, POTENTIAL AND APPLICATIONS

Alexandra Molinelli[1*], Karina Grossalber[2], Barbara Cvak[3], Michaela Helmel[4], Michael Zheng[5], Rudolf Krska[6]

[1 2 3 4 6] Christian Doppler Laboratory for Mycotoxin Research, IFA-Tulln, Austria
[5] Romer Labs Singapore Pte Ltd, Singapore, Singapore
[*] Corresponding author–E-mail: alexandra.molinelli@romerlabs.com; Phone: +4322726153310

Mycotoxins are toxic natural secondary metabolites produced by several species of fungi such as *Aspergillus* and *Fusarium* on agricultural commodities in the field or during storage.

Mycotoxins such as aflatoxins and ochratoxin A (*Aspergillus* sp.), as well as prevalent *Fusarium* toxins including the B-type trichothecenes such as deoxynivalenol (DON), the A-type trichothecenes such as the T-2 and HT-2 Toxins, the type B fumonisins, and zearalenone frequently occur and are known for their adverse effects on human and animal health.

Within the European Community maximum levels are regulated e.g. as defined in the Commission regulation EC 1126/2007 (food) and in the recommendation 2006/576/EC (feed). Next to established instrumental analytical methods, immuno-assay based tests have also increasingly been used in the food and feed sector. Amongst these, strip tests for mycotoxins which allow screening of agricultural commodities with results within a few minutes are gaining acceptance and are strongly being integrated into routine quality monitoring procedures due to the need for rapid on-site (pre)-screening.

These immunochromatographic strip tests are based on a competitive immunoassay format where an antibody-colloidal gold particle complex is mixed with sample extract in a microwell and used as signal reagent. The strip test is inserted into the well and the mixed content then migrates onto a nitrocellulose membrane, which contains a test zone and a control zone. Mycotoxin-protein conjugate coated on the test zone captures free antibody-colloidal gold particle complex, allowing color particles to concentrate and form a visible line. The intensity of the test line is dependent on the sample mycotoxin concentration and is measured with a photometric strip reader. The control line will always be visible regardless of the presence of contaminant confirming correct test development.

Quantitative strip tests for *Aspergillus* and *Fusarium* mycotoxins have been developed. The presented tests are both quantitative and rapid delivering assay results within 5 minutes. The accuracy of the strip tests was determined using naturally contaminated samples. With no sample preparation required other than an extraction step, strip tests allow fast on-site screening. Nevertheless issues ranging from suitability of antibody for the strip test format to matrix effects requiring matrix-matched calibrations for each analyzed matrix remain. Factors such as ionic strength, pH, and use of surfactant strongly affect strip test performance. Challenges in strip test development and production will be discussed. Furthermore, potential and applications of quantitative strip tests for mycotoxins will be presented.

Keywords: mycotoxins, strip tests, food safety

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G-16

DEVELOPMENT AND VALIDATION OF A MULTI-METHOD FOR THE ANALYSIS OF 36 MYCOTOXINS IN GRAPE JUICE USING UPLC-MS/MS (ESI+)

André de Kok[1]*, Ionara R. Pizzutti[2], Jos Scholten[3], Bárbara V. Noronha[4]

[1 3] VWA–Food and Consumer Product Safety Authority–Chemistry Laboratory–R&D Group, National Reference Laboratory (NRL) for Pesticides and Mycotoxins Analysis in Food, Amsterdam, The Netherlands
2 4 Federal University of Santa Maria – Chemistry Department – Center of Research and Analysis of Residues and Contaminants (CEPARC), Santa Maria, RS, Brazil
* Corresponding author–E-mail: andre.de.kok@vwa.nl; Phone: +31205244711; Fax: +31205244700

Mycotoxins are a group of toxic compounds produced by fungi that are formed during growing and/or certain storage conditions in various foods such as peanuts, soybeans, wheat, grapes and dried fruits. Brazilian laws do not set any maximum limit for mycotoxins in grape juice. In the recent European legislation, the maximum limit for Ochratoxin A in grape juice and its derivatives is 2 µg kg^{-1}.

This project aimed to develop and validate a rapid method to analyze 36 mycotoxins by UPLC-MS/MS (ESI^+). The extraction was carried out by shaking 10 g of grape juice in a centrifuge tube with 10 mL acetonitrile/1% of acetic acid, followed by the addition of 5.0 g magnesium sulphate to induce a phase separation. After centrifugation, the extract was transferred to an autosampler vial for direct analysis.

For recovery experiments, the mycotoxins were divided in 2 groups: group 1 with 17 mycotoxins at the spike levels 1, 2 and 10 µg kg^{-1} and group 2 with 19 mycotoxins at the spike levels 50, 100 and 500 µg kg^{-1}. The complete multi-residue method was successfully validated for the parameters specificity, accuracy, repeatability (precision), linearity of detector response, intrument limit of detection (LOD) and method limit of quantification (LOQ).

The validation results showed good precision (RSD%) and accuracy (as percentage recovery):

- Group 1: Recoveries were found ranging from 74.3 to 116.7% at all spike levels, with relative standard deviations (RSD) < 20% for 64.7% of the mycotoxins.

- Group 2: Recoveries at all spike levels were in the 70–120% range, except for nivalenol (> 120%) at the 50 µg kg^{-1} level and nivalenol, ß-ZAL and cyclopiazonic acid (all < 70%) at the 500 µg kg^{-1} level.

Relative standard deviations (RSD) were < 20% for 80.7% of the mycotoxins.

No significant matrix effects were observed for most of the mycotoxins.

The present method has several advantages such as to be fast, simple, sensitive and efficient. The

absence of a clean-up step reduces the analysis time considerably and the method can be performed with modern LC-MS/MS equipment commonly available in laboratories, nowadays.

Keywords: mycotoxins, grapejuice, multimethod, UPLC-MS/MS (ESI+)

CEPARC/UFSM, VWA -Food and Consumer Product Safety Authority

G-17
IMAGE AND SPECTRAL ANALYSES OF FUSARIUM-DAMAGED WHEAT GRAIN

Ondrej Jirsa[1*], Ivana Polisenska[2]

[1 2] Agrotest Fyto, Ltd., Kromeriz, Czech Republic
[*] Corresponding author–E-mail: jirsa@vukrom.cz; Phone: +420573317132

Fusarium head blight (FHB), caused by *Fusarium* spp., is a destructive disease of wheat and other small grain cereals, with negative consequences for grain quality with regard to mycotoxin contamination. Spectral methods as well as machine vision combined with statistical methods have been reported to be helpful in estimation of grain damage caused by FHB. The objective of this study was to determine suitable image deors and compare machine and visual classification. Material used in this study consisted of two winter wheat sample sets (variety Ludwig and Sulamit), grown at one location and differing only in level of FHB damage due to different fungicidal treatment or agronomic practices. Grain was evaluated visually and classified into four groups: sound; visually scabby kernels (VSK) 1 – chalky, pinkish, normal sized, VSK2 – normal coloured, shrivelled, and VSK3 – chalky, pinkish, heavily shrivelled. Deoxynivalenol (DON) content was determined using an ELISA method.

The approach based on the shape and colour analysis of kernel images was applied to discriminate selected sound and diseased kernels (VSK1, VSK3). Basic shape and colour deors were determined for each image, additional ones were calculated. A significant correlation was observed between the kernel weight and the shape deors, especially width (r = 0.877) and area (r = 0.849). Length, width, area, and perimeter were not sufficient to distinguish between sound and damaged kernels. Better performance was revealed for calculated shape deors. Very good classification has been obtained using colour deors – hue (H) and saturation (S). Both VSK1 and VSK3 kernels featured higher values of H and lower values of S than sound ones.

Reflectance spectra of bulk samples within the range from 300 to 1100 nm were acquired on an Avantes S2000 spectrophotometer. Normalised and derivative spectra were correlated to DON and VSK content yielding significant correlations. Better correlations were observed in case of more infected Ludwig variety (max r = 0.720 for VSK3), where also significant relationship between VSK sum and DON content was noticed (r = 0.873).

The best discrimination of sound and damaged kernels was obtained considering the results of shape and colour analysis. Spectral analysis in visible range could evaluate VSK content, but relationship to DON is not straightforward. Detection of *Fusarium*-damaged kernels in grain employing spectral methods and machine vision can help in more accurate grading and mycotoxin content estimation.

Keywords: wheat, FHB, spectroscopy, machine vision

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G-18

QUANTITATIVE MULTI MYCOTOXIN ANALYSIS IN CEREALS USING ACCELERATED SOLVENT EXTRACTION (ASE) OR QUECHERS-BASED SAMPLE PREPARATION BEFORE LIQUID CHROMATOGRAPHY ELECTROSPRAY IONISATION TANDEM MASS SPECTROMETRY (LC-ESI-MS/MS)

Aurélien Desmarchelier[1], Walburga Seefelder[2*]

[1 2] Nestlé Research Center, Nestec Ltd., Vers-chez-les-Blanc, 1000 Lausanne 26, Switzerland
[*] Corresponding author–E-mail: Walburga.Seefelder@rdls.nestle.con; Phone: +41 21 785 8589

Hundreds of mycotoxins with very different physicochemical properties have already been identified. Their analysis is usually performed through single compound determination or for certain classes of mycotoxin by HPLC coupled to UV or fluorescence detectors after immunoaffinity column cleanup. However to enforce the various existing and upcoming European legislations [1;2], the approach by single residue methods is rather cumbersome and tedious. Moreover, co-occurrence of mycotoxins can enhance the toxicity of contaminated cereals by synergetic interactions. Therefore, fast and easy multiresidue methods allowing the simultaneous quantification and confirmatory detection of mycotoxins are highly demanded.

The aim of this study was to develop, validate [according to 3,4] and compare two multiresidue approaches for the analysis of mycotoxins in cereal matrices (corn, rice, wheat, oat, rye, barley soja, corn gluten and infant cereals) by LC-MS/MS. The extraction procedures considered were QuEChERS (acronym of Quick, Easy, Cheap, Effective, Rugged and Safe [5]) and an automated ASE method.

Currently EC-regulated mycotoxins [1] in cereal matrices (aflatoxins B1, B2, G1 and G2; fumonisins B1 and B2; ochratoxin A; deoxynivalenol and zearalenone) were surveyed as well as T2, HT-2, nivalenol, acetyldeoxynivalenol, fusarenon-X, diacetoxyscirpenol and neosolaniol.

Both extraction procedures showed good performances for linearity [$(r)^2$ >0.99], recovery [70–110%] and precision [relative standard deviation for repeatability (RSD_r) and intermediate reproducibility (RSD_{iR}) < 20%], thus fulfilling the EU requirements [3,4]. Limits of quantitation were 1.0 µg/kg for the aflatoxins, 0.5 µg/kg for ochratoxin A and within the 5–100 µg/kg range for other mycotoxins.

Performance evaluation was further conducted through the participation to four proficiency-tests (for aflatoxins, ochratoxin A, deoxynivalenol, T2 and HT2 toxins) and by the analysis of two certified reference materials (for fumonisins and zearalenone). Accuracy data (> 80%) and z-scores values ($|Z| < 2$) indicated that both extraction procedures were suitable for the quantitative determination of mycotoxins in cereal matrices.

Details on sample clean-up, performance characteristics and advantages and disadvantages of both extraction procedures will be presented.

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[2] Commission Regulation (EC) N°1126/2007.
[3] Document N° SANCO/2007/313131/October/2007.
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[5] Anastassiades, M.; Lehotay, S.J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412-431.

Keywords: Mycotoxins, Multiresidue methods, LC-MS/MS

G-19

ANALYSIS OF SPRING BARLEY IN THE MALTHOUSE BERNARD AT RAJHRAD WITH A VIEW TO FUSARIUM INFECTION, DEOXYNIVALENOL CONTENT AND BEER GUSHING

Zdenek Nesvadba[1*], Daniel Sychra[2], Simona Horackova[3], Jiri Susta[4]

[1 3] Agrotest Fyto, Ltd., Havlickova 2787/121, 767 01 Kromeriz, Czech Republic
[2] Mendel University of Agriculture and Forestry in Brno, Zemedelska 1, 613 00 Brno, Czech Republic
[4] Sladovna Bernard, Co. Ltd.–Rajhrad, Palackeho 135, 664 61 Rajhrad, Czech Republic
[*] Corresponding author–E-mail: nesvadba.zdenek@vukrom.cz; Phone: +420 573 317 182; Fax: +420 573 339 725

Beer gushing is a serious problem in the brewing industry worldwide. This term is used to describe beer excess foaming occurring after opening a bottle or can. If the package is open, carbon dioxide suddenly and spontaneously releases, accompanied by production of a big amount of short-lasting micro-bubbles throughout drink volume, which leads to fast expansion with violent ejection of the foamed drink from the package.

At present, gushing is a considerable threat to the quality of Czech beer and malt. Gushing is a complex of interrelationships between brewing raw materials (malt) and potentially many other technological parameters. So called primary gushing belongs to the most frequent effects connected with the incidence of Fusarium head blight (FHB). Presence of FHB in barley caryopsis and malt is also connected with other side effects influencing the beer quality as off-flavour or premature flocculation of yeasts leading to precocious termination of fermentation. Gushing, however, does not always have to be related to the presence of pathogenes. Some of the production factors as for example abrasive surface of bottles, supersaturation with carbon dioxide, high concentration of some heavy metals, oxidation products and many others, can be cause of a so-called secondary gushing which is not related to the quality of malt.

Analyses were performed on samples of spring barley cultivars or lines grown at four locations in the Czech Republic and on malts produced from them in the Malthouse Bernard at Rajhrad. The experiments were modified with regard to the selection of a cultivar, location, artificial infection with *Fusarium culmorum*, analysis of deoxynivalenol (DON) content and microbiological cultivation for determining mould species. Gushing was assessed using the method according to the Research Institute of Brewing and Malting Brno.

Among the cultivars examined there was no significant evidence for apparent gushing potential. Analyses of the samples from four different locations showed significantly higher propensity to gushing in barleys from the Caslav location. In barley cultivars artificially infected with *F. culmorum,* a significant difference in comparison with the control non-infected variant was found. On the other hand, no relationship between the DON content and gushing was confirmed in the cultivars examined. There was no relationship between technological processes in the malthouse and gushing propensity.

Keywords: barley, malt, beer, gushing, deoxynivalenol,

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G-20
MONITORING OF OCHRATOXIN A CONTENT IN BEERS FROM RETAIL STORES USING THE UPLC/FLR METHOD

Sylvie Běláková[1*], Zdeněk Svoboda[2], Renata Mikulíková[3], Karolína Benešová[4]

[1 2 3 4] Malting Institute Brno, RIMB, Plc., Mostecká 7, 614 00 Brno, Czech Republic
[*] Corresponding author–E-mail: belakova@brno.beerresearch.cz; Phone: 545 214 110 /38

Mycotoxins are secondary metabolites of microscopic fibrous fungi. Ochratoxin A (OTA), their important representative, belongs to a group of ochratoxins together with ochratoxins B, C, D, and α). OTA is dominant in this group. It is produced by species of *Aspergillius* (*A. ochraceus, A. melleus*) and *Penicillium* (*P. verrucosum, P. nordicum*). OTA has carcinogenic effects, it is associated with digestive disorders and it damages the immune system and kidneys. OTA occurs in many food products both of vegetable and animal origin. Cereals and products made from them belong to significant sources of OTA in a food chain. Beer is another commodity where OTA has been detected.

The OTA occurrence in beer samples was studied. The analysis was performed in 50 selected samples of pale, dark and non alcoholic beers obtained from retail stores. Contamination with OTA mycotoxin was proved in 60% of beer samples. OTA content in beer samples moved from 0.001 – 0.051 µg/l. The Commission regulation (EC) no. 1881/2006 did not specify the maximal OTA limit in beer.

For the determination of ochratoxin A content in beer, the analytical method of ultra fast liquid chromatography with fluorescent detection (UPLC-FLR) was used. The analytical method with gradient elution was optimized. Beer samples were purified on immunoaffinity columns and afterwards analyzed on the UPLC-FLR. The mobile phase consisted of acetonitrile and deionized water was adjusted to pH 2. The UPLC-FLR method was validated (LOD 0.0003 µg/l, LOQ 0.001 µg/l, RSD 5.3%).

Keywords: beer, OTA, UPLC/FLR,

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G-21

DETERMINATION OF AFLATOXINS IN CEREAL SAMPLES BY SOLID–PHASE MICROEXTRACTION COUPLED WITH LIQUID CHROMATOGRAPHY AND POST-COLUMN PHOTOCHEMICAL DERIVATIZATION-FLUORESCENCE DETECTION

Maurizio Quinto[1*], Giuseppina Spadaccino[2], Diego Centonze[3], Taddeo Rotunno[4]

[1 2 3 4] Department of Agri-Environmental Sciences, Chemistry and Plant Protection, University of Foggia, via Napoli 25, 71100 Foggia, Italy
[*] Corresponding author–E-mail: m.quinto@unifg.it; Phone: +39 0881 589102; Fax: +39 0881 740211

Aflatoxins are secondary metabolites of mould fungi Aspergillus flavus, A. parasiticus and A. nomius [1]. These micotoxins are carcinogenic, mutagenic, teratogenic and immunosuppressive to the most animal species. Aflatoxins are generally found in feed and foodstuff, such as cereal for human consumption and all products derived from cereals, including processed cereal products. It can be assumed that about 20% of food products, mainly from vegetables (e.g., cereals) are substantially contaminated [2]. For this reason and for their toxicity European Union in the Commission Regulation (EC) No 1881/2006 have established the maximum residue limits (MRLs) of aflatoxins in cereal and their derivates of 2 μg kg^{-1} for AFB1, 4 μg kg^{-1} for the sum of the four aflatoxins. Since when aflatoxins were found to adversely affect the health of various animal species, analytical methods were developed. Among different analytical methods available, HPLC with fluorescence detection (HPLC-FD) is currently the most widely used as it offers greater versatility in the analysis of complex matrices [3]. In the present study a new method for the determination of aflatoxins B1, B2, G1, and G2 (AFB1, AFB2, AFG1, AFG2) in cereal flours based on solid–phase microextraction (SPME) coupled with high performance liquid chromatography with post-column photochemical derivatization and fluorescence detection (SPME-HPLC-PD-FD) has been developed for the first time. The extraction of aflatoxins were extracted from cereal flour samples were carried out by a methanol: phosphate buffer (PB, pH 5.8, I=0.1) (80:20, *v/v*) solution, followed by a SPME clean-up step. Different SPME and HPLC-PD-FD parameters (fiber polarity, temperature, pH, ionic strength, adsorption and desorption time, mobile phase) have been investigated and optimized. This method, which was assessed for the analysis of different cereal flours showed very good results in terms of, showed excellent LOD and LOQ (from 0.035 to 0.2 ng g^{-1} and from 0.1 to 0.63 ng g^{-1}, respectively), good reproducibility (maximum within and inter-day repeatability 2.27% and 5.38%, respectively), linear ranges (up to 20 ng g^{-1} for AFB1 and AFG1 and 6 ng g^{-1} for AFB2 and AFG2) and high efficiency in the extraction-cleanup step toward all kinds of real matrices used. The results were compared with those obtained by using conventional immunoaffinity column purification step.

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Keywords: aflatoxins, liquid chromatography, SPME

G-22

DEVELOPMENT OF A SIMPLE RAPID AND RELIABLE HPLC METHOD FOR THE DETERMINATION OF FUMONISIN B1 AND B2 IN MAIZE: ADOPTION OF INTERNAL STANDARD (BUTYL PARABEN)

Necati Bariş Tuncel[1*], Neşe Yilmaz[2]

[1 2] Onsekiz Mart University, Çanakkale, Turkey
[*] Corresponding author–E-mail: baristuncel@comu.edu.tr; Phone: +90 286 218 00 18 (2262); Fax: +90 286 218 05 41

Food safety has become a major issue of public concern. Due to being one of the most important dietary staple foods in the world, maize (*Zea mays L.*) has gained considerable attention. A validated HPLC method for detecting fumonisin B_1 and B_2 (FB_1 and FB_2) and its application to maize samples harvested at regular intervals of fortnight (29, 24, 18.5, 16 and 15% moisture level) were described in this study. Chromatographic seperation was performed with HPLC equipped with a reverse phase (C18 150 mm, 4.6 mm i.d. and 5 µm particle size) column with gradient elution using an internal standard (butyl paraben). The mobile phase was consisted of methanol and 0.1 M sodium dihydrogen phosphate (pH:3.3), and the composition of the solutions were Sol-A (72:28 (*v/v*)) and Sol-B (80:20 (*v/v*)). Fluorescence signals were recorded at λ_{em} 278 and λ_{ex} 315 nm for the first six min for the optimum absorption of butyl paraben and then it was changed to λ_{em} 335 and λ_{ex} 440 nm for FB_1 and FB_2. Stable fumonisin derivatives were formed by the reagent of *ortho*-phthalaldehyde (OPA). The intra-day repeatabilities were between 0.74-1.84 for FB_1 and 1.10-1.98 for FB_2, and the inter-day variabilities were 2.62 for FB_1 and 2.99 for FB_2 (n=3, RSD%). Good linearity was obtained with strong correlation. The LOD and LOQ values were calculated to be 5.6×10^{-8} M and 1.85×10^{-7} M, respectively. The recovery values were calculated in the range of 88-103%. The analysis time was 25 minutes per injection. The method proposed here is a simplified modification of the AOAC official method and it was succesfully applied to maize samples harvested at different times. It was determined that naturally accumulated FB_1 and FB_2 concentrations were highest (4639 ppm FB_1; 1887 ppm FB_2) at the fifth and lowest (57 ppm FB_1; 24 ppm FB_2) at the first harvest time.

Keywords: Fumonisin, HPLC, butyl paraben, maize

G-23

SIMULTANEOUS ANALYSIS OF 14 MYCOTOXINS, AND 150 PESTICIDES IN CRUDE EXTRACTS OF GRAINS BY LC/MS/MS

Kristin von Czapiewski[1*], Tomas Korba [2], Hermann Scmalstieg[3], Ingrid Bujara[4], Juergen Kunze[5], Andrea Voller[6], Birgit Schlutt[7]

[1 2 7] Applied Biosystems, Damstadt, Germany
[3 4 5 6] SGS, Hamburg, Germany
[*] Corresponding author–E-mail: stephen.lock@lifetech.com; Phone: +447720276948

Multi-component methods for the detection of different compound classes, such as mycotoxins or pesticides, have been established and are widely used to analyze a broad range of food or feed. However, there is a continuing demand to test for a larger number of compounds in shorter times. The development of a combined method for different compound classes can help to meet those new challenges.

Since typically mycotoxins and pesticides require different sample preparation a simplified sample preparation had to be established to analyze the two compound classes simultaneously in one sample. No additional clean up steps by SPE or immunoaffinity columns were possible anymore. This new simplified sample preparation helped to reduce time and cost for analysis.

The sample preparation procedure is based on a simple extraction with a mix of acetonitrile and water. The extracts were simply diluted prior LC/MS/MS analysis. The data were acquired by using a Shimadzu Prominence LC system coupled to an API 4000™ LC/MS/MS system. The method was developed for over 160 compounds. Two separate injections in each polarity where performed to match high throughput requirements. The run time of each injection was as short as 20 min.

In this paper we present a fast, robust, and reliable method, which has been validated for the detection of 14 mycotoxins and approximately 150 pesticides in the matrix grain. The LC/MS/MS method in Multiple Reaction Monitoring (MRM) detects all mycotoxins with Limits of Quantitation (LOQ) between 1 µg/kg and 10 µg/kg. The LOQ for pesticides was found to be 10 µg/kg and less. All LOQs meet the requirements of the EU.

Keywords: Pesticides, Mycotoxins, LCMSMS

G-24

USING A CLIMATIC CHAMBER FOR RAISINING OF GRAPES AS AN ALTERNATIVE TO TRADITIONAL SUN-DRYING TO MINIMIZING THE CONTENTS ON OCHRATOXIN A IN SWEET WINES

M. Jesús Ruiz Bejarano[1], M. Carmen Rodríguez Dodero[2*], Carmelo García Barroso[3]

[1 2 3] Departamento de Química Analítica, Facultad de Ciencias, Universidad de Cádiz, Puerto Real, Spain
[*] Corresponding author–E-mail: maricarmen.dodero@uca.es; Phone: +34-956-016360; Fax: +34-956-016460

Some sweet wines are produced from grapes dried into raisins after harvesting. During the process, grapes are subjected to climatic variations, which can alter the final product. If grapes are infected with *Botrytis cinerea*, the penetration of mold into weak grape berries destroys the skin, contributing to the development of ochratoxigenic fungi.

It is therefore considered important to seek alternatives to traditional sun-drying that allow raisins to be produced irrespective of climatic conditions, as well as providing better control of the process. One possibility is the use of a climatic chamber, which permits the temperature and humidity of the drying process to be adjusted, making the process independent of climatic conditions. The results obtained in a previous study showed that the use of the climatic chamber enabled the drying process to be performed in a controlled system, and gave results very similar to those of sun-drying in the content of polyphenols compounds in the resulting musts obtained from the raisins.

In Spain, the practice of making raisins by sun-drying, known as raisining, is feasible only in warm zones such as Jerez, Málaga or Montilla-Moriles in Andalusia, using white varieties, like Muscat and Pedro Ximenez. In the present work it has been studied these both varieties along the raisining of grapes in climatic chamber, as well as the Muscat wines obtained according different vinification processes. Moreover, the aging in oak cask was also monitored.

The content on Ochratoxin A in raisins and wines was determined by HPLC-FLD analysis, according to the methods developed and optimized in our research team.

In general, It has been found that the use of a climatic chamber for raisining enables wines to be produced within the normal and legal ranges for OTA content under European Union regulations (< 2 µg/L). Sensory profiles of the obtained wines have been also studied.

Keywords: Ochratoxin A, sweet wines, climatic chamber, raisining

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G-25
MOLECULARLY IMPRINTED POLYMER SOLID-PHASE EXTRACTION FOR DETECTION OF ZEARALENONE IN CEREAL SAMPLE EXTRACTS

Paolo Lucci[1], Delphine Derrien[2], Florent Alix[3], Céline Pérollier [4], Sami Bayoudh[5*]

[1 2 3 4 5] POLYINTELL, Pharma Parc II, voie de l'innovation, chaussée du vexin, 27100 Val de Reuil France
[*] Corresponding author–E-mail: sami.bayoudh@polyintell.com; Phone: +33 (0)2 32 09 32 70

Zearalenone [6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcyclic acid lactone] is a mycotoxin produced as a secondary metabolite of various Fusarium fungi. Zearalenone (ZON) is known to cause estrogenic effects at relatively low levels, including infertility, reduced serum testosterone levels and sperm counts, reduced incidence of pregnancy, and change in progesterone levels. Several analytical methods for the determination of ZON have been reported in the literature, including thin-layer chromatography, high-performance liquid chromatography, gas-chromatography and enzyme-linked immunosorbent assay. Recently, we developed a new class of intelligent polymers based on molecularly imprinted polymers as a powerful technique for detection of mycotoxin. Molecular imprinting polymer (MIP) is a synthetic material with artificially generated three-dimensional network able to specifically rebind a target molecule. The aim of this work was to develop a method for the clean-up and pre-concentration of zearalenone from corn and wheat samples employing molecularly imprinted polymer (AFFINIMIPTM ZON, POLYINTELL) as selective sorbents for solid-phase extraction. The precision and accuracy of this method were satisfactory for cereals at the different fortification levels tested and it gave high recoveries and capacities. The application of AFFINIMIPTM ZON molecular imprinting polymer as a selective sorbent material for detection of zearalenone fulfilled the method performance criteria required by the Commission Regulation (EC) No 401/2006, demonstrating the suitability of the technique for the control of zearalenone in cereal samples.

Keywords: Zearalenone, MIP, MIP-SPE, sample preparation

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G-26

DEVELOPMENT AND OPTIMIZATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF DEOXYNIVALENOL AND NIVALENOL IN CEREALS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND FLUORIMETRIC DETECTION WITH POST-COLUMN DERIVATIZATION

Marilena Muscarella[1*], Marco Iammarino[2], Donatella Nardiello[3], Carmen Palermo[4], Maurizio Quinto[5], Diego Centonze[6]

[1 2 3] Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy
[4 5 6] DiSACD–Department of Agro-Environmental Sciences, Chemistry and Plant Protection, University of Foggia, Foggia, Italy
[*] Corresponding author–E-mail: m.muscarella@izsfg.it; Phone: +39 0881 786353; Fax: +39 0881 786392

Trichothecenes are secondary metabolites produced by several fungal genera, but mainly by Fusarium species. At present, more than 140 trichothecenes are known and according to their functional groups are commonly divided into four classes (A-D). Deoxynivalenol (DON) and nivalenol (NIV) mycotoxins, belonging to the group of trichothecenes B, are spread worldwide in cereals such as wheat, corn, barley and oats. These compounds have been known for a number of years to cause toxicosis in humans as well as in farm animals, leading to food refusal, vomiting, anemia, hemorrhage and immune-suppression. Only for deoxynivalenol, European Union (EU) has set a maximum level (ML) of 1.75 mg/kg in unprocessed durum wheat, maize and oats and of 0.75 mg/kg in cereals intended for direct human consumption (Reg. 1881/2006/EC). Therefore, the development of sensitive, reliable and fast method represents an important feature for the evaluation and management of risk to public health arising from dietary exposure to Fusarium toxins. In this work, a selective and accurate analytical method was developed for the quantitative determination of DON and NIV in cereals for human and animal consumption. The method, based on high performance liquid chromatography and fluorescence detection, presents an automated 2 channel post-column derivatization, performed with sodium hydroxide, methyl acetoacetate and ammonium acetate. The sample preparation required a rapid extraction of mycotoxins with water and a purification step by hydrophilic-lipophilic balance (HLB) column clean-up. Optimal fluorescence detection was obtained by using an excitation and emission wavelength of 360 nm and 470 nm, respectively. Under the optimized experimental conditions, a complete separation of DON and NIV with a retention time repeatability below 1.3% (n=12) was obtained in less than 20 min, using a C18 column eluted with acetonitrile (10%) and 0.01% acetic acid (90%) at a flow rate of 0.4 mL/min. The on-line post-column derivatization ensures excellent results in terms of simplicity, sensitivity (limits of detection down to 0.07 mg/kg) and intra-day precision (RSD lower than 10%), demonstrating the feasibility of the method in accurate confirmation analyses.

Keywords: deoxynivalenol, nivalenol, HPLC, fluorescence detection

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G-27
DEVELOPMENT AND VALIDATION OF A MULTI-METHOD FOR THE ANALYSIS OF 33 MYCOTOXINS IN MAIZE USING UPLC-MS/MS (ESI+)

Ionara Pizzutti[1*], André de Kok[2], Jos Scholten[3], Cristiano Spiazzi[4]

[1 4] Federal University of Santa Maria – Chemistry Department – Center Of Research and Analysis of Residues and Contaminants (CEPARC), Santa Maria, RS, Brazil
[2 3] VWA–Food and Consumer Product Safety Authority–Chemistry Laboratory–R&D Group, National Reference Laboratory (NRL) for Pesticides and Mycotoxins Analysis in Food, Amsterdam, The Netherlands
[*] Corresponding author–E-mail: pizzutti@quimica.ufsm.br; Phone: 55 55 32209458; Fax: 55 55 32209458

Maize represents one of the most produced crops in the world. In 2007, 800 million tons were produced, and the USA is responsible for almost half of this amount. Brazil is the 3rd largest world producer and the largest in Latin America. Maize is widely used as primary base for human and animal consumption products, which makes a strict control of residues and contaminants in this crop necessary. One of the main risk factors is the presence of mycotoxins, potentially carcinogenic substances produced by certain species of fungus, which are commonly present in maize. The purpose of this work is to develop and validate a method capable to extract a large number of mycotoxins, and concurrently also pesticides. The analytical method performed was UPLC-MS/MS (ESI+). An acetonitrile-based extraction procedure was used and no clean-up step was applied. A slurry containing maize flour and water (1:1.5; *w/w*) was made and then extracted with acetonitrile/acetic acid (1%), followed by addition of magnesium sulfate for partitioning. The mycotoxins were divided in two groups for the fortification step: Group 1 with 15 mycotoxins (1, 2 and 10 µg kg^{-1} spike levels), and Group 2 with 18 mycotoxins (50, 100 and 400 µg kg^{-1} spike levels). Accuracy (as recovery%), precision (RSD%), method LOQ, as well as instrument LOD values and matrix effects, were determined. Group 1 of mycotoxins showed 2, 3 and 6 mycotoxins with acceptable method performance for quantification (recoveries > 70% and RDS < 20%), for the spike levels 1, 2 and 10 µg kg^{-1}, respectively. Group 2 presented 4, 5 and 5 mycotoxins which fulfilled the requirements at 50, 100 and 400 µg kg^{-1} spike levels, respectively. The matrix effects were, for most of the mycotoxins, insignificant or acceptable and did not had influence on the results. Due to the possibility to extract not only mycotoxins but also pesticides within the same procedure, the combined method proved to be very efficient in routine survey samples. Thus, the results obtained were satisfactory for the project's purpose.

Keywords: Mycotoxins, Maize, Multi-Method, Validation

G-28

EFFECT OF BUFFER, IONIC STRENGTH, AND PH ON A RAPID STRIP TEST FOR THE SEMI-QUANTITATIVE DETERMINATION OF TOTAL FUMONISINS IN MAIZE

Karina Grossalber[1*], Alexandra Molinelli[2], Michael Zheng[3], Rudolf Krska[4]

[1 4] Christian Doppler Laboratory for Mycotoxin Research, University of Natural Resources and Applied Life Sciences, Tulln, Austria
[2] Romer Labs Division Holding GmbH, Tulln, Austria
[3] Romer Labs Singapore Pte. Ltd., Singapore
[*] Corresponding author–E-mail: karina.grossalber@boku.ac.at; Phone: +43 / 2272 / 66280–456

Fumonisins are natural toxins produced by several *Fusarium* species that grow on agricultural commodities mainly in maize on the field or during storage. Cost effective and rapid on-site mycotoxin testing methods such as lateral flow devices have increasingly gained importance in the last few years. For these tests, factors such as the type of buffer used, ionic strength, organic solvent content and pH value affect the signal intensity of the test line, the strip background, as well as the flow characteristics. A balance between sufficient organic solvent content and buffering proportion has to be found to meet the optimum extraction conditions. A lateral flow device (LFD) for the semi-quantitative determination of total fumonisins (FB1, FB2 and FB3) in maize was developed and was used to test several sample extraction mixtures and test buffers with different pH values and ionic strength for optimizing the extraction and test procedure. PBS, Tris/HCl, carbonate, borate and citrate buffers each 0.1 M were used for this study as extraction buffer mixed with methanol (30/70, *v/v*) and as buffer for performing the strip test with or without Tween 20. Buffers strongly affected assay performance and signal intensity. Tris/HCl as extraction as well as test buffer containing Tween 20 turned out to be the best combination for producing high test line signal intensity and reducing non-specific background on the nitrocellulose membrane. Moreover adequate toxin recoveries were achieved with methanol/Tris/HCl (70/30, *v/v*) as extraction buffer compared to the more common extraction solvent methanol/dH_2O (70/30, *v/v*). The presented test is both semi-quantitative and rapid and does not require any sample clean-up steps after a 3 min extraction.

Keywords: fumonisin, LFD, optimization, buffer

G-29

DEVELOPMENT AND VALIDATION OF A MULTI-METHOD FOR THE ANALYSIS OF 36 MYCOTOXINS IN COCOA BEANS USING UPLC-MS/MS (ESI+)

Ionara Pizzutti[1*], André de Kok[2], Jos Scholten[3], Wagner Azambuja[4]

[1 4] Federal University of Santa Maria – Chemistry Department – Center of Research and Analysis of Residues and Contaminants (CEPARC), Santa Maria, RS, Brazil
[2 3] VWA–Food and Consumer Product Safety Authority–Chemistry Laboratory–R&D Group, National Reference Laboratory (NRL) for Pesticides and Mycotoxins Analysis in Food, Amsterdam, The Netherlands
[*] Corresponding author–E-mail: pizzutti@quimica.ufsm.br; Phone: 55 55 32209458; Fax: 55 55 32209458

Being produced almost only in tropical regions, with warm and moist climate, it's really difficult to keep moulds from growing in cocoa beans during storage. These moulds can produce highly toxic substances known as mycotoxins. Added to that, the world largest cocoa producers, Latin America and West Africa, face serious fungal diseases. As a consequence of these facts, an increase of mycotoxins found is expected, but for this moment the knowledge about the presence of mycotoxins in the harvested and fermented products is still lacking. This is mainly caused by the difficulties encountered with the analysis of mycotoxins in cocoa beans and its derived products. During this study, various clean-up procedures were tested with a modified QuEChERS method1 which was optimized to analyze mycotoxins and pesticides simultaneously. Due to unacceptable losses, a clean-up step was omitted for the final method. For validation purposes, the mycotoxins were divided in two groups, with the 17 mycotoxins which were detectable more sensitively, spiked at 1, 2 and 10 μg kg^{-1} and the 19 less sensitive ones, spiked at 50, 100 and 400 μg kg^{-1}. All mycotoxins were analyzed six times under repeatability conditions for each spike level. Linearity of analytical curves, recoveries, precision (RSD%) and matrix effects were determined. From the 33 mycotoxins analyzed, 21 presented recoveries between 70% and 120% and RSD lower than 20% (method performance criteria set by SANCO) and were successfully validated. The LOQ of 5 mycotoxins reached 1 and 2 μg $kg^{-1}$and the others showed values between 5 and 100 μg kg^{-1}. Matrix effects were significant, but had no considerable influence on the obtained results. The developed method has been applied successfully for a survey of mycotoxins in cocoa beans. The mycotoxins detected prove the relevance of the method.

[1] Pizzutti, I. R., De Kroon, M., Prestes, O. D., Rensen, P., De Kok, A., 7th European Pesticide Residue Workshop, Berlin, Book of Abstracts, 2008, 213.

Keywords: Mycotoxins, Cocoa beans, Multi-Method, Validation

G-30

MULTI-MYCOTOXIN ANALYSIS USING IMMUNOAFFINITY COLUMN CLEAN-UP AND LC-MS/MS DETERMINATION FOR DEOXYNIVALENOL, ZEARALENONE, T2 AND HT2 TOXINS IN FOODS

Rita Lorenzini[1*], Davide Garbini[2], Martino Barbanera[3], Ilaria Braschi[4]

[1 4] Department of Environmental Science and Technologies, University of Bologna, Italy
[2 3] Coop Italia, Casalecchio di Reno (BO), Italy
[*] Corresponding author–E-mail: rita.lorenzini@unibo.it; Phone: 0039-333-6745562

Mycotoxins have very low legislative limits due to their high toxicity, thus their extraction yield and LODs are critical. Analyses of single toxins are costly and time consuming, making them unappealing for large scale food control activity. Development of multiextractive and multideterminative methods, suitable for a variety of different food commodities, is an important task for many laboratories.

This study refers on a multiextractive method for deoxynivalenol (DON), zearalenon (ZON), T2, and HT2 toxins (the last two still awaiting legislative limits) proposed by R-Biopharm Rhône LTD and preliminarly tested by Central Science Laboratory (UK). The DZT PREP multiantibody immunoaffinity columns (IACs), not yet on the market at the time and kindly supplied by Biopharm Rhône, were tested. The optimised extraction method (60% MeOH-40% H_2O for 20 min followed by IAC cleanup) was then applied to the analyses of different food matrixes, as sweet and savoury snacks, dry and filled pasta, flours, bread, breakfast cereals, etc. Recoveries were 60 ± 11% for DON (200 to 400 ppb spike), 73 ± 17% for ZON (100 ppb spike), 100 ± 10% for T2 (100 ppb spike) and 96 ± 10% for HT2 (100 ppb spike), showing a good compromise between different analytes extraction conditions.

Conventional LC detectors used for mycotoxin (UV, fluorimeter) only allow the determination of one or a few analytes at the time, whereas MS/MS detector overcomes this limit. For each of the two mycotoxins groups, a dedicate multideterminative LC-MS/MS method was created. The chromatographic run lasts 14 min. The quantitation was performed setting the MRM mode on three daughter ions for each analyte.

The optimized method was used to analyse several food commodities collected from the Italian market, allowing high speed and easiness of analysis, using the same method for a variety of different food matrixes.

Keywords: mycotoxins, immunoaffinity, multiantibody, food analysis

G-31
DETERMINATION OF MYCOTOXINS IN BABY FOOD

Marta Kostelanska[1*], Jana Hajslova[2], Ivana Sosnovcova[3], Milena Zachariasova[4], Alexandra Malachova[5], Zdenka Veprikova[6], Jan Poustka[7]

[1 2 3 4 5 6 7] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: marta.kostelanska@vscht.cz; Phone: +420220443142

Mycotoxins are toxic natural contaminants, which can be often and very easily found on various types of crops, feeds and foodstuffs worldwide. To the very sensitive kind of food belong infant and baby foods, whose consumers are considered to be more susceptible to different toxins than adults, especially because of their low weight, higher metabolic rate and not fully developed ability to detoxify hazardous xenobiotics that they consume. The inevitable exposure to mycotoxins and harmful impact on this young group of consumers lead to necessity to established their health risk assessment. The outcomes of extensive projects´ surveys can help with determination of actions which would be the most effective in reducing risks and set appropriate control regulations. Unfortunately, there are not sufficient analytical methods and data available for baby food until today. Latest trends in mycotoxins analysis are to develop perfect analytical methods that can be used for multi-mycotoxins determination in single run and without any time-consuming sample pretreatment.

The aim of this study was to develop a multi-mycotoxins analytical method based on HPLC-MS/MS system for sensitive, fast and easy determination of large scale of mycotoxins in baby food. For this purpose sophisticated instrument HPLC coupled with 5500 Q-TRAP (Applied Biosystems) was used. Mentioned analytical system enabled to reduce analysis time to only few minutes and obtaining LOQs at low ppb levels. In general, the following mycotoxins were implemented into the method: trichothecenes type A and B, their conjugates and metabolites, ochratoxin, patulin, zearalenon, fumonisins and alternaria toxins. These toxins belong to the most commonly detected in various types of food and for some of them hygienic limits have been already established. Method optimization and validation was performed by means of commercial samples of baby food with cereal and/or fruit basement.

Keywords: mycotoxins, conjugated mycotoxins, Baby Food, HPLC-MS/MS

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G-32

CRITICAL ASSESSMENT OF TWO ALTERNATIVE APPROACHES IN ANALYSIS OF CYANOGENIC GLUCOSIDES IN FLAXSEED: DART-TOFMS AND LC-MS/MS

Anna Krajcova[1], Hana Novotna[2], Vera Schulzova[3], Jana Hajslova[4*], Marie Bjelkova[5]

[1 2 3 4] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[5] Agritec Plant Research s. r. o., Sumperk, Czech Republic
[*] Corresponding author–E-mail: jana.hajslova@vscht.cz; Phone: (+420) 220443185

Cyanogenic glucosides (CGs) are present in more than 2500 different plant species, including flax (*Linum usitatissimum* L.), white clover (*Trifolium repens*), cassava (*Manihot asculentum*) etc. These β-glucosides of α-hydroxynitriles derived from amino acids are produced by plants as a secondary metabolites which are considered to be a part of their chemical defense against herbivores due to bitter taste and release of toxic hydrogen cyanide (HCN) upon tissue disruption. The toxicity of HCN is caused by its affinity to terminal cytochrome oxidase in the mitochondrial pathway.

Considerable dietary source of cyanogenic compounds is flaxseed, with diglucosides linustatin and neolinustatin as a major representatives of this group and monoglucosides linamarin and lotaustralin as minor components. The levels of cyanogenic compounds occur in flax in a large range: 30-3000 mg/kg, depending on a variety, climatic conditions, and way of farming.

In this study, two analytical approaches for determination of CGs in flaxseed have been tested and subsequently compared. In the first phase of experiment, aqueous methanolic extracts of flaxseed samples were examined using LC-MS/MS by monitoring of sodium adducts of target analytes. As an alternative approach we also used unique ion source Direct Analysis in Real Time (DART) coupled to time-of-flight mass spectrometry (TOFMS) (FWHM 5000). The use of DART-TOFMS technique enabled elimination of sample preparation step thus significant improvement in sample throughput (the time needed for analysis of single sample is 30 s per run). Although limits of detection (LODs) are rather higher for DART-TOFMS analysis, two major CGs can be reliably quantified when internal standard is employed for compensation of total ion current fluctuation. As far as minor CGs, linamarin and lotaustralin, are to be determinated, either mass analyser with high resolution or tandem mass spectrometer should be used.

Keywords: cyanogenic glucosides, LC-MS/MS, DART-TOFMS

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G-33

THE BENEFITS OF ORBITRAP HIGH RESOLUTION MASS SPECTROMETRY FOR FREE AND MASKED MYCOTOXINS IN MALT AND BEER

Milena Zachariasova[1*], Tomas Cajka[2], Michal Godula[3], Marta Kostelanska[4], Alexandra Malachova[5], Jana Hajslova[6]

[1 2 4 5 6] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[3] Thermo Fisher Scientific, Prague, Czech Republic
[*] Corresponding author–E-mail: milena.zachariasova@vscht.cz; Phone: 0042220443142

The goal of this study was to demonstrate the analytical capabilities of high resolution mass spectrometer based on the Orbitrap™ technology for quantitative analysis of mycotoxins, toxic secondary metabolites of filamentary fungi, in food matrices, concretely barley, malt and beer. The Ultra High Performance Liquid Chromatography (U-HPLC) coupled to an Orbitrap (Thermo Scientific Exactive™) operating with ressolving power up to 100.000 FWHM in combination with accurate mass measurement (< 5 ppm) were used for this purpose. High resolution of the orbitrap technology implicated good separation of target analytes from co-eluting background matrix interferences resulting to very low detection limits of targeted compounds. Additionally, potential of atmospheric pressure chemical ionization in terms of sensitivity of detection when compared to electrospray ionization is also discussed.

Keywords: LC-MS, orbitrap, mycotoxins

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G-34
LC-MS/MS DETERMINATION OF CALYSTEGINES AND GLYCOALKALOIDES IN ORGANIC AND CONVENTIONAL POTATOES

Vera Schulzova[1*], Anna Krajcova[2], Jana Hajslova[3]

[1 2 3] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: vera.schulzova@vscht.cz; Phone: +420220443218

The demand for organically produced food is increasing and potatoes (*Solanum tuberosum*) are one of the main of staple commodity grown in organic farming systems. They are known for their richness of health promoting compounds and micronutrients, but they also contain a large number of biologically active constituents, commonly referred to as secondary metabolites.

Potatoes contain toxic glycoalcaloids, mainly α-solanine and α-chaconine. Typical glycoalkaloid levels in potatoes are 30–120 mg/kg. The main toxic action of glycoalkaloids is inhibition of blood and brain cholinesterase and disruption and injury of membranes in the gastrointestinal tract. Hygienic limit is 200 mg/kg (expressed as sum of α-solanine, α-chaconine and α-tomatine). In addition to glycoalkaloids, potatoes also contain biologically active calystegines. Calystegines are water-soluble biologically active northropane alkaloids, their structure resembles atropine, they are selective glycosidase inhibitors. Until now, 17 calystegines differing in number and position of hydroxyl groups in their molecule have been identified and the main potato calystegines are A_3, B_2, and B_4.

For determination of calystegine levels HPLC method with MS-MS detection in APCI+ mode was developed and validated. 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 for individual calystegines A_3, B_2 and B_4 in plant matrices was 0.4 mg/kg, 0.25 mg/kg and 0.5 mg/kg respectively. Repeatability, expressed as relative standard deviation, was in the range 4–6%. For determination of glycoalkaloid levels HPLC method with MS-MS detection in SRM mode (ESI+) was used. Limit of detection for analysed glycoalkaloids was 0.1 mg/kg, repeatability, expressed as relative standard deviation, was 4%. The possibility of determination of calystegines and glycoalkaloides within one analytical method was tested too.

In our study five common potato varieties Bionta, Karin, Marabel, Rosara and Satina were grown in two localities in organic and conventional cultivation system. Interestingly, the levels of calystegines (sum of A3, B2 and B4) were markedly higher than that of α-solanine and α-chaconine. Average level of glycoalkaloids was 92 mg/kg, while average level of calystegines was even 229 mg/kg. Levels of analysed natural toxins have been shown to be strongly affected by an individual variety and climatic conditions way of farming does not influence significantly the levels of these plant secondary metabolites.

Keywords: calystegines, glycoalkaloides, potatoes, LC-MS/MS

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G-35

NIVALENOL-GLUCOSIDE: ANALYTICAL APPROACHES TO DETERMINATION OF THIS NEW MASKED MYCOTOXIN

Alexandra Malachova[1*], Ondrej Lacina[2], Milena Zachariasova[3], Marta Kostelanska[4], Jana Hajslova[5]

[1 2 3 4 5] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: alexandra.malachova@vscht.cz; Phone: +420 220 443 142

One of the main issues in mycotoxins determination being now globally solved is the identification of their so far not well known forms called masked mycotoxins. Masked or conjugated forms of mycotoxins originate in plants as a result of detoxification reactions of plant metabolism. This detoxification process includes conjugation of parent mycotoxins to polar substances such as sugars, amino acids or sulphate. Up to now, masked forms of deoxynivalenol, zearalenon, ochratoxin A and fumonisins have been detected. This study is documenting the identification of new masked mycotoxin–nivalenol-glucoside. The identification was performed using an Acquity Ultra High Performance Liquid Chromatography coupled to orthogonal accelerated time-of-flight mass spectrometer Waters LCT Premier XE (Waters, USA), whereby mass resolving power of 12,000 FWHM and a mass accuracy measurement < 0.001 Da for masses < *m/z* 200 allows sufficiently accurate mass identification of peak of interest. Confirmation of nivalenol-glucoside presence was carried out applying specific fragmentation measurement in both positive and negative modes. In addition, various sample preparation procedures, chromatographic separations and conditions must have been developed and tested during nivalenol-glucoside detection as discussed in the study.

Reference:
Engelhardt D., Ruhland M.: Metabolism of Mycotoxins in Plants, Adv. Food Sci., 21 (1999) 71-78

Keywords: masked mycotoxins, nivalenol-glucoside, LC-TOF MS

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G-36

TECHNOLOGIES FOR REPLACEMENT OF RODENT BIOASSAYS IN SENSITIVE DETECTION OF TOXINS IN FOODS

John Mark Carter[1*], David Brandon[2], Luisa Cheng[3], Laurie Clotilde[4], Xiaohua He[5], Reuven Rasooly[6], Miles Scotcher[7], Larry Stanker[8]

[1 2 3 4 5 6 7 8] Agricultural Research Service, US Dept Agriculture, Albany, CA, USA
[*] Corresponding author–E-mail: j.mark.carter@ars.usda.gov; Phone: 510-610-9235; Fax: 510-559-6429

Rapid sensitive assays for biothreat toxins that can be used to detect intentionally contaminated foods are now typically performed via bioassay in live mice. While bioassay provides essential data on bioavailability, animal models are technically, fiscally, and ethically challenging. Through careful application of state-of-the-art techniques for immunization and screening, we created new monoclonal antibody reagents (MAb) specific for detection of botulinum neurotoxin (BoNT). These MAbs bind BoNT so tightly that, in a sandwich ELISA, they are more sensitive than the rodent bioassay. These reagents are also useful for sample preparation and production of portable tests for field use. Through a CRADA we used these MAbs to develop a simple “dipstick” assay that can detect BoNT in food at levels well below the human oral LD_{50}. We also used the new MAbs to develop sample preparation methods based on immunomagnetic beads. In liquefied food extracts these beads rapidly and irreversibly bind all toxin present in a large sample. Sequestering the beads with a magnet effectively concentrates the toxin into a small volume suitable for laboratory testing. While the toxin is still bound to the beads, we can detect its highly specific peptidase activity using a fluorescence (FRET) based substrate, for detection of sublethal amounts of BoNT in foods.

Keywords: botulinum neurotoxin, ricin, immunoassay, antibody

G-37
MULTI RESIDUE SCREENING ANALYSES BY INTEGRATED FOOD CONTAMINANT ELISA KITS MANAGED BY AN INNOVATIVE ROBOT

Francesca Diana[1*], Giulia Rosar[2], Paola Curto[3], Lidija Persic[4], Maurizio Paleologo[5]

[1 2 3 4 5] Tecna S.r.l, Area Science Park, Padriciano 99, 34012 Trieste, Italy
[*] Corresponding author–E-mail: rd@tecnalab.com; Phone: +39 040 3755341; Fax: +39 040 3755343

Nowadays, a large number of food contaminants have to be analysed by industries and state control laboratories. For this purpose, several ELISA kits are available on the market. Despite the validation data for these screening methods, the reliability of routine analysis is sometimes questionable. In this context, automation could be a way to standardize results and help in the multiresidue screening of foodstuffs. MK-Robot is a robotic workstation for the full automation of enzyme immunoassays. The whole ELISA protocol can be carried out automatically: from standard and sample dilution, dispensing, shaking, incubation, washing and plate reading, to analysis of data and creation of the test report. The MK-Robot can host up to six microtitre plates, and, when incubation time and washing procedure are the same, more than one assay can be run on a single plate. Up to 160 samples can be managed and analysed for one as well as for multiple contaminants. A detector assures that the correct amount of liquid is dispensed, and high precision pumps determinate highly precise liquid handlings (CV < 1%). Special washing programs and liquid handlings can be created to optimize the timing of a method and to counterbalance dispensing and incubation time. The robot performance was validated on Tecna's EIA kits for mycotoxins as well as for veterinary drugs residues. The results obtained were compared with those from manual test implementation in terms of time requirement and performance. Calibration curves were always compliant with assay kit specifications. The dosage of spiked and incurred certified samples was as accurate as in manual implementation, and the specificity for negative samples was also similar. The work time for the operator is highly reduced, because after programming the work session and preparing all the necessary reagents, no further operations are required: MK-Robot is a completely "walk away" automatic system. Moreover, the automation prevents errors that can occur to a human operator, such as reagents mismatch, dispensing errors and wrong incubation times. This fact enhances quality of results and consequently saves money. The validation results demonstrate that MK-Robot is an accurate, precise, time-sparing and error-free system for multiple ELISA tests, convenient even for medium throughput laboratories.

Keywords: ELISA, automation, robot, multiresidue, mycotoxin

G-38
CHANGES IN MYCOTOXIN CONTENT DURING MALTING AND BREWING

Zdenka Veprikova[1], Milena Zachariasova[2], Marta Kostelanska[3], Alexandra Malachova[4], Jan Poustka[5], Jana Hajslova[6*]

[1 2 3 4 5 6] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: jana.hajslova@vscht.cz; Phone: + 420 220 443 185

Fusarium mycotoxins, the secondary toxic metabolites produced by microscopic filamentary fungi belonging to the *Fusarium* genera, are natural contaminants of cereals. Food processing technologies, especially fermentation process, may significantly influence their levels in foodstuffs. Transfer of the most frequent *Fusarium* mycotoxin deoxynivalenol (DON) and its main metabolite deoxynivalenol-3-glucoside (DON-3-Glc) from raw barley into the malt and beer perform a serious problem for malting and brewing industry, and on this account, maximal effort to monitor content of those mycotoxins during the production technology chain have been developed. The aim of this work was to clarify the dynamic of DON and its conjugates during the malting and brewing technologies. The raw barley, malting intermediates (steeped barley, green malt and malt germs), malt, brewing intermediates (mush, sweet wort, young wort, young beer) and final beer were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Generally, it can be concluded that during malt and beer production significantly increase the DON-3-Glc content (DON-3-Glc / DON molar ratio is mostly ≥ 1). The reason is probably the high enzymatic activity during germination of barley and mashing of malt which implicates the releasing of DON-3-Glc from bound forms.

Keywords: mycotoxins, fusarium, beer, malt, deoxynivalenol

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G-39
DUTCH SURVEY ON THE OCCURRENCE OF PYRROLIZIDINE ALKALOIDS IN ANIMAL FORAGE

Patrick Mulder[1*], Babette Beumer[2], Efraim Oosterink[3], Jacob de Jong[4]

[1 2 3 4] RIKILT–Institute of Food Safety, Wageningen, The Netherlands
[*] Corresponding author–E-mail: patrick.mulder@wur.nl; Phone: 0031 317 480 361

Pyrrolizidine alkaloids (PAs) are secondary plant metabolites produced by a number of plants from the *Asteraceae*, *Boriginaceae* and *Fabaceae* families. Many of these alkaloids are toxic, causing hepatic veno-occlusive disease, liver cirrhosis and ultimately death. Many PAs have also mutagenic and carcinogenic potential. Amongst livestock, cattle and horses are especially known to be susceptible to the toxic effects of PAs. Humans may also be at risk by the consumption of milk of livestock fed with PA-contaminated forage. The European Food Safety Authority (EFSA) has recently published a scientific opinion on the presence of PAs as undesirable substances in animal feed (EFSA Journal, 447: 1 (2007)). The EFSA report states the need for suitable analytical methods for the detection and quantification of PAs in animal feed and the importance of collecting monitoring and survey data.

Members of the ragwort and groundsel genus (*Senecio*) are common weeds occurring world-wide in pastures, (marginal) grasslands, along the borders of rivers, roads and highways. *Senecio* species have been often implicated in intoxications of livestock and sometimes humans.

At RIKILT a (semi)quantitative LC-MS/MS method has been developed for the determination of 40 macrocyclic PAs in animal feeds. The included PAs comprise tertiary bases and N-oxides representative for ragwort species. The method has been used for the analysis of 147 forage samples (grass silage, dried grass, hay, alfalfa) collected in 2006–2008 in the work of the Dutch National Monitoring Program on animal feedingstuffs. In 31 forage samples (traces of) PAs could be detected (LOD: 10 µg/kg). In nine samples the total PA content exceeded 100 µg/kg. In three instances the total PA content exceeded 1 mg/kg. A relatively low incidence of PA contamination was observed for grass silage, dried grass and hay. One hay sample from a nature reserve area contained 549 µg/kg PAs and one dried grass sample contained 288 µg/kg. In contrast, alfalfa was found to be often contaminated with PA residues as 23 samples (74%) contained at least traces of one or more PAs. Three samples (10%) contained high amounts of PAs, between 3.5 and 5.4 mg/kg

In order to link the observed PA profiles in the contaminated samples with a particular *Senecio* species, reference samples of Tansy ragwort (*Senecio jacobaea*), Common groundsel (*Senecio vulgaris*) and Narrow-leafed ragwort (*Senecio inaequidens*) were collected in the vicinity of Wageningen, The Netherlands. Average PA profiles were constructed for these three species. Comparison with these reference profiles revealed that in most instances the forage samples were contaminated with Common groundsel. Only in two occasions there was evidence for other *Senecio* species present in the forage.

Keywords: pyrrolizidine alkaloids, animal forage, survey

G-40

EVALUATION OF TOTAL AFLATOXIN LEVELS IN LAYER FEED SAMPLES OF COMPANIES PRODUCING THEIR OWN FEED IN EDINCIK AND BANDIRMA PROVINCE OF TURKEY

Nurcan Degirmencioglu[1*], Hüseyin Eseceli[2], Ergün Demir[3], Songül Sentürklü[4]

[1 2 3] Balıkesir University Vocational High School Bandırma, Balıkesir, Turkey
[4] Canakkale Onsekiz Mart University Vocational High School Biga, Canakkale, Turkey
[*] Corresponding author–E-mail: nurcan412004@yahoo.com; Phone: +902667210610; Fax: +902667210613

Feed contamination to fungi can lead to nutrient losses and detrimental effects on animal health and production. Mycotoxins are secondary metabolits produced by fungi which contaminate a large variety of feeds, with toxic effects in animals. In this study, a total of 100 layer feed samples were collected randomly during the years 2007 and 2008 from different 25 companies producing their own feed in Edincik and Bandırma province of Turkey. Feed samples were stored in plastic bags at 40°C until the analysis. Samples were analyzed for contamination with aflatoxins using enzyme-linked immunoabsorbent assay (ELISA) method. The RIDASCREEN® (Art.No: R5202) test kits (R-Biopharm AG; Germany) were used for the analyses. The mean total aflatoxin concentration were found 4.38 ± 0.96 (n=50) and 5.21 ± 1.24 (n=50) ppb in first and second periods, respectively and the incidence of total aflatoxin in the layer feeds was 8%. In conclusion, the levels of aflatoxin found in the samples could not be considered a risk to poultry health and productivity.

Keywords: layer hen, mycotoxin level, feed

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PROCESSING AND PACKAGING CONTAMINANTS

(H-1 – H-32)

H-1
FOOD CONTACT MATERIALS PROFICIENCY TESTING PROVIDES AN ESSENTIAL QC ROLE

Mark Sykes[1*], Elaine Leach[2], Paul Hauk[3], Emma Bradley[4]

[1 2 3 4] Fera, York, UK
[*] Corresponding author–E-mail: mark.sykes@fera.gsi.gov.uk; Phone: +44 (0)1904 462697

Packaging materials are intended to keep the foods within fresh and protected from external contamination. Media concern and legislation on contamination from the food packaging materials themselves is increasingly evident. Rapid alerts issued on food contact materials raise awareness of the issue of contamination of foods from their packaging. The analysis of contact materials that migrate into foods poses a number of challenges. The Community Reference Laboratory for Food Contact Materials publishes various methods, both for overall migration and specific analytes. Laboratories participating in proficiency testing for packaging materials are instructed to follow a particular methodology for the migration step. The determination method would then be the laboratory's own routine one. The Food Analysis Performance Assessment Scheme (FAPAS®) runs proficiency tests for overall migration, as well as specific analyte migration, of food contact materials. Despite the use of CEN standard procedures and certified reference materials, there tends to be a low number of satisfactory results for such proficiency tests. Generous target standard deviations are applied, to match the large tolerance of uncertainty for this type of analysis. Nevertheless, overall migration proficiency tests are a valuable source of quality assurance for participating laboratories.

FAPAS® specific analyte proficiency tests for contact materials concentrate on those chemicals of particular current interest plus those which are frequently documented. Phthalates and bisphenol A are examples of the latter, especially in relation to infant food packaging. Primary aromatic amines (PAAs) have been introduced into proficiency testing rounds, in response to more recent concern raised in rapid alerts. Similarly, epoxidised soy bean oil (ESBO) migrating from jar lid gaskets into oily foods, has been tested for only in the last couple of years.

Here we present examples of typical FAPAS proficiency testing results for food contact materials. The spread of z-scores is an indication of the difficulty of the analysis. Proficiency testing in this area is relevant to the media interest and legislation in food contamination. Participation in such proficiency testing is a requirement of accreditation for laboratories. Proficiency testing provides an essential quality control service, even for those laboratories without accredited methods.

Keywords: proficiency testing, food contact materials

H-2

RAPID ANALYSES OF INK PHOTOINITIATORS WITH A MULTI METHOD IN FOOD PACKAGING MATERIALS AND FOODSTUFFS

Tina Richter[1*], **Thomas Gude**[2]

[1 2] Swiss Quality Testing Services (SQTS), Switzerland
[*] Corresponding author–E-mail: tina.richter@sqts.ch; Phone: +41 (0)44 277 3154

In the food packaging industry, photoinitiators are used to trigger the radical polymerisation of UV cured inks and lacquers, whilst drying the liquid film on the outer surface of the packaging materials. Photoinitiators, like benzophenone and its derivates, are found in fragrances, toiletries, pharmaceuticals and insecticides. In cosmetic products they are used as photostabilisers and have a photoprotective effect on skin. Exposure from all these source are also possible. One of the most well researched photoinitiators is isopropyl-9H-thioxanthen-9-one (ITX), but many other substances can be used as substitutes. Benzophenone is until now the only one regulated in the European Commission Directive 2002/72/EC, with a specific migration limit of 0.6 mg/kg food.

Inks applied to food packaging materials are currently not specifically regulated in the EC legislation, only in the general Regulation 1935/2004 and GMP Regulation 2023/2006. Switzerland however, has a more detailed regulation for food contact materials, including the printing inks used. The ink manufactures will be required to provide the necessary data for the Swiss authorities by the end of March 2010. Key issue will be beside toxicological profiles also the analytic of printing ink components.

Along with the toxicological profiles, the quantification of these substances in packaging material, as well as in food, will be required. To reduce the analytical costs, a rapid multi method is used to determine the most common photoinitiators including derivates. The possible migration will be simulated with the modified polyphenylenoxid (MPPO), a suitable simulant also known as Tenax®. In this rapid multi method, the extracting agent is acetonitrile and the analysis uses a UPLC-MSMS, with a detection limit of 0.1 µg/dm^2 for packaging material and 10 µg/kg for food. In addition, all results will be verified and compared using the UPLC-TOF and will be presented upon completion.

Keywords: UVinks, photoinitiators, migration, food packaging

H-3
DIETARY INTAKE OF THE FOOD PROCESS CONTAMINANT FURAN

Arvid Fromberg[1*], Sisse Fagt[2], Kit Granby[3]

[1 2 3] National Food Institute, Technical University of Denamrk, Søborg, Denmark
[*] Corresponding author–E-mail: arfr@food.dtu.dk; Phone: +45 35 88 74 72

Furan has been found to be formed in canned, jarred and roasted food items and high levels of furan have been found in coffee. As furan is carcinogenic in animal experiments, attention has been drawn to the presence in commercial and home-cooked foods. Home cooked foods having high levels of carbohydrates are most likely to form furan, probably due to Maillard browning reactions of the food. For ready-to-eat food with an initial level of furan, cooking reduced the level of furan in the probably due to evaporation of the furan during heating. Nevertheless furan is relatively stable in food items and the loss of furan from heated ready-to eat foods left for cooling may be disregarded as levels do not decrease significantly until the food is reheated. On basis of analysed data, a few supplementary furan data from the literature and intake of foods and beverages from the Danish National Survey of Dietary Habits and Physical Activity 2000–2004, the exposure of furan has been calculated. In the present analysis data from adults 15v75 years (n=4692) has been used. An estimate of the furan intake for adults revealed that 95% was from consumption of coffee. This estimate was, however based on the Danish consumption data and as Danes like other adults from Northern Europe has an average consumption of more than 0.6 L of coffee the contribution to the intake is high. As furan contributes to the sensoric expression of the food items consumed, recommendations to reduce the levels in food might affect the sensoric quality. Nevertheless risk communication to the consumers recommending heating ready-to-eat products properly as well as not toasting bread dark brown may improve the food safety without hampering the sensoric quality.

Keywords: furan, dietary intake, coffee, adults

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H-4

APPLICATION OF THE STANDARD ADDITION METHOD FOR THE DETERMINATION OF ACRYLAMIDE IN HEAT-PROCESSED FOODS BY LIQUID CHROMATOGRAPHY TANDEM MASS-SPECTROMETRY

Eva Muñoz[1*], Antoni Rúbies[2], Francesc Centrich[3]

[1 2 3] Servei de Química. Àrea d'anàlisi orgànica. Agència de Salut Pública de Barcelona. Av. Drassanes 13, 080001 Barcelona, Spain
[*] Corresponding author–E-mail: emunozca@aspb.es; Phone: 0034 93 443 94 00

Acrylamide is a naturally occurring by-product of the cooking process. Several studies have examined the link between dietary intake of acrylamide and various forms on cancer.The wide variety of matrices to analyze, not to have blank samples (without acrylamide), the low-level study to determine (µg/kkg) and the molecular characteristics (high polarity and low molecular weight) makes its quantification difficult.

In this contribution we present a validated method for the analysis of acrylamide residues by extraction with water, purification of extract using a solid-phase extraction cartridge, and then determination with liquid chromatography coupled to mass spectroscopy LC-ESI-MS/MS. In order to obtain clean extracts an improved purification procedure based on the coupling of two highly cross-linked polystyrene–divinylbenzene polymeric sorbents were used. Sufficient retention in the chromatographic method was achieved using a Synergy Hydro-RP column (250 $\times$ 4.6 mm, 4 µm; Phenomenex) at a column temperature of 40°C with water/ACN as eluent at a flow rate of 0.5 mL min^{-1}. The detection and confirmation was in MS/MS-mode, monitoring the quantification transition *m/z* = 72 to 55 and the ion 72 to confirm for acrylamide and the transition *m/z* = 75 to 58 for deuterated acrylamide. The difficulty of finding blank representative matrices to do the calibration curves and the different matrix effect for each sample, signal suppressions were observed due to coeluting substances from the column, led us to use the standard addition to quantify. A four-point standard addition protocol was used to quantify acrylamide in food samples. A series of foodstuff samples was spiked with acrylamide at levels around the expected concentration range of the sample. Deuterated acrylamide was added to the sample prior to the extraction step at a constant level as internal standard. The limit of quantification (LOQ) was estimated to be 10 µg/kg. Validation and quantification results demonstrated that the method should be regarded. Acrylamide was determined in several of the most frequently eaten foodstuffs as potato crisps and chips, biscuits, crisp breads, pastry, breakfast cereals, dried fruits, frankfurt, babyfood and coffee.

Studied samples showed different levels of acrylamide ranging from <10 to 1500 µg/kg. The lowest values obtained were for bread and babyfood on the order of <10 µg/kg to 500 µg/kg, whereas french fries, potato chips and biscuits presented the highest values reaching to 1500 µg/kg.

Keywords: acrylamide, LC-MS/MS, standard addition

H-5

IMPACT OF INORGANIC SALTS ON L-ASPARAGINASE EFFECTIVITY OF ACRYLAMIDE ELIMINATION IN CEREAL PRODUCTS

Kristína Kukurová[1*], Zuzana Ciesarová[2], Alena Bednáriková[3], Lucie Marková[4], Vural Gökmen[5], Özge Çentikaya Açar [6]

[1 2 3] Food Research Institute, Priemyselná 4, 824 75 Bratislava, Slovak Republic
[4] Brno University of Technology, Faculty of Chemistry, Purkyňova 118, 612 00 Brno, Czech Republic
[5 6] Department of Food Engineering, Hacettepe University, 06800 Beytepe, Ankara, Turkey[*] Corresponding author–E-mail: kukurova@vup.sk; Phone: +421 2 50237 208

Acrylamide is a suspected carcinogen that is formed during heat-induced reaction between reactive carbonyls and amino acid asparagine known as the Maillard reaction. It has been proved that acrylamide formation in foods can be effectively avoided up to 90% by the appropriate application of L-asparaginase enzyme. The unambiguous advantage of this enzyme treatment seems to be the sustainability of expected nutritional properties of final products, as well as the browning and taste aspects [1]. Mechanism of L-asparaginase action consists in the conversion of free asparagine into aspartic acid which does not form acrylamide. The activity of enzyme strongly depends on pH and temperature values while L-asparaginase produced by *Aspergillus oryzae* has a pH optimum at neutrality with activity range from pH 4.0 to pH 8.0 at 37°C [2].

The aim of presented study was to determine the impact of different leavening agents on the effectivity of enzyme action during the cereal product processing and consequently in acrylamide mitigation. It was affirmed that selected inorganic salts significantly influenced the final pH value of dough which led to the reduced efficiency of L-asparaginase. The enzyme treatment at optimized conditions (pH 6.8, temp. 37°C) resulted in 75% of acrylamide decrease in final cookies. The increase of pH value from 6.8 in control sample (without leavening agent addition) to 8.1 with sodium hydrogen carbonate addition caused the decrease of asparaginase efficiency up to 65%. The prolonged time of enzyme incubation from 15 to 60 min subtilized the negative effect of higher pH since the decline of enzyme efficiency was observed just in the range of 25–30%.

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Keywords: acrylamide, asparaginase, inorganic salts, cookies

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H-6
OCCURRENCE OF FURAN FROM FOODSTUFFS IN THE BELGIAN MARKET

Georges Scholl[1*], Marie-Louise Scippo[2], Guy Maghuin-Rogister[3], Gauthier Eppe[4], Edwin De Pauw[5], Claude Saegerman[6]

[1] CART, Mass Spectrometry Laboratory, Faculty of Sciences, and Epidemiology and Risk Assessment, Faculty of Veterinary Medicine, University of Liège
[2 3] CART, Laboratory of food analysis, Faculty of Veterinary Medicine, University of Liège
[4 5] CART, Mass Spectrometry Laboratory, Faculty of Sciences, University of Liège
[6] Epidemiology and Risk Assessment, Faculty of Veterinary Medicine, University of Liège
[*] Corresponding author–E-mail: g.scholl@ulg.ac.be; Phone: +32(0)43663433

Food safety is a matter of a steadily increasing concern. To rely on a scientific assessment, the Authorities need an improved knowledge of the emerging contaminants [1]. Besides methods for their identification and quantification, their effect on human health should be evaluated. This requires extensive epidemiological studies including toxicokinetic and toxycodynamic data, dose-response, toxicological reference value, occurrence and exposure. To allow an estimation of the mean human exposure, a food occurrence assessment that cover as much as possible different matrices from basic food items to complex mixture is mandatory.

This assessment was carried out on furan, a food toxicant classified by IARC as possibly carcinogenic to human since 1995, and known as carcinogenic to rats [2,3]. Furan is a little heterocyclic molecule known to be found in foods that undergo heat treatment like canned and jarred food, but also in coffee and baby food [4]. The formations pathways are not yet well known and research about are going on.

We developed a sampling plan as exhaustive as possible with a limited number of items (n=500). This plan does not only take into account the matrices known to be contaminated, but also various matrices across the food chain. The items were distributed into 30 different groups of the food pyramid (e.g.: coffee, fats, meat, meat products and substitutes, fruits, vegetables, dairy based products, fish and fishery products). The distribution takes into account the geographic variation (different fabrication place and local product), the different supermarket companies and brands (different process and basic foodstuff), and also the consumption frequency (greater concern about the most consumed foodstuff).

The study reveals the presence of furan, from background to higher levels, in all the food chain. The ubiquitous contamination helps to estimate the ground level. Some specific food items exhibit high levels of furan: the coffee (the most contaminated item), the baby food, the breakfast cereals, and the ready to eat meals.

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Keywords: Furan, Occurrence, Food, Consumption, Belgium

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H-7
VALIDATION OF A SUB-ROOM TEMPERATURE ID-SPME-GC-MS METHOD FOR THE ANALYSIS OF FURAN IN FOOD

Georges Scholl[1*], Marie-Louise Scippo[2], Jean-François Focant[3], Edwin De Pauw[4], Gauthier Eppe[5]

[1 3 4 5] CART, Mass Spectrometry Laboratory, Faculty of Sciences, University of Liège
[2] CART, Laboratory of food analysis, Faculty of Veterinary Medicine, University of Liège
[*] Corresponding author–E-mail: g.scholl@ulg.ac.be; Phone: +32(0)43663433

Furan is a toxicant found in many food items that undergo heat treatment like canned and jarred food [1]. It is a little heterocyclic molecule classified by the IARC in 1995 as a possible carcinogenic to humans (group 2B) [2]. In 2004, the US Food and Drug Administration (FDA) published a report about its occurrence in food [1]. More recently, the European Food Safety Authority (EFSA) called for more information about its presence in food. Development of fast, sensitive and reliable analytical methods are needed to provide actual levels of furan in food in order to allow a more sound dietary exposure assessment of the European population.

We already reported the development of a headspace–solid phase microextraction (HS-SPME) coupled to gas chromatography–mass spectrometry (GC/MS) method [3]. The HS-SPME parameters were optimised by experimental design and the major finding resulted in a sub-room optimal extraction temperature [3]. The current study focuses on the validation of the HS-SPME for hot drink, juices, sauces and baby food according to the Commission Decision 2002/657/EC. To estimate the Limit of Detection (LOD) and the Limit of Quantification (LOQ), the standard-deviation/slope ratio approach was used instead of the signal to noise (S/N) approach. Indeed, the HS-SPME-GC-MS method has a limited linearity response range (3 orders of magnitude), The assessment of the LOQ by the S/N approach (S/N = 10) did not provide an acceptable accuracy at the LOQ. The CC_α and CC_β approach gave more reliable limits. Since no maximum limits for furan in food have already been enforced, the *Minimal Required Performance Level* (MRPL) methodology was applied. The CC_β were close to, or lower than, 1 ppb (e.g. 0.18 ppb, 1.02 ppb, 1.57 ppb and 0.32 ppb for the juices, the hot drinks, the sauces and the baby foods, respectively).

The intermediate precisions and the trueness were evaluated using juices, sauces and hot drinks homemade matrices,. The intermediate precisions RSDs (3 days, n=20) were 3.4% (at 0.65 ppb), 7.8% (at 1.37 ppb), and 12.6% (at 1.4 ppb), respectively. The mean relative biases (same conditions) were 9.8, 5.8, and 12.3%. In addition, the baby food matrix was evaluated through the participation at an interlaboratory exercise. The z-score (22 participants) was 0.7 (assigned value: 44.2 ppb; SD: 9.7 ppb) [4] and the intermediate precision RSD (2 days, n=4) 9.0%.

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Keywords: Furan, Validation, Analysis, SPME, GC-MS

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H-8

SCREENING OF FURANS IN AROMATIZED COFFEE SAMPLES BY SPME-GC-MS: COMPARISON WITH CONVENTIONAL COFFEE

Catarina Petisca[1*], Olívia Pinho[2], Isabel Ferreira[3]

[1 2 3] REQUIMTE- Serviço de Bromatologia, Faculdade de Farmácia da Universidade do Porto
[*] Corresponding author–E-mail: catarinapetisca@fcna.up.pt; Phone: +351222078929; Fax: +351222003977

Furan derivatives have traditionally been used as flavouring additives to food. In addition these compounds are formed during heating of carbohydrate rich food both during the industrial processing of food and at home during cooking. For some furan compounds like 5-hydroxymethylfurfural (HMF, 5-hydroxymethyl-2-furancarboxaldehyde) the levels exceed 1 g/kg in several food items, namely coffee. Furan itself has been regarded as a prioritised substance by EU. However, there are very little knowledge a number of furan related contaminates, like for example HMF and HMF derivatives, when comes to food sources, levels in food and toxicity. A risk assessment of HMF for use as a flavouring substance was performed by EFSA in 2005, and the use of HMF as a flavouring agent was put on hold because of potential genotoxicity. Several other HMF related substances are also used as flavouring substances and the knowledge of toxicity of these substances is very limited. In addition a number of potentially genotoxic furan related contaminates are produced in food and only a few of these compounds are investigated regarding occurrence in food. Various furan contaminants in heated and flavoured foods have only been studied to a limited extent, thus, analyses of furan derivatives such as 2-furfuraldehyde, 5-methylfurfuraldeyde, 2-furan-3-carboxaldehyde and furylacrolein, among others is very important. A brand-new line of aromatized espresso coffee, mixed the aroma and the scent of vanilla, caramel, almond liquor and irish cream to offer the connoisseurs a new fragrance and a pleasant form of expression is in the market, however, no studies were published concerning its furan composition or comparison with conventional espresso coffee. A simple and sensitive method for the screening of furans in espresso coffee was optimized using headspace solid-phase microextraction (SPME) and gas chromatography with mass detection. The SPME fiber, adsorption and desorption parameters were chosen to obtain the maximum m Carboxen-PDMS sensitivity for furan compounds. Headspace SPME using a 75 fiber provided effective sample enrichment. The optimized methodology was used to compare furan composition from four aromatized espresso coffee samples (vanilla, caramel, almond liquor and irish cream coffee samples), an Arabica and a blend of Arabica and Robusta espresso coffee. Twenty eight furans were identified in coffee samples. The furans were found to be the most predominant group of compounds amongst the coffee aromatics, except for almond liquor and irish cream espresso coffee samples. Depending of the flavour added, the furan profile and quantity of compounds detected changed.

Keywords: furans, aromatized, espresso coffee

H-9
THE MONITORING OF ACRYLAMIDE LEVELS IN FOOD IN POLAND

Miroslaw Jarosz[1], Hanna Mojska[2*], Iwona Gielecińska[3], Katarzyna Malecka [4], Katarzyna Stoś[5]

[1 2 3 4 5] National Food and Nutrition Institute, Warsaw, Poland
[*] Corresponding author–E-mail: hmojska@izz.waw.pl; Phone: 48 22 550 96 56; Fax: 48 22 842 11 28

Acrylamide is formed as a result of a reaction between amino acids and reducing sugars during the heating of carbohydrate-rich foods and occurs in high levels in food such as potato crisps, French fries, cookies, cereals etc. Acrylamide is classified as a probable human carcinogen (IARC, 1994). In regard to its demonstrated neurotoxic, genotoxic and carcinogenic effects, acrylamide may cause human`s health risk (WHO, 2002; EC, 2002). Therefore, in accordance to Commission Recommendation 2007/331/EC on monitoring of acrylamide levels in food of 3 May 2007, the Member States should to perform annually the monitoring of acrylamide levels in certain foodstuffs in 2007, 2008 and 2009.

The purpose of our study was to determine acrylamide levels in food randomly selected from all over the Poland according to the Commission Recommendation (2007/331/EC). The samples were brought in local stores and restaurants by sanitary inspection employees, in 2007. The selected food groups were as follow French fries (ready-to-eat) (7), pre-cooked French fries for home cooking, prepared in laboratory (7), potato crisps (7), wheat-rye bread (14), corn flakes (14), biscuits, including infant biscuits (14), roasted coffee (14), jarred baby food (14), processed cereal-based baby food (14) and salted sticks (14).

The analysis was performed by gas chromatography tandem mass spectrometry (GC-MS/MS) using Finnigan GCQ instrument. Deuterium–labeled (d_3 labelled) acrylamide was used as internal standard. The ions monitored for identification were acrylamide dibromo derivative precursor ion *m/z* 152 and product ion *m/z* 135 and d_3 labelled acrylamide dibromo derivative precursor ion *m/z* 155 and product ion *m/z* 137. To quantify the acrylamide content in food the ratio of area of ion peaks *m/z* 135 and *m/z* 137 was estimated.

The highest level of acrylamide was found in pre-cooked French fries prepared in laboratory with a average concentration of 796 µg/kg (347 ÷ 2175 µg/kg). Other food groups contained lower amounts: potato crisps with a median of 484 µg/kg (113 ÷ 1673 µg/kg), roasted coffee – 364 µg/kg (227 ÷ 699 µg/kg), French fries ready-to-eat – 333 µg/kg (134 ÷ 518 µg/kg), salted sticks – 293 µg/kg (71 ÷ 879 µg/kg), biscuits – 265 µg/kg (75 ÷ 672 µg/kg), corn flakes – 157 µg/kg (105 ÷ 189 µg/kg) and processed cereal-based baby foods – 153 µg/kg (52 ÷ 296 µg/kg). The lowest level was found in wheat-rye bread – 55 µg/kg (38 ÷ 99 µg/kg) and in jarred baby food– 79 µg/kg (18 ÷ 162 µg/kg).

Keywords: acrylamide, food, monitoring

H-10
DETERMINATION OF FURAN IN SAMPLES OF BABY-FOOD FROM THE BRAZILIAN MARKET

Adriana Arisseto-Bragotto[1*], Eduardo Vicente[2], Maria Cecília Toledo[3]

[1 2 3] Institute of Food Technology, Italy
[*] Corresponding author–E-mail: adriana.arisseto@ital.sp.gov.br; Phone: +55 19 37431772

In May 2004, the US Food and Drug Administration reported the occurrence of furan in several heat-treated foods, including canned and jarred foods, at levels ranging from non-detectable to 125 µg/kg. This discovery has attained considerable public concern, since furan is classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer. Gas chromatography coupled to mass spectrometry (GC-MS) has been used to determine the content of furan in foods, preceded by headspace sampling (HS) or solid phase microextraction (SPME).

So far, no data on the level of furan in foods from Latin America are available in the literature. Therefore, the objective of this study was to perform an in-house validation of a SPME-GC-MS method and determine the content of furan in jarred baby-food available on the Brazilian market. The SPME was carried out by using a 75 µm carboxen-polydimethylsiloxane (CAR/PDMS) fiber, under the previously optimized conditions, i.e. extraction temperature: 25ºC and extraction time: 30 minutes. Furan-d_4 was used as internal standard. The method was validated according to the guidelines laid down by the Brazilian Institute of Metrology, Standardization and Industrial Quality.

Good linearity over the range 0-100 µg/kg was obtained (r^2 = 0,998). A comparison between curves set on standard solutions and on matrix by applying the *F*-test and *t*-test revealed a non-significant matrix effect. Limit of detection (LOD) and limit of quantitation (LOQ) were 0.7 and 2.4 µg/kg, respectively. Recovery, repeatability and within-laboratory reproducibility were evaluated by spiking blank samples with furan at 2.5, 10 and 50 µg/kg (seven replicates for each concentration level). Mean recoveries ranged from 80 to 107%, and coefficients of variation ranged from 5.6 to 9.4% for repeatability and from 7.4 to 12.4% for within-laboratory reproducibility. The method was applied to 31 samples of jarred baby-food and the results indicated furan levels from <LOQ to 95.5 µg/kg. Samples containing vegetables and meat showed higher furan levels than samples containing only fruits. These results are comparable to data reported in the literature by European and North-American countries.

Keywords: Furan, baby-food, SPME-GC-MS

FAPESP (Proc. 2008/50095-0) and CNPq (Proc. 474267/2008-3)

H-11

TARGETED MULTIDIMENSIONAL GAS CHROMATOGRAPHY USING A HEART CUTTING DEVICE AND CRYOGENIC FOCUSING FOR THE DETERMINATION OF BENZOPHENONE DERIVATIVES IN FOODSTUFFS

Aurélie Bugey[1*], Yves Janin[2], Patrick Edder[3], Stefan Bieri[4]

[1 2 3 4] Official Food Control Authority of Geneva, Switzerland
[*] Corresponding author–E-mail: aurelie.bugey@etat.ge.ch; Phone: +41 22 327 39 81; Fax: +41 22 327 39 89

Amongst the many potential chemical contaminants from food packaging materials, photo-initiators are in the forefront as they can easily migrate into foodstuffs by direct diffusion or contamination from outer to unprinted inner layers of the packaging during a set-off process. Benzophenone derivatives are commonly used photo-initiators which promote polymerization of UV printing inks and varnishes, but unfortunately they are also suspected to possess carcinogenic activities. Recently, benzophenone (BZP) and 4-methylbenzophenone (4-MeBZP) have been flagged by the Rapid Alert System for Food and Feed (RASFF) that noticed several food contaminations by those derivatives. Shortly after these announcements, the European Food Safety Authority (EFSA) was urged to reassess the toxicological evaluation of BZP and examine the case of 4-MeBZP, but so far the specific migration limit of 0.6 mg BZP/kg foodstuffs still has legal force.

In order to tackle this problematic, a fast and selective analytical method was developed for the determination of BZP and 4-MeBZP in cereal-based foodstuffs as well as in cardboard packaging. Food samples or packages were rapidly extracted by accelerated solvent extraction (ASE) with acetonitrile. The resulting extracts were then washed with n-hexane and analyzed without further sample handling by multi-dimensional gas chromatography-mass spectrometry (MDGC-MS). The heart-cutting approach served to reduce background and eliminate potential interferences from sample matrices. The sensitivity of the method was markedly increased by additionally refocusing target analytes on the short second dimension column by means of a longitudinally moving cryogenic trap. Commercial and in-house synthesized deuterated internal standards were used for accurate quantification over the entire concentration range investigated. Our survey of BZP and 4-MeBZP levels in chocolate-derived “muesli” from the Swiss market and packaged in cardboard boxes revealed some products which did not comply with Swiss and European regulations.

Keywords: multi-dimensional gas chromatography, packaging, contaminants

H-12

DETERMINATION OF PVC PLASTICIZERS IN PRESERVED THAI HERBS IN OIL AND THAI CURRY PASTES BY GAS CHROMATOGRAPHY

Natchanun Leepipatpiboon[1*], Chanchira Chanprasert[2], Siripastr Jayanta[3], Maurus Biedermann[4], Sumalee Tangpittayakul[5], Duanghathai Pentrakoon[6]

[1 2 3] Chromatography and Separation Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Road, Bangkok 10330, Thailand
[4] Office Food Control Authority of Canton of Zürich (Kantonales Labor), Fehrenstrasse 15, CH-8032, Zürich, Switzerland
[5] Department of Science Service, Ministry of Science and Technology, Rama VI Road, Bangkok 10400, Thailand
[6] Department of Material Science, Faculty of Science, Chulalongkorn University, Phayathai Road, Bangkok 10330, Thailand
[*] Corresponding author–E-mail: natchanun.l@chula.ac.th; Phone: +662 2187608; Fax: +662 2541309

Forty one samples of preserved Thai herbs in oil and Thai curry pastes packed in glass jars with metal lids were analyzed for amount of tainted plasticizers migrated from jar PVC gaskets. Quantitative data of clean gaskets imply that the gaskets contain 27.1 to 49.9% (w/w) of diverse plasticizers. Jar gaskets can be classified into four groups based on the contamination patterns observed. Fifty-six percent of the test samples used ESBO based gaskets, 27% used polyadipates based gaskets, 10% used ATBC based gaskets, and approximately 7% used DINCH based gaskets. Contamination occurs when gasket is in contact with food. Forty-one percent of the samples were contaminated above the overall migration limit set by the European Union at 60 mg/kg food. A few plasticizers were detected above the EU OML, e.g. maximum contamination of ESBO was detected at 543.9 mg/kg; DINP at 713 mg/kg; DBS at 201 mg/kg; and DINCH at 2,358 mg/kg, respectively. Parameters affecting the contamination level were determined to be the original amount of plasticizers in gaskets; amount of free oil in the foods; and the molecular size of individual plasticizer molecule.

Keywords: plasticizers, jar gasket, food contamination

The financial support was provided by The Innovations for the Improvement of Food Safety and Food Quality for New World Economy Project, Chulalongkorn University, Bangkok, Thailand and Center of Petroleum, Petrochemicals and Advanced Materials. Analytical instruments were provided by the Department of Science Service, Ministry of Science and Technology, Bangkok, Thailand.

H-13

COMPARISON OF HPLC AND LC/ESI-MS/MS METHODS FOR DETERMINATION OF ACRYLAMIDE IN WHEAT, BARLEY AND RYE MALTS

Zeynep Küçük[1], Necati Bariş Tuncel[2*], Neşe Yilmaz[3]

[1 2 3] Onsekiz Mart University, Çanakkale, Turkey
[*] Corresponding author–E-mail: baristuncel@comu.edu.tr; Phone: +90 286 218 00 18 (2262); Fax: +90 286 218 05 41

The presence of acrylamide in heat treated foods was recognised in 2002 and found out that it was formed mainly from asparagine and reducing sugars through Maillard reaction. Acrylamide formation in food depends on food composition and processing conditions. Enzymatic activity during malting, leads to an increase in the reducing sugar content in malt and these conditions are favourable for acrylamide formation. The materials of this work are wheat, barley and rye malts which were obtained at laboratory conditions by roasting at constant temperature of 250°C for 10, 15, 20, 25, 30, 35 and 40 minutes.

Acrylamide analysis was performed with HPLC and LC-ESI/MS/MS chromatographic methods. Strong correlation (0.96 for wheat, 0.89 for barley and 0.85 for rye malts) was found between the data obtained from the two methods. It was observed that prolonged processing time caused a decrease of acrylamide levels and the lowest acrylamide amount was observed in malts roasted for 40 minutes for all cereal species. Also acrylamide amount of wheat malt was found significantly higher than barley and rye malts.

Keywords: acrylamide, malt, HPLC, LC-ESI/MS/MS

H-14

CORRELATION OF METHODS FOR DETECTION OF BACTERIA PRODUCING BIOGENIC AMINES IN CHEESE

Eva Standarová[1*], Ivana Borkovcová[2], Marta Dušková[3], Lenka Vorlová[4]

[1 2 3 4] Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
[*] Corresponding author–E-mail: standarovae@vfu.cz; Phone: +420 541 562 720; Fax: +420 541 562 711

Biogenic amines (BA) are natural antinutrition factors and are important from hygienic point of view as they have been implicated as the causative agents in a number of food poisoning episodes. BA found in foods are synthetized by the microbial decarboxylation of certain amino acids (AA). The most implicated food groups concerning high BA contents is fermented food such as cheese. Cheese represents an ideal environment for BA production because of the great variability of AA and the presence of bacteria.

Rapid and simple methods are needed for the analysis of the ability to form BA in order to evaluate the potential risk of bacterial occurring in some food products. Screening plate method for the detection of AA decarboxylase positive microorganisms (especially lactic acid bacteria, LAB) was developed. Modification of previously described methods included pyridoxal-5-phosphate as decarboxylase cofactor for its enhancing effect on the AA decarboxylase activity. The suitability and detection level of the designed medium were quantitatively evaluated by confirmation of amine-forming capacity using by HPLC procedures. Analytical chromatographic methods used for routine BA analysis of food substrates have been applied to bacterial cultures. The potential to produce the most toxicologically important BA histamine and tyramine in a LAB isolated from cheeses sheep's milk and goat cheeses was investigated. The presence of bacterial genes encoding tyramine and histamine was determined also by PCR method. Good correlations with results of the chemical analysis and both plate and PCR methods were obtained. Tyramine was the main BA formed by LAB strains investigated. Enterococci (*E. faecalis, E. faecium, E. casseliflavus)* and *Lactobacillus* spp. were the most intensive tyramine producers. Contents of tyramine for more strains reached 903–2363 $mg.l^{-1}$. No significant histamine production has been detected for any of the strains tested.

Keywords: Biogenic amines, Cheeses, Decarboxylase activity

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H-15
DETERMINATION OF CHOLESTEROL OXIDES CONTENT IN SELECTED ANIMAL PRODUCTS BY GC/MS

Dorota Derewiaka[1*], Ewa Sosińska[2], Mieczysław Obiedziński[3]

[1 2 3] Warsaw University of Life Sciences, Faculty of Food Sciences, Poland
[*] Corresponding author–E-mail: dorota_derewiaka@sggw.pl; Phone: +48(22)59376784;
Fax: +48(22)8478698

Sterols present in foods undergo oxidation process as the result of many factors (high temperature, oxygen, presence of free radicals and peroxides, dyes etc.) The main products of oxidation are hydroxy-, keto- and epoxy- compounds, which form derivatives of sterol triol as the result of hydratation. The processes of sterol oxidation might, therefore, take place along the whole food chain from storage and manufacturing of products through their processing, distribution and preparation for consumption especially during thermal processing.

According to the literature cholesterol oxidation products (COPs) contribute to disorders of circulatory system e.g. arteriosclerosis and are characterised, among others, by mutagenic and cancerogenic activities.

A gas chromatography/mass spectrometry (GC/MS) technique was applied by us for COPs identification and quantitation. Additionally validation of the method was made.

This technique was used to determine COPs in selected Polish food samples mainly animal origin e.g. pates, dry fermented sausages, minced meats, fish and Cordon- blue and turkey chops. The samples were analyzed as purchased in local supermarkets or as commonly consumed after thermal processing (e.g. pan frying with rapeseed oil).

The results of the research shows that COPs content in analyzed products was between 7–718 μg/ 100 g of products. The highest contents of COPs was discovered in thermally processed meats and chops.

The results are comparable to cholesterol oxidation products contents reported in literature. The findings of the research illustrate the content of COPs in Polish foodstuffs and amounts that are formed during thermal processing of food. The results of the experiment can be used in evaluation of COP's intake in human diet.

Keywords: animal products, oxysterols, GC/MS

H-16

3-MCPD-ESTERS ANALYSIS IN EDIBLE OILS AND FATS: A FEASIBILITY STUDY INTO THE USE OF LARGE VOLUME INJECTION AND COMPREHENSIVE GC×GC-TOF MS

Sjaak de Koning [1*], Zuzana Zelinková [2], Karel Hrncirik [3], Hans-Gerd Janssen [4]

[1] LECO Instrumente, Mönchengladbach, Germany
[2] Institute of Chemical Technology, Prague, Czech Republic
[3 4] Unilever R&D, Vlaardingen, The Netherlands
[*] Corresponding author–E-mail: sjaak.dekoning@leco.de; Phone: +49–(0)2166–687 104;

Recently there has been considerable interest in the formation of 3-chloropropane-1,2-diol (3-MCPD)-fatty acid esters during edible-oil processing. In model systems the 3-MCPD-esters have been shown to yield free 3-MCPD as a result of lipase activity. Because of the suspected toxicity of the free 3-MCPD, methods for the analysis of its fatty acid esters have recently been developed.

The current methods for 3-MCPD ester analysis in edible oils and fats actually measure the total 3-MCPD content of the oil or fat after hydrolysis. The procedures consist of a number of subsequent steps starting with the hydrolysis, removal of the fatty acids (as their FAMEs), extraction of the free 3-MCPD with salting out, derivatisation with phenylboronic acid, preconcentration by the solvent evaporation and finally GC-MS analysis. Deuterium labelled 3-MCPD-d_5 or esters thereof, are used as internal standards. Potential problems in the procedure are degradation of the 3-MCPDs during (alkaline) hydrolysis resulting in high detection limits, and the formation of additional 3-MCPDs if chloride salts are used in the salting out extraction.

In this work a feasibility study is presented that focuses on the use of comprehensive GC×GC–TOF MS with large volume injection for faster, more reliable and more sensitive 3-MCPD analysis in oils and fats. Sample aliquots up to 25 µl could be injected. As a result of this, the salting out step can be less critical: low detection limits were obtained even at low extraction recoveries and thus the side reaction with the chloride can be eliminated. Additionally, the final preconcentration step could be skipped making the method faster and reducing the manual sample handling. A clear advantage of the use of comprehensive GC×GC–TOF MS is the substantially improved resolution of the GC separation leading to the elimination of interferences even at very low 3-MCPD levels.

Keywords: 3-MCPD, GC/MS, processing contaminants

H-17

RAPID MULTI-ANALYTE QUANTIFICATION OF BENZOPHENONE, 4-METHYLBENZOPHENONE AND RELATED DERIVATIVES FROM PAPERBOARD FOOD PACKAGING

Riitta Koivikko[1*], Sarah Pastorelli[2], Ana Rodríguez-Bernaldo de Quirós[3], Rafael Paseiro-Cerrato[4], Perfecto Paseiro-Losada[5], Catherine Simoneau[6]

[1 2 6] Joint Research Centre, IHCP, CAT, TP 260, 21020 Ispra (VA), Italy
[3 4 5] Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain
[*] Corresponding author–E-mail: riitta.koivikko@jrc.ec.europa.eu; Phone: +39 0332 786682

Photo-initiators are used in printing inks hardened with UV light and one of the most commonly used photo-initiator is benzophenone (BP). Recent notifications under the Rapid Alert System for Food and Feed have shown migration of 4-methylbenzophenone (4MBP) from packaging into cereals [1]. A specific migration limit exists for BP of 0.6 mg/kg for its use as an additive in plastics. There is no specific European legislation covering cardboard boxes and/or printing inks for food contact use. However, due to the high levels detected, the European Food safety Authority (EFSA) published recommendations and the Standing Committee for the Food Chain and Animal Health endorsed a limitation to 0.6 mg/kg for the sum of BP and 4MBP [2].

While studies have been published on photo-initiators in the past, there is a fundamental lack of data on 4MBP especially for its combined analysis with others. We present a high performance liquid chromatography analytical method with diode array detector to determine simultaneously the levels of BP, 4-MBP as well as 7 other possible derivatives from secondary packaging for food applications. This method was then tested and applied to 46 samples of paperboard for secondary packaging collected both from supermarkets and directly from paperboard supplier. In addition, a survey on recycled paperboard (n= 19) collected from a supplier was conducted in order to evaluate the background quantity of BP and other derivatives in recycled board.

The most abundant photo-initiator found in the survey was BP which we found in 61% of samples whereas from 30% of the samples we found 4MBP. It seems that these compounds are used to replace one another. Other derivatives were found in minor quantities. Further, we found traces of BP from 42% of the samples of recycled, unprinted board. We are conducting further investigations to these respects.

[1] European Food Safety Authority (2009) EFSA statements on the presence of 4-methylbenzophenone found in breakfast cereals. The EFSA Journal RN-243: 1-19.
[2] European Commission (2009) Standing Committee on the Food Chain and Animal Health Section Toxicological Safety, Conclusions of the meeting of 06 March 2009, SANCO – D1(2009)D/410408.

Keywords: photo-initiators, UV inks, HPLC-DAD, migration

H-18

DETERMINATION OF THE PARTITION COEFFICIENTS ($K_{P/F}$) OF THREE MODEL SUBSTANCES IN SELECTED FOODS IN CONTACT WITH SPIKED LDPE FILM

Joaquim Maia[1*], Raquel Sendón[2], Roland Franz[3], Annika Seiler[4], Laurence Castle[5], Emma L. Bradley[6], I. Leon[7], Giorgia Beldi[8], V. Spinosi[9], Sarah Pastorelli[10]

[1 2] Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy. University of Santiago de Compostela, E-15782 Spain
[3 4] Fraunhofer Institut fürVerfahrenstechnik und Verpackung, Giggenhauser Str. 85354 Freising, Germany
[5 6 7] The Food and Environment Research Agency, Department for Environment, Food and Rural Affairs, Sand Hutton, York, YO41 1LZ, UK
[8 9] European Commission, DG Joint Research Centre, Institute for Health and Consumer Protection, Unit Chemical Assessment and Testing, T.P. 260, 21020 Ispra (Va), Italy
[10] Nestlé Research Center, Q&S; PO Box 44, CH-1000 Lausanne 26
[*] Corresponding author–E-mail: joaquim.feiteira@rai.usc.es; Phone: 0034981598450

Migration is a process where mass transfer of mainly low weight substances (plastic additives, monomers and reaction by-products) occurs from packaging material into foodstuff. This is a subject of great interest due to possible harmful effects on human health.

One task in the EU-FP7 project FACET is to develop enhanced diffusion models of this migration process. Migration modelling needs a number of input parameters including partition coefficients. The aim of this study is to determinate partition coefficients ($K_{P/F}$) of three model substances in a low density polyethylene (LDPE) package/food system at different temperatures. The model substances are: benzophenone (photo-initiator for UV-cured inks), diphenylbutadiene (DPBD; model substance widely used in food migration studies) and Uvitex® OB (optical brightening agent). The foods are selected to have a range of different properties and are: cheese (fatty solid food), turkey (non-fatty solid food) and orange juice containing pulp (acidic and aqueous liquid food).

For each food/temperature combination 10 LDPE strips (1×10 cm^2), previously spiked with benzophenone, DPBD and Uvitex® OB, were used. With cheese and turkey, a LDPE strip was placed between two food slices with the same dimension, wrapped in aluminium foil (as a barrier layer) and sealed it in a polyamide/polyethylene bag (to prevent the food drying-out). For liquid foods, the LDPE strip was placed in a flask with 50 ml of orange juice and closed tightly. The LDPE/food systems were incubated at selected temperatures (20, 40 and 60ºC for cheese and orange juice; 20 and 40ºC for turkey).

At selected times, samples were removed and the LDPE strip was separated from the food. The extraction of the three substances from LDPE was achieved by extracting with ethanol for 6 hours at 70 ºC. The extract was analysed by HPLC-DAD in order to quantify the model substances. The value of $K_{P/F}$ was calculated using the measured concentration in the exposed film and the concentration in the food, calculated from the difference between the amount of model substance in the film before exposure and the amount after the exposure.

It was found that the temperature does not affect significantly the $K_{P/F}$ values, while the food nature affects it greatly ($K_{P/F}$ in cheese<turkey<orange juice for all migrants). The migrant nature also deeply affects the $K_{P/F}$ values being lower for the benzophenone and higher for Uvitex® OB. In all cases $K_{P/F}>1$, except for benzophenone in cheese were $K_{P/F}<1$ for the three temperatures.

Keywords: migration, modelling, food packaging, $K_{P/F}$

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H-19

DEVELOPMENT OF A RAPID AND SIMPLE ANALYTICAL METHOD FOR THE DETERMINATION OF BENZOPHENONE, DIPHENYLBUTADIENE AND UVITEX® OB IN SPIKED LDPE FILMS

Joaquim Maia[1*], Roland Franz[2], Annika Seiler[3], Emma L. Bradley[4], I. Leon[5], Catherine Simoneau[6], Giorgia Beldi[7], Sarah Pastorelli[8], José Manuel Cruz[9], Perfecto Paseiro[10]

[1 10] Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy. University of Santiago de Compostela, E-15782 Spain
[2 3] Fraunhofer Institut fürVerfahrenstechnik und Verpackung, Giggenhauser Str. 85354 Freising, Germany
[4 5] The Food and Environment Research Agency, Department for Environment, Food and Rural Affairs, Sand Hutton, York, YO41 1LZ, UK
[6 7] European Commission, DG Joint Research Centre, Institute for Health and Consumer Protection, Unit Chemical Assessment and Testing, T.P. 260, 21020 Ispra (Va), Italy
[8] Nestlé Research Center, Q&S; PO Box 44, CH-1000 Lausanne 26
[9] Department of Chemical Engineering, E.T.S.E.I. University of Vigo, Spain
* Corresponding author–E-mail: joaquim.feiteira@rai.usc.es; Phone: 0034981598450

Packaging is an important procedure in food manufacturing and distribution. Plastic is one of the packaging materials most widely used by the food industry. This is not an inert material and is able to interact with the surrounding environment, allowing food-packaging interactions, such as sorption, permeation and migration. Plastic additives, residual monomers and oligomers, are not chemically bound to the polymer molecules and can, therefore, move freely within the polymer matrix. In this way, substances from food packaging are able to migrate into the foodstuff. This phenomenon, known as migration, claims special attention in the aspect of food safety because the chemicals that migrate into food may be potentially harmful to human health. The aim of this study is to develop a quick and simple method for the simultaneous analysis of three chemicals in low density polyethylene (LDPE) film. The chemicals chosen for this work were: benzophenone (one of the most commonly used photo-initiator in inks that are cured with UV light), diphenylbutadiene (model substance widely used in food migration studies) and Uvitex® OB (optical brightening agent). The addition of the chemicals into the LDPE film took place during the extrusion process. The three chemicals were spiked into LDPE and are to be used as model substances for subsequent work in measuring migration kinetics and developing enhanced migration models as part of the EU-FP7 project FACET. A weighed sample of spiked LDPE film (1×6.5 cm^2) was placed in a glass flask with 50 ml of ethanol and extracted for 6 hours at 70ºC. A second extraction was made, using the same procedure. Both extracts were analysed directly by HPLC-DAD (C_{18} column, water/tetrahydrofuran/methanol mobile phase, run time 17 min) in order to quantify the three model substances. The resulting chromatograms showed three well resolved peaks. The first extraction proved to be enough to extract the three model migrants completely. A control sample showed that there was not any interference during this analytical process. Calibration lines, ranging 0.05-10.0 $\mu g.ml^{-1}$, showed a good linearity (r^2=0.9999). It is concluded that the method is fast and accurate. The method will be used extensively within FACET to provide data on the initial concentration of the model substances in the plastic, Cp,o, which is an important parameter in migration modelling. The method will also provide migration concentration data in foods and equilibrium partition coefficients, estimated as the mass fraction lost from the film after a migration experiment.

Keywords: migration, food packaging, analytical method

This work was co-funded by the European Union under Grant Agreement 211686 (Project FACET–Flavours, Additives and food Contact material Exposure Task) and by Xunta de Galicia (Proj. nº INCITE08PXIB203096). The findings and the conclusions in this poster are the responsibility of the authors alone and they should not be taken to represent the opinion of sponsors. The authors are also grateful to C. Casal, P. Blanco and G. Hermelo for their excellent technical assistance.

H-20
STUDY OF THE MIGRATION OF PHOTOINITIATORS THROUGH THE VAPOR PHASE

Ana Rodríguez-Bernaldo de Quirós[1*], Rafael Paseiro-Cerrato[2], Sarah Pastorelli[3], Riitta Koivikko[4], Catherine Simoneau[5], Perfecto Paseiro-Losada[6]

[1 2 6] Department of Analytical Chemistry, Nutrition and Food Science.Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain
[3] Nestlé Research Center, Q&S; PO Box 44, CH-1000 Lausanne 26
[4 5] Joint Research Centre (JRC), IHCP, Chemical Assesment and Testing unit, T.P. 260, 21020 Ispra (VA), Italy
[*] Corresponding author–E-mail: ana.rodriguez.bernaldo@usc.es; Phone: 0034981598450

Benzophenone and other related derivatives are components of UV inks widely used as photoinitiators in the printing of food packaging. Due to their low molecular weight and physicochemical properties, these substances can migrate from the packaging to the food and consequently may cause a potential danger for health.

Several studies have demonstrated the migration of benzophenone from the paper and board to the food via direct contact. However, data concerning the migration through the vapour phase is very limited. Moreover, there has been recentely a notification concerning the migration of 4-methylbenzophenone from cardboard to certain breakfast cereals on the Rapid Alert System for Food and Feed (RASFF). Consequently the migration of these photoinitiators into foodstuffs has attracted the interest of the scientific community.

Kinetics of migration of seven benzophenone-related derivatives (4-hydroxybenzophenone, methyl-2-benzoylbenzoate, 2-hydroxybenzophenone, 4-methylbenzophenone, 4-benzoylbiphenyl, 4,4'-bis(diethylamino) benzophenone and benzophenone) through the vapour phase were investigated. The parameters affecting to the migration process were also evaluated.

Polyethylene wax enriched with benzophenone-related derivatives was used as source to release photoinitiators.. Wax and food were placed without direct contact into hermetically closed glass containers and stored at 70ºC (to simulate accelerated conditions) up to 360 hours.

Keywords: vapour-phase migration, benzophenone, 4-methylbenzophenone, inks.

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H-21

UPDATED REVIEW OF THE CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF POLYFUNCTIONAL AMINES USED IN FOOD PACKAGING MATERIALS

Rafael Paseiro-Cerrato[1], Ana Rodríguez-Bernaldo de Quirós[2*], Raquel Sendón[3], Juana Bustos[4], María Eugenia Cirugeda[5], José Juan Sánchez[6], José Manuel Cruz[7], Perfecto Paseiro-Losada[8]

[1 2 3 8] Department of Analytical Chemistry, Nutrition and Food Science.Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain
[4 5 6] National Center for Food, Spanish Agency for Food Safety and Nutrition, 28.220 Majadahonda, Spain
[7] Department of Chemical Engineering, E.T.S.E.I. University of Vigo, Spain
[*] Corresponding author–E-mail: ana.rodriguez.bernaldo@usc.es; Phone: 0034981598450

Polyfunctional amines are group of substances commonly used as additives or monomers in the manufacture of food packaging materials. These compounds can migrate from the material to food and may be a potential danger for health. In order to verify the migration of these compounds into foodstuffs, sensitive and accurate techniques are required. The majority of published procedures are single-component methods, being very scarce methods for the simultaneous analysis of several polyfunctional amines. The techniques most commonly used to analyze amines are liquid and gas chromatography. In order to achieve a suitable sensitivity a derivatization step is essential in many cases. Different derivatizing agents have been used including dansyl chloride, o-phthaldialdehyde and fluorescein among others.

In this communication an updated review of the chromatographic methods available in the literature for the determination of polyfunctional amines commonly used as monomers and additives in food packaging materials is presented.

Since the knowledge of chemical structures and physicochemical properties are essential to select a suitable analytical method, a table with the chemical structures and chemical properties is also reported.

Keywords: polyfunctional amines, review, food packaging

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H-22

DETERMINATION OF HYDROXYMETHYLFURFURAL IN OILS FROM ROASTED SEEDS AND NUTS

Gökhan Durmaz[1*], Burce Atac Mogol[2], Vural Gokmen[3]

[1] Inonu University, Department of Food Engineering, 44280, Malatya, Turkey, and Hacettepe University, Department of Food Engineering, 06800, Beytepe, Ankara, Turkey
[2 3] Hacettepe University, Department of Food Engineering, 06800, Beytepe, Ankara, Turkey
[*] Corresponding author–E-mail: gdurmaz@inonu.edu.tr; Phone: +90 312 297 7100; Fax: +90 312 299 2123

Hydroxymethylfurfural (HMF) is one of the Maillard reaction and sugar pyrolysis intermediates. The presence of HMF in foods is undesirable because it is suspected as a potentially harmful substance. It has been evaluated as an indicator for the severity of heat processing in several foods including spirits and honey, wine and other alcoholic beverages, fruit juices, vinegars, ultrahigh-temperature-treated milks, coffee, breakfast cereals, breads, and baby cereals. However there is no report showing the presence of HMF in oils obtained from roasted seeds.

This study describes a novel and simple analytical method for the determination of HMF in oils. The method entails a liquid-liquid extraction of HMF from oils and reversed phase liquid chromatography. Accuracy and reliability of the method was tested by analyzing both stripped and non-stripped oils spiked with different amounts of HMF.

HMF was extracted from oil in a vortex mixer with 70% methanol. HMF was completely recovered from the spiked samples using two steps extraction. Almost 90% of HMF was recovered in the first extraction.

In order to confirm the presence of HMF in oils obtained from roasted seeds, seven commercially important oily nuts and seeds were roasted at 180°C for 30 min. The oil was obtained from the roasted samples by extracting with organic solvents having different polarities. HMF was detected in all of the extracted oils at certain concentrations. Increasing the polarity of extraction solvent also increased the rate of HMF transferred to oil from the roasted samples. HMF concentrations of oils extracted with different solvents were in the order of ethyl acetate > diethyl ether > *n*-hexane.

Keywords: HMF, Oil, Seed, Nut, Roasting

H-23
MONITORING OF FURAN LEVELS IN MALT AND BEER USING HS-SPME/GC-MS

Katerina Riddellova[1*], Tereza Valentova[2], Monika Cerna[3], Tomas Cajka[4], Vladimir Kocourek[5], Jana Hajslova[6]

[1 2 3 4 5 6] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: katerina.riddellova@vscht.cz; Phone: +420220 44 3218

Since 2004, when the first report on the levels of furan in food was published by the US Food and Drug Administration (US FDA), research has been focused on monitoring the presence of furan in food, as well as on understanding the ways it is formed. The most recent report on the occurrence of furan in food, covering the period from 2004 to 2009, was published by the European Food Safety Authority (EFSA) in June 2009. The highest values of furan were found in coffee (thousands of μg kg^{-1}), followed by cereal and meat products, soups and sauces (hundreds of μg kg^{-1}). Levels exceeding 100 μg kg^{-1} were also detected in jarred baby food, the average furan content in this food category being 25 μg kg^{-1}. Based on an assessment of adult food consumption in Europe, a tentative median furan exposure of up to 0.78 μg kg^{-1} b.w. was calculated.

This work, conducted within the work of the TRUEFOOD project (Traditional United Europe Food), is focused on monitoring furan levels in malt and beer. To assess what happens to furan during beer production, we also analyzed samples representing the whole beer production chain from raw material (malt, water, hop) to finished filtered beer.

We used head-space solid phase micro-extraction followed by gas chromatography–time-of-flight mass spectrometry to analyse furan in samples of malt and beer, and quantified the results using the standard addition method.

Using a PDMS/CX/DVB fibre, the estimated limit of quantification in malt and beer was 0.5 μg kg^{-1} and 0.2 μg kg^{-1}, respectively. Repeatability, expressed as RSD, was 14% (malt, level 5 μg kg^{-1}) and 5% (beer, level 1 μg kg^{-1}).

We analyzed two sets of Czech beer samples (54 samples in total) in 2008 and 2009. Furan was detected in all samples, but the levels were generally low (under 5 μg kg^{-1}).

Within the samples representing three different beer production chains, the highest amounts of furan were found in the malt samples (up to 1519 μg kg^{-1}). In the other samples furan levels generally did not reach 10 μg kg^{-1}. Furan was not detected in the water and hop samples. The results did not indicate any correlation between the malt used and the level of furan in the finished beverage.

Keywords: Furan, beer, malt, HS-SPME, GC-TOFMS

This study was carried out with support from (i) TRUEFOOD project – Traditional United Europe Food (FOOD-CT-2006-016264) and (ii) MSM 6046137305 project supported by the Ministry of Education, Youth and Sports of the Czech Republic.

H-24
MONITORING OF ACRYLAMIDE LEVELS IN VARIOUS BEERS USING LC–MS/MS

Katerina Valenzova[1], Veronika Bartackova[2], Katerina Riddellova[3], Vladimir Kocourek[4], Jana Hajslova[5*]

[1 2 3 4 5] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: jana.hajslova@vscht.cz; Phone: +420220443185

The potential presence of acrylamide in malt, which belongs to the group of 'heat processed food products prepared from raw materials rich in starch/reducing sugars', may result in transfer of this toxic processing contaminant into beer. Acrylamide levels depend on the temperature and the time applied for the preparing of food, amount of precursors in barley is important too. In this study, we focused on the assessment of possible transmition of acrylamide thorough the beer production chain, from the primary commodities into the finished beer.

To monitor acrylamide levels, a new analytical strategy enabling direct determination of acrylamide in this commodity has been developed. The procedure consists of cleanup step employing solid-phase extraction (SPE), with column Isolute Multimode and ENV+, followed by liquid chromatography coupled to tandem mass spectrometry with a triple quadrupole mass analyser (LC–MS/MS). The isotope dilution technique employing use of $^{13}C_3$-acrylamide as an internal standard was used to quantify native acrylamide. The performance characteristics of the new validated method are following: limit of detection 0.2 μg L^{-1}, limit of quantification 0.5 μg L^{-1} and repeatability (RSD) 5%. A wide range of beers obtained at the Czech market has been examined within this monitoring study. The highest levels (up to 15 μg L^{-1}) of acrylamide were found in dark beers. While almost only traces of this contaminant were found in light beers. Supposing that most of acrylamide is transferred from malt into the beer then this commodity should be also considered as an acrylamide dietary source.

Keywords: acrylamide, malt, beer, LC–MS/MS

This study was carried out with support from: (i) TRUEFOOD project – Traditional United Europe Food (FOOD-CT-2006-016264), (ii) MSM 6046137305, (iii) The development of analytical method was founded by The Ministry of Education, Youth and Sports, Czech Republic – project 2B06168.

H-25

COMPARISON OF PERFORMANCE CHARACTERISTICS OBTAINED IN ACRYLAMIDE ANALYSIS IN VARIOUS MATRICES EMPLOYING LC-MS/MS AND UPLC-TOF/MS

Veronika Bartackova[1], Ondrej Lacina[2], Katerina Valenzova[3], Katerina Riddellova[4], Jana Hajslova[5*]

[1 2 3 4 5] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: jana.hajslova@vscht.cz; Phone: +420220443185

Occurrence of acrylamide in heat processed foodstuffs represents an issue of health concern. To monitor levels of this processing contaminant in various matrices, reliable analytical methods are needed. Currently, the most routinely used technique for acrylamide quantification is high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). Typically, relatively laborious sample preparation procedure employing two solid phase extractions is used prior to instrumental analysis. Our significantly simplified sample preparation procedure approach employs (*i*) addition of acetonitrile to primary aqueous extract and (*ii*) induction of phases separation by addition of salts ($MgSO_4$ and NaCl). Most of matrix co-extracts transferred into organic phase together with acrylamide are removed by dispersive solid phase extraction ($MgSO_4$ and basic Al_2O_3 are used for this purpose).

In our study, two alternative MS systems were tested. In addition to well established

LC-MS/MS method, acrylamide analysis was also performed by UPLC-TOF/MS. Comparable or even better results (LOQ´s, repeatability) were obtained by the later approach. The trueness of results generated by both tested methods was demonstrated via analyses of FAPAS® test samples (Rounds 3018, 3021).

Keywords: acrylamide, LC–MS/MS, UPLC-TOF/MS

This study was carried out with support from: (i) MSM 6046137305 (ii)The development of analytical method was founded by The Ministry of Education, Youth and Sports, Czech Republic – project 2B06168.

H-26

DETERMINATION OF BISPHENOL A IN INFANT FORMULA BY AUTOMATED SAMPLE PREPARATION AND LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

Yang Shi [1*], Catherine Lafontaine[2], Francois Espourteille[3], Yolanda Fintschenko[4]

[1 2 3 4] Thermo Fisher Scientific, Franklin, USA
[*] Corresponding author–E-mail: yang.shi@thermofisher.com; Phone: 508-520-5575

2,2-bis(4-hydroxyphenyl) propane, commonly known as Bisphenol A (BPA), is one of the primary chemicals used to make plastics. It is also heavily used in the production of various types of food and drink containers. Because BPA has been known to leach from the plastic lining of metal canned food, the potential risks of exposure to BPA have been a great concern over the past years. There is a consensus that infants are at the greatest risk of harm due to exposure to extremely low levels of BPA. The maximum acceptable or "reference" dose for BPA is 50 µg/kg body weight/day established by the U.S. Environmental Protection Agency.

Liquid chromatography-mass spectrometry (LC-MS) technique has been recently described for the determination of BPA in food. Current strategies employ sample preparations for the detection of BPA in canned infant formula that involve complicated extraction steps such as solid phase extraction, solvent-based extraction and some micro-extraction techniques, all of which require additional sample concentration and reconstitution in appropriate solvent. These sample preparation methods are time-consuming and are more vulnerable to variability due to errors in manual preparation.

A quick, automated online extraction LC-MS/MS method using TurboFlow technology has been developed here that is sensitive enough to detect as low as 7.80 µg/kg (ppb) dry powder limit of detection and quantify 31.3 µg/kg (ppb) dry powder (LLOQ) of BPA (background-adjusted) in infant formula powder for screening purposes. Compared to offline liquid/liquid or solid phase extractions, this method eliminates the need for time-consuming sample preparation procedures. The TurboFlow method also shows the advantage of fast separation over other online sample treatment techniques, such as RAM technology. The LC-MS/MS method run time is only 5.6 minutes, and the sample throughput can be improved by multiplexing on an Aria TLX-2 (or TLX-4) system.

Keywords: BPA, Turboflow, LC-MS/MS

H-27
SOLUTION OF ANALYTICAL PROBLEMS EMERGING FROM LC-ESI-MS-MS ACRYLAMIDE DETERMINATION

Alena Bednáriková[1], Zuzana Ciesarová[2*], Kristína Kukurová[3]

[1 2 3] VUP Food Research Institute, Bratislava, Slovak Republic
[*] Corresponding author–E-mail: ciesarova@vup.sk; Phone: 421 2 50237197

Acrylamide is a suspected carcinogen that is created when starchy foods are baked, roasted, fried or toasted. It is proved to be formed as a result of the Maillard reaction between specific amino acids, especially asparagine, and saccharides found in foods reaching high temperatures.

Among various methods of acrylamide determination in foods which are used, the LC-ESI-MS-MS method appears to be a very effective, reliable and precious one. On the other hand, due to the complexity of food samples and low levels of acrylamide, efficient clean-up steps are required to avoid interferences from co-extractives. The common strategies necessary to achieve clean extracts consist in some sample preparation steps (extraction with water, centrifugation, clean-up procedure with organic solvent or with solid phase extraction, filtration).

The present study is focused on the selected critical points of sample preparation procedure (single or multi-stage extraction with water, salts removal, SPE clean-up procedure) before the LC-ESI-MS-MS acrylamide analysis itself; and then on the validation of the acrylamide determination method in real food matrices.

Keywords: acrylamide, LC-MS determination

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H-28
TRANSFER OF PRINTING INK CONSTITUENTS FROM PACKAGING INTO FOOD SIMULANTS

Zdenka Dupakova[1*], Kamila Klaudisova[2], Lenka Votavova[3], Jaroslav Dobias[4], Michal Voldrich[5]

[1 2 3 4 5] Institute of Chemical Technology, ague, Czech Republic
[*] Corresponding author–E-mail: zdenka.dupakova@vscht.cz; Phone: +420 220 443 015

Printing inks contain many substances, the use of which is not allowed in food contact materials. The substances originated in printing have been several times found in packaged foods in consequence either their migration through the packaging material or via the set-off transfer phenomenon.

The aim of presented study was to quantify the transfer of selected printing ink plasticizers (acetyl tributyl citrate, diethylhexyl adipate, tributoxyethyl phosphate, ethylhexyl diphenyl phosphate, diethylhexyl phthalate) from printing of packaging materials into food simulants. Two polymer packaging films, i.e. polyethylene film (LDPE, thickness 50 μm) and laminated polyethylene terephthalate and polyethylene film (PET/LDPE 12 μm/ 35 μm) were printed with flexography technique to obtained packaging material with defined content of free above mentioned substances in printing layer (0.05 mg/dm^2 and 0.5 mg/dm^2). The migration of tested additives through the packaging films into distilled water and 95% ethanol was studied at 6°C and 25°C. The set-off transfer of plasticizers into LDPE film in contact with printing was tested at 40°C.

The results confirmed the possibility of migration of all tested additives through the both tested films. In the case of LDPE film 15–80% of total amount of plasticizers in printing were extracted into stimulant during 30 day storage at 25°C. For PET/LDPE the slightly lower migrations of plasticizers were determined, i.e. 5–65%. As expected the migration into distilled water was more than 10 times slower and set-off transfer about 50 times slower compared with migration into 95% ethanol.

The work will discuss the detailed results of above mentioned experiments as well as the comparison of obtained experimental data with the mathematical model. The results of the tests on transfer of selected photoiniciators from printing of packaging materials into food simulants, which are at present in progress, will be also mentioned.

Keywords: food packaging, inks, plasticizer, migration

This study was carried out in the framework of the research project no. 525/07/1153 supported by Czech Science Foundation.

H-29
FURAN LEVELS IN FOOD AND DRINKS

Jan-Willem Wegener[1*], Jacco Koekkoek[2], Patricia López- Sánchez[3]

[1 2] Institute for Environmental Studies, VU University Amsterdam, The Netherlands
[3] IRMM, Joint Research Centre, Geel, Belgium
[*] Corresponding author–E-mail: jwegener@ivm.vu.nl; Phone: 0031 20 5989517; Fax: 0031 20 5989553

Furan is a monocyclic heterocyclic aromatic compound containing one oxygen atom. It is a suspected carcinogen, IARC Group 2B classification, “possibly carcinogenic to humans”. A possible furan source is the heat treatment of food especially if treatment is in the final closed packing (like bottles or cans).

Its toxic action mechanisms have been investigated in an EU supported research project, “Role of Genetic and Non-Genetic Mechanisms in Furan Risk”, EU contract SSPE-CT-2006-44393, project acronym Furan-RA.

Part of the research project deals with the analysis of food and drink samples for furan levels by means of a validated head-space gas chromatographic method with mass-selective detection.

Prior to sampling, the existing literature was searched for data on furan levels in food and drinks. The well over one thousand existing data were collected in an Excel-based database, that has been made public on the project website. The data were screened for food categories with unexpected high or contradicting furan levels and these categories were selected for sampling and subsequent analyses for furan:

- (baby) fruit and vegetable juices;
- pharmaceutical and convenience nutrition drinks;
- bakery products other than bread.

If product availability allowed, three different lots per product were analysed to investigate the variation in furan levels between lots. All results have been added to the afore-mentioned database. The method has been validated by analyses of the samples in triplicate (precision) and by recovery experiments with furan-spiked samples (trueness).

An attempt has been made to correlate furan levels with ingredients or production methods. For (notably baby) fruit and vegetable juices high furan levels can be related to relatively high pH values. A higher pH value renders the drink more acceptable for little children but requires a more severe heat treatment procedure as compared to more sour tasting drinks of low pH value. For bakery products it was found that wholegrain products contain the highest furan levels.

Keywords: furan, determination, analysis, food, drinks

European Commission contract number SSPE-CT-2006-44393

H-30

EXTRACTION OF MELAMINE FROM VARIOUS MATRICES USING RESIN-BASED MIXED-MODE CATION EXCHANGE SPE AND ANALYSIS WITH LC-MS/MS

Lee Williams[1*], Rhys Jones[2], Helen Lodder[3], Steve Plant[4], Steve Jordan[5], Richard Calverley[6], Claire Desbrow[7], Gary Dowthwaite[8], Joanna Caulfield[9]

[1 2 3 4 5 6 7 8 9] Biotage GB Ltd, Ystrad Mynach. UK

[*] Corresponding author–E-mail: lee.williams@biotage.com; Phone: (0) 1443 864459; Fax: (0) 1443 816512

Melamine is traditionally used in making plastics, however, it's low cost and high nitrogen content has led to exploitation in various sections of the food industry, most notably involving dairy products. The standard test for estimating protein content is based on measurement of nitrogen levels, therefore, addition of melamine to sub standard or watered down milk results in the protein levels appearing higher. Sustained melamine exposure can result in kidney stones and renal failure, with the young being most susceptible. This poster will demonstrate the use of a new resin-based mixed-mode strong cation exchange SPE sorbent, EVOLUTE CX 50 µm, for the extraction of melamine from various dairy products and human biological fluids. All extractions were performed using EVOLUTE CX 50 µm in the 50 mg/ 3 ml format. Pasteurised milk (semi skimmed), infant milk (powdered and liquid forms), human urine and plasma were spiked with melamine and processed using a variety of standard cation exchange protocols to investigate optimum extraction conditions. Extracts were evaporated to dryness and reconstituted in mobile phase for LC-MS/MS analysis. Chromatographic separation was achieved using HILIC chromatography on a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer. Melamine was monitored using positive ion electrospray in the MRM (multiple reaction monitoring) mode. SPE method optimization was investigated using a variety of different pH based conditions. All matrices (1 mL) were pre-treated 1:1 with buffer. Powdered baby milk was made up with boiling water as per the suggested procedure and left to cool before pre-treatment. SPE protocols were based on either 50mM ammonium acetate at pH 5, 50 mM ammonium acetate at pH 6, or 2% formic acid methods. Recovery data shows that pre-treatment with 50 mM ammonium acetate at pH 6 resulted in lower recoveries on the majority of matrices. Since melamine is a small polar base with a pKa of around 8.0 we believe this low recovery to be due to inadequate pH sample pre-treatment. Recoveries using the 50 mM ammonium acetate at pH 5 and the formic acid method both showed results greater than 85%, however, substantial signal suppression was observed with the latter. Whilst quantitative internal standards were not used, simple signal to noise experiments showed that resulting limits of quantitation were worsened by the use of the formic acid method. Full results, discussion and conclusions will be shown in the final poster.

Keywords: Melamine, SPE, LC-MS/MS

H-31
ANALYSIS OF HMF AND HMFA IN FOOD AND URINE

Michael Murkovic[1*]

[1] Ínstitute of Biochemistry, Graz University of Technology, Graz, Austria
[*] Corresponding author–E-mail: michael.murkovic@tugraz.at; Phone: +43 316 8736495; Fax: +43 316 8736971

5-Hydroxymethylfurfural has become a substance of interest since recent results show a possible carcinogenic potential in consequence of a metabolic activation by sulfotransferases. 5-Hydroxymethylfurfural is formed either by acid catalysed degradation of reducing sugars or via the Maillard reaction. This work provides an overview of foods potentially containing high amounts of 5-hydroxymethylfurfural. It comprises dried fruits with a high sugar content that were exposed to heat for a long time. The concentration ranges from very low in e.g. figs (1 mg/kg) to plums that contained up to 2200 mg/kg. Several types of roasted coffee were analysed which contained from 300 to 2900 mg/kg of 5-hydroxymethylfurfural. In a small human study with 7 healthy volunteers the urine excretion of unmetabolised 5-hydroxymethylfurfural was investigated. After uptake of 20 g of plum jam containing 24 mg of 5-hydroxymethylfurfural 163 µg (mean) were excreted within 6 hours being equivalent to 0.75% of the ingested 5-hydroxymethylfurfural.

Keywords: 5-Hydroxymethyl-2-carboxaldehyde, HMF, dried fruits

H-32
LC/MS/MS ANALYSIS OF BIOGENIC AMINES IN FOODS AND BEVERAGES

Brent Lefebvre[1], Takeo Sakuma[2], Andre Schreiber[3*], Michael Quilliam[4]

[1 2] MDS Analytical Technologies
[3] Applied Biosystems
[4] National Research Council Canada
[*] Corresponding author–E-mail: andre.schreiber@lifetech.com; Phone: +1-905-660-9006

Biogenic amines are a group of biologically active organic compounds produced by decarboxylation of free amino acids. They are found in bacterially contaminated food, particularly in fish and are therefore potential quality indicators. They can, in sufficient concentrations, pose a threat to human health. Histamine is the main causative agent in Scombroid fish poisoning. The other biogenic amines such as putrescine, cadaverine and tyramine are also of great interest as their presence enhances the toxicity of histamine. Biogenic amines can also react with nitrites to form potentially carcinogenic nitrosamines. Analysis of these amines is usually carried out by ELISA at a detection limit of low – medium ppm. Analysis by traditional RP-HPLC is difficult because of poor retention. Derivatization methods are time consuming, ion-pairing agents can inhibit LC/MS analyses, and both can adversely affect method reproducibility. In 2006 we investigated the use of cation-exchange column coupled with tandem mass spectrometry detection to analyze biogenic amines in seafood. Although the method worked well, it requires column regeneration, ion suppressor, etc., and is not suited for high throughput analysis. With the introduction of new LC phases, we wanted to see if HILIC or fluorinated packing material can handle the direct analysis of these polar biogenic amines: cadaverine, histamine, 2-phenylethylamine, putrescene, serotonin, spermidine, spermine, tryptophan, tryptamine, tyramine and urocanic acid. We examined several LC columns and found that Pinnacle® DB PFPP works fine with 0.05% trifluoroacetic acid for all of these amines with detection limits (based on S/N=10) of low ppb. The current Food Standards Code indicates the permitted level for histamines to be 200 mg/kg or 200 ppm. Therefore, our new method meets the requirement for routine screening for these compounds. The analysis time including column regeneration is 6 minutes using a 1.9-µm, 50 × m, 150 × 2.1 mm PFPP column requires 12 minutes 2.1 mm PFPP column, whereas 5- or less per sample. Over 200 various foods and beverages have been tested in triplicate injections for reproducibility and robustness of this new method. This method was also applied to a study of time, storage condition and concentration of these biogenic amines.

Keywords: biogenic amines, LC/MS/MS, fish

BIOTECHNOLOGY BASED METHODS

(I-1 – I-5)

I-1
BIOASSAY SCREENING: THE ADDED VALUE

Toine Bovee[1*], Ron Hoogenboom[2], Jeroen Rijk[3], Michel Nielen[4]

[1 2 3 4] RIKILT-Institute of Food Safety
[*] Corresponding author–E-mail: toine.bovee@wur.nl; Phone: +31-317-480391; Fax: +31-317-417717

Recently we constructed yeast cells that either express the human estrogen receptor α or the human androgen receptor in combination with a consensus ERE or ARE repeat in the promoter region of a green fluorescent protein (yEGFP) read-out system. These bioassays were proven to be highly specific for their cognate agonistic compounds and were fully validated for calf urine and animal feed. An inter-laboratory test was performed with the yeast estrogen bioassay for screening estrogenic activity in calf urine.

Here we show the value of these yeast bioassays for analysing compounds with antagonistic properties as well. Several pure antagonists, selective estrogen receptor modulators (SERMs), selective androgen receptor modulators (SARMs) and plant-derived compounds were tested. Many of these compounds will find their use in therapeutic treatments. However, some of them will also have a high potential for misuse in veterinary practice and the sporting world.

In addition, we demonstrate the added value of bioassay screening by screening eighteen different dietary supplements, already analysed by a liquid chromatography tandem mass spectrometry method (LC-MS/MS) for the presence of anabolic steroids, for androgenic activity. Eleven samples containing at least one anabolic steroid, with a concentration that was around or above 0.01 mg unit-1 according to LC-MS/MS, were also positive in the bioassay. Seven samples did not contain any of the 49 compounds screened for in LC-MS/MS. In contrast two of them were positive in the bioassay. Bioassay-directed identification, using the bioassay as an off-line LC-detector and LC-time of flight-MS with accurate mass measurement was carried out in these two samples and revealed the presence of 4-androstene-3β,17β-diol and 5α-androstane-3β,17β-diol in the first and 1-testosterone in the second supplement, showing the added value of the bioassay in comparison with a LC-MS/MS screening method alone.

We also report our findings with a 60-year old man who was surgically treated for gynaecomastia. It is shown that the effect was most probably caused by an orally taken herbal supplement, marketed on the internet for 'prostate problems'. The supplement showed a strong effect in a yeast estrogen bioassay. Using LC/TOFMS, the responsible synthetic compound was identified. This case demonstrates that physicians need to be aware of the use of supplements with illegal components that may be responsible for unwanted side-effects.

Keywords: hormones, agonists, antagonists, supplements, screening

I-2
MYELOPEROXIDASE–MEDIATED OXIDATION OF ORGANOPHOSPHOROUS PESTICIDES

Tamara Lazarevic Pasti[1], Ljubodrag Vujisić [2], Vesna Vasic[3*]

[1] Vinča Institute of Nuclear Sciences, Belgrade, Serbia
[2] Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia
[3] Vinča Institute of Nuclear Sciences, Belgrade, Serbia
[*] Corresponding author–E-mail: evasic@vinca.rs; Phone: +381 11 3408 287; Fax: +381 11 244 7207

The flow injection-type biosensor (FIA) consisting of a reactor with immobilized acethylcholinesterase (AChE) and spectrophotometric detector (λ = 412 nm) was used for determination of organophosphorous pesticides (OPs). In order to examine the possibility of enhancing the sensitivity of the sensor by converting OPs from thio- to oxo-forms, which are more potent AChE inhibitors, enzyme myeloperoxidase (MPO) isolated from human blood was used as an oxidant.

The influence of MPO concentration (from 5–100 nm) and incubation time between MPO and OPs (from 1–30 min) on OPs (concentration from 1×10^{-4}–1×10^{-6} M) was investigated, in order to find out the experimental conditions for the most efficient OPs oxidation. The aqueous solution samples of spiked water with some model thioorganophosphorous compounds (diazinon, malathion, chlopyrifos and commercial OPs control mixture) were used.

All oxidation experiments were verified using UPLC and GC-MS analysis. The products were identified as oxon derivatives (phosphates), where the sulfur atom from thioate group was substituted by an oxygen atom. No hydrolysis products were detected after enzymatic oxidation of these pesticides. Compounds of interest were quantified by using standard solutions of thio-OPs and identification of formed oxo-OPs was based on MS spectra.

The formed oxo-analogs were also detected using two AChE bioassays – native enzyme and FIA manifold. In FIA system inhibition of AChE was determined by comparing the enzyme activity before and after passage of an oxidized pesticide solution through the reactor for a given period of time. The activity of AChE exposured to the oxidized diazinon samples decreased with the increasing the oxidation time, as well MPO concentration, yielding the lowest value after 5 min exposure to 100 nM MPO.

A calibration curve for diazinon, as the model compound for OPs was constructed. While the lower detection limit of diazinon using native enzyme (10% AChE inhibition, 10 min incubation time) before oxidation was ca. 1×10^{-5} M, the detection limit after oxidation was below 1×10^{-7} M. However, the detection limits for FIA system depended on the flow rate, yielding the value below 1×10^{-9} M flow rate below 0.5 ml/min. Therefore, it was safely concluded that, due to the greater inhibitory power of OPs after oxidation, MPO may be regarded as an excellent oxidant for improving sensitivity of bioanalytical methods for OPs determination in water.

Keywords: organophosphates, oxidation, biosensor

I-3
HISTAMINE DETERMINATION IN WINE: AN APTAMER-BASED DETECTION SYSTEM.

Elisa Jimenez[1*], Sandra Rainieri[2], Oscar Martinez de Ilarduya[3], Kepa Escuredo[4]

[1 2 4] Food Research Division, AZTI-Tecnalia. Bizkaiko Teknologi Parkea. 48160 Derio, Bizkaia. Spain
[3] Universidad de A Coruña, A Coruña, Spain
[*] Corresponding author–E-mail: ejimenez@azti.es; Phone: +34946574000-ext293

The presence of histamine and other biogenic amines in wine is due to bacterial metabolism. Histamine presence in food may involve a relatively serious toxicity problem when present at high levels. Therefore, histamine determination has become one of the main control parameters during wine production.

Concentrations of 5 mg/L of histamine may provoke headache after the consumption of 0.5 L of wine (Battaglia and Frölich 1978). Some countries have established maximum limits for histamine in wine like Switzerland 10 mg/L, France 8 mg/L, Belgium 5 mg/L and Germany 2 mg/L (Lehtonen, 1996; Lima and Glória, 1999).

Nowadays, histamine content determination in wine is usually performed employing High Performance Liquid Chromatography (HPLC) which is a time consuming task that requires sample preparation prior to analysis. On the other hand, the ELISA systems commercialized for this purpose require prior acylation of the sample due to the nature of the available antibodies.

Aptamer molecules are short oligonucleotides capable of binding specifically to molecules of interest against which they have been previously selected. Therefore, they have become promising capture molecules that can be coupled to multiple biosensors and they serve as excellent detection tools.

The present work investigates the applicability of aptamer molecules to histamine detection and the development of a simple easy-to-handle biosensor that could be used as histamine detection tool.

Aptamers were selected from an oligonucleotide bank through the SELEX procedure (Systematic Evolution of Ligands by Exponential Enrichment) described by Ellington and Szostak (1990). We employed a modified protocol that uses magnetic particles for separation and fluorescent molecules for labelling as described by Stoltenburg *et al*. (2005).

After fifteen SELEX selection cycles followed by PCR amplification, cloning and sequencing, we selected two aptamer molecules that showed high affinity for histamine. The fluorescence emitted by aptamers bounded to histamine molecules was measured on ABI Prism 7000 Sequence Detection System (Applied Biosystems, USA). An interaction analysis through Surface Plasmon Resonance was also performed using BiacoreX System (Biacore Life Sciences, GE Healthcare, USA).

Based on the selected aptamers, our goal was the development of an easy-to-use, quick and semi-quantitative detection method that could be used during wine production procedure with minimal sample preparation. The detection system under development is based on lateral-flow membranes containing gold nanoparticles functionalized with selected aptamers. In the absence of histamine, aptamers form a stable purple colour aggregate. The presence of histamine disrupts the formed aggregate causing red colour deposits visible with naked eye.

The proposed aptamer-mediated lateral-flow detection system would allow histamine determination in wine with minimal sample preparation.

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Keywords: Histamine, Aptamer, Nanoparticles, Lateral-flow

I-4
EXPRESS IMMUNOCHROMATOGRAPHIC ASSAYS FOR THE CONTROL OF TOXIC CONTAMINANTS IN FOODSTUFFS

Boris Dzantiev[1], Nadezhda Byzova[2], Alexander Urusov[3], Anatoly Zherdev[4*]

[1 2 3 4] Institute of Biochemistry Russian Acad. Sci., Moscow, Russia
[*] Corresponding author–E-mail: zherdev@inbi.ras.ru; Phone: +7 495 954-28-04; Fax: +7 495 954-28-04

The presented investigations were directed to the creation of express systems for the detection of a row of biologically active compounds (mycotoxins, pesticides, antibiotics). The systems are based on immunochromatography principle, i.e. flow of a sample to be analyzed and specific reactants along porous membrane carriers that is accomplished by a formation of colored zones.

A series of colloidal gold preparations of different sizes was obtained for their use as detecting carriers for the immunochromatographic assays. Concentration dependences of immobilization of antibodies on colloidal particles were studied, optimal protocols of immobilization and the corresponding loading of antibodies were determined. Affinities of the interactions between antigens and colloidal conjugates of antibodies of different composition were compared with the use of biosensoric system Biacore, and recommendations for the choice of the preparations for bioanalytical application were elaborated.

For toxicants of different nature the conditions of antigen-antibody reaction on the porous membrane carriers were chosen including optimal values of pH, immunoreactants concentrations, size of the colloidal particles and membranes pores, number of antibody molecules in their conjugates with colloidal gold nanoparticles etc. Immunochromatographic tests for express detection of mycotoxins (ochratoxin A, aflatoxin B1), pesticides (atrazine and its derivatives) and antibiotics (chloramphenicol, streptomycin, ampicillin) in foodstuffs were developed. Regimes of quantitative registration of the analyses results by a specially developed portable video-digital detector were elaborated.

The developed test-systems allow detecting toxic compounds with sensitivities up to 10 ng/mL and the assay duration not more than 10 min. Due to high rapidity and low labor immunochromatography is an efficient approach for analyses under laboratory and on-site conditions.

Keywords: immunochromatography, mycotoxins, pesticides, antibiotics

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I-5

DEVELOPMENT OF GEL-BASED IMMUNOCHROMATOGRAPHIC TEST FOR SIMULTANEOUS DETECTION OF 2,4,6-TRICHLOROPHENOL AND OCHRATOXIN A IN RED WINE

Natalia Beloglazova[1*], Irina Goryacheva[2], Tatyana Rusanova[3]

[1] Lomonosov Moscow State University, Chemistry Faculty, Department of Chemical Enzymology, Moscow, Russia
[2 3] Saratov State University, Chemistry Institute, Department of Common and Inorganic Chemistry, Saratov, Russia
[*] Corresponding author–E-mail: beloglazovanv@gmail.com; Phone: +79175862610

Ochratoxin A (OTA) is a secondary metabolite of the mould fungi genera Aspergillus and Penicillium. It is one of the most widespread mycotoxins and one of the most dangerous for human health. It is characterized as nephrotoxic, hepatotoxic, teratogenic and immunotoxic to several animal species. According to the IARC, OTA has been included in group 2B as an agent 'possibly carcinogenic to humans'. It is "frequent and undesirable guest" in foodstuff, such as grain, coffee, wine grapes and dried grapes. Red wine is one of the sources of OTA penetration to human organism. Also one of the most daunting problems in the enological industry is connected with a serious defect in bottled wine perceived as a musty and earthy off-flavour. The cork taint is related to the presence in wine of certain chloroanisoles and chlorophenols (one of them is 2,4,6-trichlorophenol (TCP)) at the ng/l concentrations. A problem of control of wine quality is actual today, but it is very complicated, because object is characterized by complex matrix. Consequently, methods which are used for wine pollutants detection usually consist of complicate and long-term procedure of clean-up. The frequently used methods for wine analysis are liquid chromatography techniques with fluorescent detection (HPLC-FLD) and -tandem mass spectrometry (LC-MS/MS). But all chromatographic methods are laborious, time-consuming, require sophisticated equipment and/or trained personnel, organic solvents and can not be used in situ. The new test-method which allows to detect different kinds of analytes (2,4,6,-trichlorophenol (TCP) and ochratoxin A (OTA)) in red wine in one step was developed. It was based on application of three separate immunolayer: two of them are test ones, third is a control layer. Each layer corresponded sepharose gel coupled with anti-OTA (for OTA-detection layer) or anti-TCP (for TCP-detection layer) antibodies. The mixture of OTA–HRP and TCP-HRP conjugates was used as competitor. Cut-off value for both analytes was 2 ng/ml For analytes screening in red wine samples combination of clean-up column and test column was developed and applied for real samples analysis. Chromatography was used as a confirmation method.

Keywords: 2,4,6-trichlorophenol, ochratoxin A, wine

AUTHENTICITY, TRACEABILITY, FRAUD

(J-1 – J-50)

J-1
FISH IDENTIFICATION IS A NOVEL PROFICIENCY TEST

Laura Prenton[1], Mark Sykes[2*], Janet Kelly[3]

[1 2 3] Fera, York, UK
[*] Corresponding author–E-mail: mark.sykes@fera.gsi.gov.uk; Phone: +44 (0)1904 462697

Identification of whole fish species is relatively straightforward. Various schemes exist to train and/or assess competency in fish identification, for fisheries or ecologists, for example. Once a fish has been skinned and minced, however, identification must be done by advanced analytical means, not simply by observation. Legislation such as the UK Food Standards Agency Food labelling guidance 2003 places particular requirements on the clear labelling of fish and fish products.

Each year, the Food Analysis Performance Assessment Scheme (FAPAS®) runs a proficiency test for the identification of fish species. This is a purely qualitative assessment whereby participating laboratories are required to identify the species of three minced fish test materials, from a list of possibilities. The number of participating laboratories is approximately 40. The percentage of satisfactory scores (ranging from 83 to 100%) is an indication that proficiency testing in this field is an essential part of quality assurance.

A test material comprises a 10 g aliquot of minced fish. The identity of the fish was confirmed by isoelectric focussing prior to dispatch of the test materials. Laboratories may then use their own method of choice, either DNA-based or non-DNA. Electrophoresis is commonly used among the non-DNA methods, although isoelectric focussing is unusual. The entire stock of test material may come from a single fish, unless the test species is small. In either case, homogeneity testing is not appropriate for this type of proficiency test. Since the test is qualitative, z-scores are not issued.

Here we present fish identification proficiency testing data, gathered over a five year period. The methodology is documented and the novel context of the test is discussed.

Keywords: proficiency testing, fish identification

J-2
ANALYTICAL AND ECONOMICAL ASPECTS OF GLAZING OF FROZEN FISH

Lynn Vanhaecke[1], Wim Verbeke[2], Hubert De Brabander[3*]

[1 3] Ghent University, Faculty of Veterinary Medicine, Department of Veterinary Public Health and Food Safety, Lab Chemical Analysis, Salisburylaan 133, B-9820 Merelbeke, Belgium
[2] Ghent University, Faculty of Bioscience Engineering, Department of Agricultural Economics, Coupure Links 653, B-9000 Ghent, Belgium
[*] Corresponding author–E-mail: Hubert.DeBrabander@Ugent.be; Phone: 32-9-264 7460; Fax: 32-9-264-7491

Frozen fish fillets are protected from dehydration, oxidation and quality loss by glazing: a thin layer of ice on the surface of the fish fillet (> 6%). Excessive glazing (> 10-12%) may significantly affect the economic value and "end user" satisfaction. Determination of the ice-glaze content of fish fillets is therefore an important asset. This study describes the application of a gravimetric procedure for the quantification of the ice-glaze content of frozen fish fillets: the determination of the glazing percentage of multiple batches (n=50) of 11 different fish species and products over a period of 5 years. Average glazing percentages were 8.7 ± 2.0% for the pooled sample (n=673), and ranged between 6.6 ± 2.2% (salmon/cod) and 10.6 ± 1.6% (plaice). The lower threshold value of 6% glazing was violated in only one batch, whereas none of the batches exceeded the 12% glazing threshold for rejection of consignment. Despite falling generally within the contractually negotiated range of 6–12% glazing, glazing percentages varied widely between and within species, as well as over time. It was however noticed that the determination of the glazing percentage must be carried out on a whole bag or batch of frozen fish and not on one of two single pieces: the variation between the glazing percentages within one bag is too large. The annual market place value of one%-point glazing is estimated at 1 million euro in a "low to moderate" fish consumption market like Belgium. Extrapolation to world market level yields an estimate of 3 to 4 billion euro/year. The large variability of glazing, combined with this technology's possible implications with respect to end product quality and economics urge for better monitoring and more controlled application of glazing in the frozen fish industry.

Keywords: Glazing, Frozen fish, Belgium, Determination

J-3
AUTHENTICITY OF EXTENDED SHELF LIFE (ESL) MILK SAMPLES IN AUSTRIA

Helmut K. Mayer[1*]

[1] BOKU University, Vienna, Austria
[*] Corresponding author–E-mail: helmut.mayer@boku.ac.at; Phone: 0043-1-47654-6106; Fax: 0043-1-4789114

The recent trend towards a longer keeping ability of pasteurized milk, without the negative flavour change normally associated with UHT, has resulted in development of extended shelf life (ESL) milk. The currently used methods to produce ESL milk are microfiltration, direct heat treatment such as injection or infusion, or in some cases also indirect heat treatment. However, heating causes a significant loss of organoleptic and nutritional quality (e.g., vitamin destruction, precipitation of calcium phosphate, denaturation of whey proteins, Maillard reaction). Therefore, different time temperature integrators have been used to evaluate the heat load of ESL milk products (e.g., the milk enzymes *alkaline phosphatase* and *lactoperoxidase*, the native whey protein ß-lactoglobulin, hydroxymethylfurfural, lactulose, and furosine).

The objective of this study was to improve published RP-HPLC methods for the analysis of furosine and native ß-lactoglobulin soluble at pH 4.6 in liquid milk using a Symmetry™ 300 column (Waters). Native polyacrylamide gel electrophoresis (Native PAGE) and SDS-PAGE were also used to assess the impact of a thermal process on milk and to distinguish different categories of heat-treated liquid milk samples to control authenticity of ESL milk.

The established RP-HPLC method enabled the separation of whey proteins within 21 minutes and was used for quantitative determination of ß-lactoglobulin. Furosine was analyzed by ion-pair chromatography RP-HPLC within 7 minutes. Native PAGE and SDS-PAGE were well suited for screening purposes to evaluate different heat loads of heat-treated milk samples. About half of the samples designated as ESL milk product (n=72) showed ß-lactoglobulin contents lower than 1.800 mg per litre of milk, which had been discussed as threshold level in Austria. Most of these ESL milk samples with excessive heat-load had a surprisingly low amount of native, non-denatured ß-lactoglobulin (< 500 mg/L) and a high furosine content (> 40 mg/100 g protein), which was almost comparable to that of UHT milk.

Thus, electrophoresis of whey proteins and HPLC of furosine and native, non-denatured ß-lactoglobulin offer fast and reliable tools to evaluate and control the heat load of milk samples to minimize the loss of nutritional quality of authentic ESL milk.

Keywords: ESL milk, authenticity, ß-lactoglobulin, furosine

J-4

FOOD AUTHENTICITY AND CONTAMINATION STUDIES OF PAPRIKA SAMPLES USING ISOTOPIC AND ELEMENT PATTERN VIA (MC)-ICP-MS

Marion Brunner[1], Robert Katona[2], Zsolt Stefanka[3], Thomas Prohaska[4*]

[1 4] Natural Resources and Applied Life Sciences, Department of Chemistry, Division of Analytical Chemistry, VIRIS Lab, Vienna, Austria
[2 3] Department of Radiation Safety, Institute of Isotopes, Hungarian Academy of Sciences, Budapest, Hungary
[*] Corresponding author–E-mail: thomas.prohaska@boku.ac.at; Phone: 0043/1/36006/6092

Food traceability ('from farm to fork') and food authenticity have become a major concern in anti fraud and consumer protection during the past few years. Several methods and analytical techniques are currently under investigation for proper determination of the geographical origin. The combined analysis of selected isotopic and elemental fingerprints has emerged as promising tool in food provenance.

Isotopic pattern of 'bio elements' like carbon, nitrogen oxygen, hydrogen or sulphur measured by GS-IRMS are known to undergo seasonal variations effected e.g. by humidity, dryness or onset of winter. Additionally, environmental or anthropogenic impacts (e.g. fertilizer) may significantly change the isotopic signature of food. Heavy isotopes (e.g. Sr, Pb, etc.) are mainly influenced by geogenic sources. Annually variances are expected to be less pronounced and therefore they are suitable stable tracers for the determination of geographical origin of food and beverages. The combination with elemental pattern (e.g. rare earth elements) improved the correlation to the geographic origin, too.

This poster will present the recent application of multielement and strontium isotopic analyses including soil studies using (MC)-ICP-MS. They are applied for food authenticity and traceability studies which will be shown on the basis of food stuff which had undergone processing after harvesting. We selected paprika powder and investigated both the origin of sources of the elements as well as the influence of the production processes on the final product.

Keywords: element/isotopic pattern, contamination, (MC)-ICP-MS

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J-5
DNA AND PEPTIDES AS MOLECULAR MARKERS FOR ASSESSING FOOD AUTHENTICITY

Stefano Sforza[1*], Tullia Tedeschi[2], Alessandro Calabretta[3], Mariangela Bencivenni[4], Francesca Lambertini[5], Gianni Gaalverna[6], Arnaldo Dossena[7], Rosangela Marchelli[8]

[1 2 3 4 5 6 7 8] University of Parma, Parma, Italy
[*] Corresponding author–E-mail: stefano.sforza@unipr.it; Phone: +39-0521-905406; Fax: +39-0521-905472

In recent years many foods have gained in reputation and marketing value by claiming special sensory properties to be due in part to their geographical origin and/or way of production. In view of these claims, authenticity of food, in terms of adherence to a defined technology and the true deion of its origin, has thus become a concern for both food chemists and legislators. In order to assess authenticity, one common approach is the use of molecular markers, i.e. molecules which can be linked to a particular ingredient/technology/region of production. One of the most used molecular marker is DNA, but almost every compound in food (protein, peptides, secondary metabolites and so on) can be used as such, providing that a clear-cut correlation between the declared characteristic and the analysed molecule is clearly demonstrated. The applied methodologies are of paramount importance, since they must ensure the possibility to specifically detect defined compounds, sometimes present in trace amounts, in complex molecular mixtures as foods.

In the present communication several approaches for assessing food authenticity based on DNA and peptides as molecular markers will be presented. Preliminar studies for the discrimination of tomato varieties and olive oil cultivars will be presented, based on the detection of single nucleotide polimorphisms (SNPs), in the extracted and amplified DNA, through specific binding with Peptide Nucleic Acid (PNA) probes, oligonucleotide analogues with a pseudopeptide backbone. The advantages and the limitations of the different approaches which can be used with PNA technology (HPLC, microarray, microfluidics) will be discussed. In the second part of the communication it will be shown how the complex peptide mixtures generated by the proteolytic events which take place during cheese manufacturing and ripening can be rapidly investigated by using LC/MS methodologies. The informations on the peptides present in the cheeses can be used in order to detect frauds (such as cows' milk addition in sheep cheeses), to determine the technology of production and to assess the lenght of ripening. Moreover, several peptides can be linked to the flavour properties of the cheeses, thus providing the ground for studies aimed at finding objective markers for food sensory quality.

Keywords: DNA, Peptide,s PNA, LC/MS, Authenticity

J-6
ISOTOPES AS TRACERS OF GREEN COFFEE

Carla Rodrigues[1*], Rodrigo Maia[2], Marion Brunner[3], Magdalena Lang[4], Thomas Prohaska[5], Cristina Máguas[6]

[1 2 6] University of Lisbon, Faculty of Sciences, Centre for Environmental Biology, Stable Isotopes and Instrumental Analysis Facility (SIIAF), Ed. ICAT, Campo Grande, 1749-016 Lisbon, Portugal
[3 4 5] University of Natural Resources and Applied Life Sciences (BOKU), Division of Analytical Chemistry (VIRIS Laboratory), Vienna, Austria
[*] Corresponding author–E-mail: cirodrigues@fc.ul.pt; Phone: +351960082532; Fax: +351217500172

The importance of the coffee market and its globalization arise increased concern about its origin and producers respond by offering products with origin labelling to the consumer. The quality of coffee has a high variation according to its geographical origin and several attempts have been made to discriminate the origin of green and also roasted coffee beans. Analytical methods such as gas chromatography mass spectrometry, near infrared spectroscopy, as well as the determination of organic compounds, and stable isotope analysis of specific compounds extracted from the green coffee bean have been studied extensively with promising results. The stable carbon and nitrogen isotopic ratios are related to the plants' climatic conditions during growth, mainly water and nutrient availability along with light intensity and temperature, and can be useful as indicatives of their origin, providing tools to limit their potential cultivation areas if the conditions differ significantly. So the analysis of stable isotopes is a useful and promising tool for creating isotopic fingerprints in food matrices to trace back their provenance. Heavy isotopic systems such as strontium are mainly influenced by geological processes, weathering and cycling processes in nature. A combination of these isotopic systems (bio-elements and heavy isotopes) in food and in related environmental samples (e.g. soil, ground water, precipitation, etc.) provides an isotopic pattern, which allows the determination of geographical origin of agricultural and processed food.

The aim of this work was to apply stable isotope analysis (EA-IRMS; Elemental Analysis – Isotope Ratio Mass Spectrometry) for carbon, nitrogen and oxygen isotopic composition determination of green coffees derived from Central America, Pacific, South America, Africa, Asia and Oceania. Strontium isotopic composition was also determined by MC-HR-ICP-SFMS (Multicollector high resolution inductively coupled plasma sector field mass spectrometry). Multivariate analysis of data was done in order to discriminate between green coffee samples. Non-parametric measure of correlation between edaphic-climatic factors and analytical data was performed to determine the relevant factors for green coffee origin discrimination based on isotope composition analysis. A scale-down to the state of Minas Gerais, Brazil allowed us to observe that for some of the elements studied, isotopes may record important ecological processes occurring during the coffee bean development.

Keywords: green coffee, isotopes, IRMS, MC-ICP-MS

J-7
SEAFOOD AUTHENTICITY TESTING SYSTEM USING PCR-RFLP AND BIOANALYZER TECHNOLOGY

Scott Happe[1*], Harini Ravi[2], Natalia Novoradovskaya[3], Robert Kincaid[4], Scott Basehore[5], Jeff Braman[6], Rachel Formosa[7], Steve Garrett[8]

[1 2] Agilent Technologies, Cedar Creek, TX, USA
[3 5 6 7] Agilent Technologies, La Jolla, CA, USA
[4] Agilent Technologies, Santa Clara, CA, USA
[8] Campden BRI, Chipping Campden, Gloucestershire, UK
[*] Corresponding author–E-mail: scott.happe@agilent.com; Phone: 512-321-3031

As the global demand for seafood increases and governmental regulations regarding product labeling and sustainability become stricter, seafood operators are under greater pressure to authenticate the fish species used in their products. Current methods require expert morphological identification for raw fish or protein-based methods for processed foods. Protein-based methodologies require a skilled analyst, have difficulty distinguishing closely related species, and are unsuitable for heavily processed foods due to protein degradation. In contrast, DNA methods are less subjective, more specific, and highly robust, even in over-processed samples. Agilent Technologies, in collaboration with Campden BRI, has developed a DNA-based system to identify fish species in fresh and processed seafood. The method combines DNA extraction, polymerase chain reaction (PCR), restriction fragment length polymorphism assay (RFLP), and fragment surveillance using the Agilent 2100 Bioanalyzer lab-on-a-chip technology. First, DNA is rapidly isolated from small amounts of fish tissue using a convenient spin column method, resulting in pure DNA ready for analysis. Next, a *cytochrome b* target fragment, common to most fish species, is PCR-amplified, followed by digestion with 3 different restriction enzymes. The result is a unique DNA fingerprint that is easily decoded using the Bioanalyzer lab-on-a-chip system and RFLP Matcher software. The pattern generated by the test sample is compared to an expanding list of species in an integrated database to identify the species based on probability scores. Currently, the methodology has successfully identified over 30 species of common fish and has been implemented in a commercial fish processing environment. The results of a validation study will be described, demonstrating accuracy, reproducibility, and ease-of-use. With routine implementation, this seafood authenticity testing system has the potential to enhance the quality of seafood products, reduce the environmental impact of illegal fishing activities, and assist in maintaining accurate records suitable for compliance with governmental regulations.

Keywords: seafood, Bioanalyzer, PCR, RFLP, fish

J-8

ADVANCED DIAGNOSTIC TEST SYSTEMS FOR THE PROOF OF FOOD AUTHENTICITY: ISOTHERMAL AMPLIFICATION STRATEGIES

Ilka Haase[1*], Felix Focke[2], Markus Fischer[3]

[1 2 3] University of Hamburg, Institute of Food Chemistry, Hamburg, Germany
[*] Corresponding author–E-mail: ilka.haase@chemie.uni-hamburg.de; Phone: +49-40-42838-4379; Fax: +49-40-42838-4342

Desoxyribonucleic acid (DNA) is a very stable molecule which enables the detection of qualitative important food components also in highly processed food products where classical methods sometimes fail.

Apart from that, the DNA sequence – as carrier of the genetic information – is individual for every organism and can therefore be used for the differentiation of even highly related plant and animal species. Thus, DNA based methods are highly suited for food quality assessment and for proof of authenticity.

The so called “loop mediated isothermal amplification (LAMP)” (Notomi et al., 2000, Nucleic Acids Research, 28, e63) is a relatively young DNA amplification technique. In contrast to state-of-the-art PCR techniques LAMP is performed under isothermal reaction conditions using a polymerase with strand displacement activity. Moreover, four up to six (loop LAMP) primers are used for every template which results in an increased specificity. Due to this sophisticated primer design the DNA amplification with LAMP results in a species specific DNA-fragment pattern. This is in contrast to classical PCR experiments yielding just one specific DNA-fragment. The LAMP technology could also be used for quantitation (real-time LAMP). Due to the pronounced specificity compared to other DNA based methods, the LAMP strategy is best qualified for the identification of closely related foodstuffs e.g. spices like black and white pepper or black and white mustard.

Usually spices are characterized on the basis of their morphological differences, e.g. characteristic cells or tissues. For that purpose, trained staff is necessary and the quantity of a certain spice can only be estimated. Especially in fine cut products or in processed food characteristic attributes could not longer be detected and consequently identification is not possible.

Keywords: authenticity, DNA, LAMP, specificity, spices

J-9
COPPER CHLORIDE CRYSTALLIZATION WITH ADDITIVES– APPLICATION ON FOOD QUALITY ANALYSIS

Johannes Kahl[1*], Nicolaas Busscher[2], Angelika Ploeger[3]

[1 2 3] University of Kassel, Witzenhausen, Germany
[*] Corresponding author–E-mail: kahl@uni-kassel.de; Phone: ++495542981715; Fax: ++495542981713

The quality of organic produce is determined by the process and not the product itself. Increasing demand for organic produce requires methods that show which production process has been used in the product (authenticity test). A survey of the literature for the present work revealed that crystallization of copper chloride with additives seems to be particularly suitable in this respect. In this method a mixture of sample and copper chloride crystallizes on a glass dish to a pattern that can be evaluated both visually and using a computer program. First, all steps in the laboratory were documented and corresponding standard work instructions drawn up. A new computer programme allows to record the conditions during the sample preparation and crystallization for each sample and pattern. A texture analysis can help to evaluate the large number of patterns created for this work, and the results can be statistically evaluated. In this way it is possible to describe the process and methods for wheat and carrot samples. Various factors of influence were examined. The procedure is especially sensitive to changes in the sample preparation (e.g. mixing ratio between the sample and the copper chloride). The repeatability of the method as well as of single steps in the procedure was investigated. Evaporation and crystallization represent the most important factors on the variation. A laboratory comparison test showed that the method thus documented and characterized can be transferred successfully in other labs. The process was used for the nominal differentiation of wheat, carrot, apple and milk samples from different cultivation and processing steps. It was possible to significantly differentiate selected samples from defined cultivation and processing steps. The influence of juice production, heating and ageing could also be shown significantly. Furthermore, the process can also applied to other sample types. The process works in a way that individual substances are not determined analytically, but rather the result is a pattern. Although the mechanism of the pattern formation is still under investigation, the texture and structure properties of this pattern can be evaluated using standardized methods.

Keywords: organic food, authenticity, crystallization, additives

J-10

DNA BARCODES UNIVERSALITY AND POLYMORPHISM IN FOOD AUTHENTICITY. APPLICATION TO OLIVE OILS AUTHENTICATION

Miguel Faria[1*], Eugénia Nunes[2], Beatriz Oliveira[3]

[1 3] REQUIMTE, Faculty of Pharmacy, University of Porto, Portugal
[2] CIBIO, CIBIO – Faculty of Sciences, Universitiy of Porto, Portugal
[*] Corresponding author–E-mail: mfaria@ff.up.pt; Phone: +351222078910; Fax: +351222003977

Food authentication methodologies are increasingly applying molecular biology techniques. This is in part justified by the high specific resolving power of these techniques, mostly based on DNA analysis, and by the relative stability of the molecule. In the recent years efforts have been made to develop universal markers capable of discriminating all species by sequencing relatively short fragments of the genome of animals, plants and fungi. This is the case of DNA barcodes that are standardized *loci*, short and highly polymorphic. Furthermore, once they are located on plastid genomes greater yields of DNA can be recovered from processed food samples.

DNA barcodes have been proposed by plant molecular biologists to assist biodiversity studies, identify juveniles, and in taxonomy. Other applications as in forensic analyses have also been proposed.

The barcode region for members of the animals kingdom have already been defined as the cytochrome c Oxidase I (COI) gene (Hebert et al. 2003). Concerning plants the definition of a barcode is being evaluated by the CBOL (Consortium for the barcoding of Life) Plant Working Group with several candidate regions being studied (Lahaye et al. 2008).

In this study we evaluated the possibility of using DNA barcodes in the authentication of plant derived foods namely olive oils, which can be adulterated with hazelnut oils. For that purpose we have studied polymorphisms at 6 *loci* (rbcL, matk, nrITS, rpoC1, trnL and trnH-psbA) for the species Olea europaea and Corylus avellana.

Fragments of the referred loci were amplified and sequenced. When available, sequences were also retrieved from public databases as NCBI or BOLD (Ratnasingham and Hebert 2007) for comparison. Alignments were performed using the software MEGA (Tamura et al. 2007) based on CLUSTALW.

Sequence alignments have confirmed the high polymorphism of the referred regions which permits the discrimination and identification of the species in study. Experiences were based on taxon specific endonucleases recognition sites and HRMA (High Resolution Melting Analysis) of amplified barcode regions to evaluate the possibility for using DNA barcoding as a mean of surveying the composition of olive oils.

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Keywords: DNA barcodes, Authenticity, Food, PCR

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J-11
THE USE OF TWO-DIMENSIONAL GAS CHROMATOGRAPHY IN FOOD ANALYSIS

Antónia Janáčová[1*], Ivan Špánik[2]

[1 2] Institute of Analytical Chemistry, Faculty of Food and Chemical Technology Slovak University of Technology, Bratislava, The Slovak Republic
[*] Corresponding author–E-mail: antonia.janacova@stuba.sk; Phone: +421 593 25 276

New EU regulations about identification of food-stuffs origin requires use of exact analytical methods, based on minimal sample preparation, that are able to differentiate complicated food matrix. This problematic requires determination of many analytical parameters therefore chromatography has important influence in food authentication. For many years analytical techniques based on different chromatographic systems were used for determination of food-stuffs origin and production process. Also gas chromatography methods, especially identification of volatile organic compounds (VOC) profile or specification of suitable marker are used.

One dimensional gas chromatography does not have a sufficient separation power that results into frequent coelution of analytes from matrix. The solution of this problem is the use of two-dimensional chromatographic system with different column combination (nonpolar-polar, nonpolar-chiral, polar-chiral and more other). In two-dimensional GC we distuinguish heartcut gas chromatography with analysing only small part of 1D efluent in second dimension or entire two dimensional separation of whole sample – two-dimensional comprehensive gas chromatography.

Aim of our study was to investigate the possibilities of the use of two-dimensional GC systems in authentication of selected food commodities.

Keywords: food authenticity, two-dimensional GC

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J-12
DIFFERENTIATION OF MEAT FROM SELECTED PIG BREEDS BY USING PCR-RFLP TECHNIQUE

Anna Jánosi[1*], Gabriella Ujhelyi[2], Erika Szabó[3], Éva Gelencsér[4]

[1 2 3 4] Central Food Research Institute, Food Safety Department, Budapest, Hungary
[*] Corresponding author–E-mail: a.janosi@cfri.hu; Phone: 00-36-1-3558-244/618; Fax: 00-36-1-225-3342

To meet requirements in the field of food analysis, application of specific, reliable, quick and sensitive methodologies are of increasing importance. Beside the traceability document it is necessary to identify breed of the meat or other animal product through the whole pork-chain by using different analytical methods. Our research group started to work on the field of DNA -based breed-specific identification of meat origin with Mangalica pig, which breed is one of the indigenous protected animals of Hungary. At the end of 90's Mangalica was rediscovered for food producing due to the excellent gastronomic value of meat (high intramuscular fat and dry material content combined with deep red colour) The Polymerase Chain Reaction – Restriction Fragment Length Polymorfism (PCR-RFLP) was applied for differentiation of Duroc, Mangalica, Hungarian Landrace, Hungarian Large White and Cornwall – Mangalica F1 crossbreed pigs. The selected sequences for amplification were two fragments of coat colour gene and mitochondrial cytochrome b gene as the template DNA. Reaction mixture contained RedTaq Ready PCR mix, DNA template (purified using the Wizard resin) and a pair of primers in a final volume of 50 µl. 8 µl of the PCR product was digested by addition of 10 U of restriction endonuclease enzyme (AccII, Bsph1, HaeIII, HaeII). Samples were digested for 2h at 37°C and fractionated on a 10% polyacrilamide gel electrophoresis. The gels were performed in TRIS-Borate-EDTA puffer for 1h at 200 V. The bands were visualised by staining with ethidium-bromide and photographed under UV transilluminatior. On the basis of the different PCR-RFLP assays we could getting up a decision tree for the differentiation of meat from white coat colour pigs (Hungarian Landrace and Hungarian Large White), Duroc, group of Mangalica colour variants (red, blond and swallow-bellied), and Cornwall – Mangalica F1 crossbreed pigs.

Keywords: breed identification, Mangalica, meat, PCR-RFLP

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J-13
AUTHENTICITY OF FRUIT SAUCE IN ICE CREAM

Kirstin Gray[1*], Michael Walker[2], Peter Colwell[3]

[1 2 3] LGC, Teddington, England
[*] Corresponding author–E-mail: kirstin.gray@lgc.co.uk; Phone: +44 (0)20 8943 7309

Food authenticity has many aspects. Authenticity claims can influence purchasing decisions and include for example the geographical origin, production method (e.g. organic or conventional, farmed or wild), whether natural or otherwise and the species present. When such claims are false or a cheaper ingredient is substituted for a more expensive one it jeopardises consumers' ability to make informed choices about what they want to buy and eat. It often means poor value for money for consumers and in extreme cases can be fatal – such as when an allergen (e.g. peanut) is fraudulently added to improve profit margins without honest labelling. Simple mistakes can also arise.

In the UK the Government Chemist is required to act as the national focus of technical appeal in specified areas where there is an actual or potential dispute between food businesses and regulator. After an anonymous "tip off" a Local Authority found that raspberry sauce had been substituted by strawberry sauce in an ice cream product. The food business disputed the official findings and the Government Chemist was called in to resolve the issue and the reference portions of six formally taken samples were referred to us.

Three analytical approaches were used: microscopy, DNA and anthocyanins.

Microscopy–Bright field high power microscopy looking for the characteristic trichomes (plant hairs) that differ in morphology and abundance in the two fruits

DNA -Polymerase Chain reaction DNA (PCR-DNA) used to amplify microsatellite markers (short tandem repeats) described in the peer reviewed scientific literature and specific to or characteristic of the fruit and

Anthocyanins – specific anthocyanins predominate in the pigments of many fruit and are a basis for discrimination. Extracted anthocyanins analysed by High Performance Liquid Chromatography with UV detection (HPLC-UV) and by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) which also confirmed the molecular identity of the relevant anthocyanins present.

Based on results from the three analytical approaches the Government Chemist confirmed that three samples contained strawberry and three raspberry. The findings broadly confirmed those of the public analyst, that different tubs of the ice cream contained different sauces – some strawberry and some raspberry. This can only have occurred because a container of strawberry sauce was erroneously fed into the production process at some point.

Keywords: Authenticity, Fruit, Microscopy, PCR-DNA, Anthocyanins

J-14
GC ISOLINK: A NEW CONCEPT FOR COUPLING GAS CHROMATOGRAPHY WITH ISOTOPE RATIO MASS SPECTROMETRY

D. Juchelka[1], M. Krummen[2], A. Hilkert[3], J.B. Schwieters[4], M. Godula[5*]

[1 2 3 4] Thermo Fisher Scientific (Bremen) GmbH, Hanna-Kunath-Str. 11, 28199 Bremen, Germany
[5] Thermo Fisher Scientific Praha, Slunecna 27, 100 00 Praha 10, Czech Republic
[*] Corresponding author–E-mail: michal.godula@thermofisher.com; Phone: +420777114430

irm-GC/MS has revolutionized *i*sotope *r*atio *m*ass *s*pectrometry (IRMS) within the last 20 years. While GC/MS provides information on structural elucidation and compound quantitation, isotope ratio monitoring GC/MS (irm-GC/MS) reveals the history and origin of compounds by reading their isotopic signature with ultra-high precision. Small variations of the natural isotope abundances cannot be detected with GC/MS systems. Only the combination of a GC and an isotope ratio mass spectrometer is capable of routinely achieving the information and required precision.

Irm-GC/MS has entered into widespread applications like environmental studies, forensics, tracers and metabolite studies, biogeochemistry, doping control, authenticity control of food and flavors. Isotopic fingerprints analyzed by irm-GC/MS may still provide information where classical GC/MS methods based on compound quantitation cannot. The growing interest and appreciation in isotope ratio applications requires new features and functionalities of the instrumentation.

The GC IsoLink follows a new concept for an automated multi-element irm-GC/MS. It includes automated switching between different modes in order to determine isotope ratios of the bioelements H, C, N or O automatically. This hyphenated technique allows the determination of the isotope ratios of all individual compounds in a complex mixture. All fields of application using GC and GC/MS can benefit from isotope ratio monitoring GC/MS.

The principle of the device is discussed with respect to the dynamic range, precision, accuracy together with the requirements on sample size. Examples for multi-element and multi-component isotope analysis will be shown.

Keywords: irm-GC/MS, food authenticity, isotope ratios

J-15

SIMULTANEOUS QUANTITATIVE DETERMINATION OF MELAMINE AND CYANURIC ACID IN FOODSTUFFS BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONISATION TANDEM MASS SPECTROMETRY

Aurélien Desmarchelier[1], Thierry Delatour[2*]

[1 2] Nestlé Research Center, Nestec Ltd., Vers-chez-les-Blancs, 1000 Lausanne 26, Switzerland
[*] Corresponding author–E-mail: Thierry.Delatour@rdls.nestle.com; Phone: +41 021 785 9220

Particular concern has recently raised against melamine (MEL) and cyanuric acid (CA). Several events triggered the role of these chemicals in cases of renal pathology in animals fed with contaminated feed and in Chinese infants bottle-fed with infant formulae tainted with MEL.

Hence, there is a need for effective and reliable methods to monitor MEL and CA in infant formula and control raw material with high risk of adulteration i.e. cow milk, egg powder and soya powder. An isotope dilution liquid chromatography–electrospray ionization tandem mass spectrometry is described for the simultaneous determination of MEL and CA in cow milk (range 0 – 0.3 mg/kg) and infant formula (ranges 0–0.3 mg/kg and 0–2.0 mg/kg). The sample preparation of this quantitative method encompasses a protein precipitation in acetonitrile:water prior to centrifugation and direct injection of the supernatant. Selected reaction monitoring of two diagnostic transition reactions for each analyte and each corresponding ^{13}C,^{15}N-labeled compounds enables selective and confirmatory detection. Validation of the method was conducted according to the European Union criteria (2002/657/EC) [1].

Internal standard-corrected recoveries were within the 99–116% range for both analytes in cow milk and infant formula, along with repeatability and intermediate reproducibility values ≤ 12.3% and ≤ 31.2%, respectively. LODs were 0.025 and 0.050 mg/kg for MEL and CA respectively, while LOQs, set arbitrarily at the lowest fortification level, were 0.05 and 0.10 mg/kg for MEL and CA, respectively. $CC\alpha$ and $CC\beta$, at the 1 mg/kg maximum limit set by WHO [2] for infant formula, were 1.05 and 1.07 mg/kg for MEL and 1.11 and 1.19 mg/kg for CA.

Performance evaluation based on 3 proficiency tests (including matrices like milk powder, backing mix, egg powder and soja powder) indicates that the method is suitable for the quantitative determination of MEL and CA for a broad variety of food. Robustness of the method was demonstrated during the analysis of >2000 routine samples.

[1] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. Communities* 2002, L221, 8-36.
[2] World Health Organization/Food and Agriculture Organization of the United Nations. *Expert Meeting to Review Toxicological Aspects of Melamine and Cyanuric Acid*; 2008; pp 1-10.

Keywords: Melamine, Cyanuric acid, Foodstuffs, LC-MS/MS

J-16

IDENTIFICATION OF VOLATILE COMPOUNDS IN OXIDIZED ALMOND OIL BY HS-SPME-GC-MS. APPLICATION TO CULTIVAR AUTHENTICITY

Ana Beltrán Sanahuja[1*], M. Carmen Garrigós Selva[2], Nuria Grané Teruel[3], M. Luisa Martín Carratalá[4]

[1 2 3 4] Department of Analytical Chemistry, Nutrition and Food Science, University of Alicante, Alicante, Spain
[*] Corresponding author–E-mail: ana.beltran@ua.es; Phone: 965903400 Ext 1190; Fax: 965903527

Oxidized lipids can accelerate deterioration of sensory quality in almonds and decrease consumer acceptances [1]. Lipid oxidation is influenced by factors including antioxidants, metals, photosensitizers and oxygen content, heat intensity and light energy and number of double bonds in fatty acids. One of the parameters indicating the degree of oxidation is oxidized volatile compounds. Concentration of hexanal has been monitored for determining the degree of oxidation in foods [2].

The aim of this study was to propose a method based on HS-SPME coupled with GC-MS for the rapid analysis and characterization of volatiles resulting from lipid oxidation from 3 different cultivars almond oils (Guara, Marcona and Butte) in order to obtain a set of parameters for discrimination between Spanish and American almond cultivars.

A divinilbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibber coating was used. 1 g almond oil samples was placed in a closed dark 20 mL vial and kept under thermally treatment at 100ºC for 20 days. Oxidative stability of samples was evaluated by measuring oxidized volatiles content at 1, 3, 5, 7, 10, 15 and 20 days, in duplicate; to obtain an oxidation kinetic profile. All samples were immersed in a water bath at 60ºC and extraction was carried out with stirring for 60 min. The SPME needle was inserted through the septum and left in the headspace. After extraction, the fibre was immediately transferred into a Perkin Elmer TurboMass Gold GC-MS with a split/splitless injector and a SPB-5 capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 µm). The column temperature program was from 50ºC (10 min) to 280ºC (5 min) at 4ºC/min. Injector and detector temperatures were 270ºC. The fibre desorption time was 10 min and helium was used as carrier gas (1 ml/min). Detection was performed in scan mode.

Several volatile compounds were found in all samples: hexanal, 2-heptenal, 2-octenal, nonanal, 2-nonenal, 2,4-nonadienal and 2,4-decadienal. Highlighted differences between Spanish and American almond oils were found in samples kept under thermally treatment for 7 days.

HS-SPME has proved to be a valuable methodology for determination of volatile compounds associated with almond oil oxidation. It has shown to be a reliable, rapid, sensitive and repeatable technique. Finally, obtained results proved the suitability of the proposed method for discrimination between different almonds cultivars.

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[2] Iglesias J; Lois S; Medina I. J. Chorm. A 2007, 1163, 277.

Keywords: SPME, volatiles, almond, authenticity, characterization

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J-17
CHARACTERISATION OF BRANDY DE JEREZ DURING ITS AGING

Mónica Schwarz[1*], Mª Carmen Rodriguez[2], Dominico Guillen[3], Carmelo G.Barroso[4]

[1 2 3 4] Centro Andaluz de Investigaciones Vitivinicolas, Universidad de Cadiz, España
[*] Corresponding author–E-mail: monica.schwarz@uca.es; Phone: 0034956016363

Brandy de Jerez is aged in wooden casks following the traditional dynamic system (Soleras and Criaderas). This dynamic aging involves the periodic racking of part of the contents of each barrel into another containing older Brandy, over the course of several years. The barrels are arranged in a series of scales, known as criaderas, ranked according to the age of the wine contained. The final scale of the system is known as the solera, from which the fully aged Brandy is drawn off periodically for bottling and sale. It has long been known that the wood of which the barrels are made has a considerable influence in the aging of wines and liquors. The wooden cask serves both as a container during the period of aging and as an active contributor to the organoleptic properties of the product by the extraction of compounds from the wood. One of the differentiating characteristics of Brandy de Jerez is that the casks in which it is aged have previously contained Sherry wine of one or other type: fino, oloroso, Pedro Ximénez, etc. This process is known as the "wining" of the casks, and it constitutes another route whereby polyphenols are incorporated into Brandy de Jerez. The role of polyphenolic compounds in creating the flavour and aroma of the brandy has been fully and clearly proven by many authors.

Because of the increase in counterfeit products and consumer demands for protection, food industry professionals need to be able to guarantee the authenticity of their products. Consequently, the characterisation of Brandy de Jerez has acquired considerable commercial interest because this makes it possible to differentiate a particular product analytically from all other similar products on the market, thus protecting its authenticity.

This work studies the kinetics of polyphenol and acid organics extraction from oak wood to distillate, as well as the possible correlations between both parameters in brandy and its age or the commercial type it belongs to (Solera, Solera Reserva, or Solera Gran Reserva). The determination of phenolic compounds was performed by Ultra-performace-liquid-chromatography (UPLC), and the determination of Short-Chain Organic Acids was performed using an ION-300 ion-exclusion column. Furthemore, total polyphenolic index and color following Cielab parameters were measured.

Keywords: Brandy Jerez, aging, characterisation, authenticity

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J-18

APPLICATION OF FLUORESCENCE SPECTROSCOPY AND CHEMOMETRICS TO THE EXPLORATORY ANALYSIS OF JUNIPER-FLAVOURED SPIRIT DRINKS

Pavel Májek[1*], Jana Sádecká[2], Ľubomír Píš[3]

[1 2 3] Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia
[*] Corresponding author–E-mail: pavel.majek@stuba.sk; Phone: +421259325311; Fax: +421252926043

Juniper-flavoured spirit drinks are spirit drinks produced by flavouring ethyl alcohol of agricultural origin and/or grain spirit and/or grain distillate with juniper berries (*Juniperus communis* L. and/or *Juniperus oxycedrus* L.). Geographical indications shall be reserved for spirit drinks, which acquire their quality, reputation or other characteristics during the production stage in a certain geographic area. Some geographic indications include Spišská borovička, Slovenská borovička Juniperus, Slovenská borovička, Inovecká borovička, and Liptovská borovička (Slovakia) (Regulation EC No 110/2008). The use of geographical indications allows producers to obtain a premium price and market recognition. In the case of premium spirits, there is a large economic interest to mix or completely substitute one brand with another less expensive brand. For this reason, there is a need for a rapid method for determining the geographical origin of products to protect regional designations. Although juniper-flavoured spirit drinks are well known and widely consumed, there are only few studies on their volatile/semivolatile composition and sensory profiles available. Recently, information about the sensory profile of four London Dry Gins and two gins with geographic indications was presented. In addition, the sensory results were in agreement with the composition of gin volatile fraction obtained by gas chromatography/mass spectrometry. Only limited data on the nonvolatile (aromatic) components of leaves, twigs, and berries of juniper are reported to date. Eugenol, methoxyeugenol, flavonoids, biflavonoids, coumarins, and chlorophyll are the best known aromatic molecules in juniper berries. These components are also possible fluorescent molecules in juniper-flavoured spirit drinks.

This work shows that front face fluorescence spectroscopy and multivariate statistical methods (principal component analysis, hierarchical cluster analysis, and parallel factor analysis) can be used for distinguishing between commercial samples of juniper-flavoured spirit drinks. The studies were performed on 44 drinks from 5 different producers. Fluorescence emission spectra were recorded from 250 to 700 nm, repeatedly, at excitation wavelengths from 200 to 500 nm, spaced by 5 nm interval in the excitation domain. It appears that front face fluorescence spectroscopy offers a promising approach for the authentication of juniper drinks as neither sample preparation nor special qualification of the personnel are required, and data acquisition and analysis are relatively simple.

Keywords: fluorescence spectroscopy, chemometrics, juniper, authenticity

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J-19

DEVELOPEMENT AND APPLICATION OF A GC-MS/MS METHOD FOR MEASURING MELAMINE AND ITS DEGRADATION PRODUCTS IN MILK-BASED PRODUCTS

Bruno Veyrand[1*], Sophie Durand[2], Philippe Marchand[3], Jean-Philippe Antignac[4], Bruno Le Bizec[5]

[1 2 3 4 5] LABERCA, ENVN, Nantes, France
[*] Corresponding author–E-mail: veyrand@vet-nantes.fr; Phone: 33 2 40 68 78 80

Melamine is an organic synthetic chemical compound rich in nitrogen most commonly found in the form of white crystals. It is commercially used for whiteboards, floor tiles, kitchenware, fire retardant fabrics and filters. In September 2008, this compound was found in powdered infant formulae and caused four children to die and about 13,000 children to be hospitalized in China. It was found that melamine was added to raw milk which was previously diluted with water in order to increase the nitrogen content of the milk and therefore its apparent protein content.

Decision 2008/921/EC was made by the European Commission on 9th December 2008 imposing special conditions governing the import of products containing milk or milk products originating in or consigned from China, and fixing a maximum concentration of 2.5 mg kg^{-1} of melamine in food products. Then, suitable analytical methods dedicated to melamine had to be developed, in order to control these food products.

The developed extraction procedure was derived from a method previously described [1]. This purification step without the use of SPE clean-up, generally based on cation-exchange (for melamine analysis) or anion exchange (for cyanuric acid analysis), enables a simultaneous detection of all target compounds (including melamine, cyanuric acid, ammelide and ammeline). If most of described method in the literature use LC-MS/MS detection, it has been chosen to use GC-MS/MS after a derivatisation step with MSTFA, which present numerous advantages in term of specificity (multiple fragment ions used as diagnostic signals) and sensitivity.

Performances of the method have been evaluated and present very good results, allowing the detection of compounds in the range of concentrations requested by European reglementation. Identification criteria fixed by the 2002/657/EC decision criteria were properly fulfilled, thanks to the MS/MS detection which provides at least two specific transitions for each compound, guaranteeing reliable analyte identification.

Use of GC-MS/MS after a derivatization step allows for the simultaneous detection of melamine, ammelide, ammeline and cyanuric acid with very good sensitivity, and finally a signal not affected by matrix effect (ion suppression) as sometimes observed in LC-(ESI)-MS/MS.

[1] "GC-MS Screen for the Presence of Melamine, Ammeline, Ammelide and Cyanuric Acid". (FDA method) – http://www.fda.gov/cvm/GCMSMelamine.htm

Keywords: melamijne, GC-MS/MS, milk products

J-20

VIBRATIONAL SPECTROSCOPY CHARACTERISATION OF HIGH QUALITY OLIVE OILS AROMATISED WITH GARLIC

Josu Trebolazabala[1], Irantzu Martinez-Arkarazo[2], Alberto de Diego[3*], Eneko Atxa[4], Juan Manuel Madariaga[5]

[1] Department of Analytical Chemistry, University of the Basque Country, P. O. Box 644; 48080 Bilbao, Basque Country, e-mail: josutreb@hotmail.com
[2 3 5] Department of Analytical Chemistry, University of the Basque Country, P. O. Box 644; 48080 Bilbao, Basque Country
[4] Azurmendi jatetxea, Legina auzoa, 48195 Larrabetzu, Basque Country
[*] Corresponding author–E-mail: alberto.dediego@ehu.es; Phone: 0034946015550;

The Mediterranean countries are responsible for 90% of global production of olive oil. Olive oil exhibits exceptional nutritional properties, and its taste and flavour are highly appreciated all over the world, specifically, among cooks and top chefs. The use of high quality olive oil aromatised with different flavours and aromas is nowadays a constant in top level restaurants, and becomes a powerful tool in the hands of an experienced and imaginative chef. Moreover, some people ask for these kinds of gourmet like products in order to use at home for special events.

Current production of aromatised oils, however, requires long maceration processes (more than 1 month) and the quality and characteristics of the resulting product highly depends on variables which are of difficult control. Only oils of the maximum quality should be used in the production of aromatised olive oils, which results in an expensive product with a reduced but sophisticated potential market.

Within this scenario, it becomes evident the need of tools for the characterisation of (aromatised) olive oils. Chromatographic techniques (both liquid and gas chromatography) have been mainly used in the analysis and characterisation of edible olive oils, but the analytical procedures are complex and time consuming, thus reducing the possibility to perform a great number of measurements in a reasonable time. Vibrational spectroscopic techniques have gained popularity in the last years, due to their simplicity and potentiality for non-destructive analysis. Techniques like Near Infra-Red spectroscopy (NIR), Attenuated Total Reflection Fourier Transform Infra-Red spectroscopy (ATR-FTIR), Diffuse Reflectance Fourier Transform Infra-Red spectroscopy (DRIFT-FTIR) provides important information on oil composition. Raman spectroscopy (Raman), in addition, adds the possibility of remote and portable analysis.

In this work, we report preliminary results on the production and characterisation of a high quality olive oil aromatised with garlic. Several approaches have been investigated in order to shorten the maceration process and to obtain products of the maximum quality and with different potential uses in the elaboration and creation of dishes. The possibilities of vibrational spectroscopic techniques such as NIR, ATR-FTIR, DRIFT-FTIR and Raman to *i)* characterise the final products, *ii)* discern among aromatised oils macerated in varying conditions and *iii)* monitor the maceration process are critically discussed in comparison with the taste observations of an expert panel of olive oil testers.

Keywords: olive oil, garlic, vibrational spectroscopy

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J-21
EVALUATION OF THE ORIGIN OF ESTONIAN HONEYS BY AMINO ACID CONTENT

Riin Rebane[1*], Koit Herodes[2]

[1 2] University of Tartu, Tartu, Estonia
[*] Corresponding author–E-mail: riin.rebane@ut.ee; Phone: 372 5052696; Fax: 372 5052696

The honey market is a large part of the economy for many countries, but honeys of various geographical and botanical origins are differently valued. Due to this difference, cheaper honeys are more commonly labeled as more expensive types of honeys. Traditional method used for honey verification has been melissopalynology but search for alternative method is ongoing. It has been shown that there is a relationship between the amino acid composition of honey and its origin, most commonly botanical origin. The free amino acid content of 61 honey samples (seven types of unifloral honeys and polyfloral honeys) from Estonia was determined. Method for amino acid analysis was developed. For sample preparation strong cation exchange solid phase extraction was used. Amino acid analysis was by HPLC-UV with precolumn derivatization with diethyl ethoxymethylenemalonate. The most abundant amino acids in Estonian honeys were proline and phenylalanine. The resulting data has been analyzed by t-test and principal component analysis (PCA). t-test revealed that some amino acids (α-alanine, β-alanine, asparagine, γ-aminobutyric acid, glutamine, glycine, histidine, ornithine, phenylalanine, proline, serine and tryptophan) are more potent for assigning honey botanical origin than others. In PCA, the first principal component is a function of all amino acid concentrations almost equally and therefore, all amino acids must be taken into account for PCA. Determination of the amino acid composition of various Estonian honeys shows that even though honeys have similar amino acid profiles, the t-test and PCA can bring out some differences. In the space of the two first principal components in PCA, heather honeys form a cluster which is clearly separable from, for example, polyfloral honeys. Heaher honey has higher arginine and proline concentrations and also total amino acid contents than other Estonian uni- and polyfloral honeys. The t test makes it possible to distinguish some honeys types from each other. It is concluded that analysis of free amino acid profile may serve as useful tool to assess the botanical origin of Estonian honeys.

[1] Rebane, R; Herodes, K. Evaluation of the botanical origin of Estonian uni- and polyfloral honeys by amino acid content. J. Agric. Food Chem. 2008, 56, 10716-10720.

Keywords: honey authenticity, amino acids, HPLC

J-22

MULTI-ELEMENT STABLE ISOTOPE ANALYSIS AS A POTENTIAL TOOL FOR VERIFYING THE GEOGRAPHIC ORIGIN OF BEEF

Rumiko Nakashita[1*], Yaeko Suzuki[2], Takashi Korenaga[3]

[1 2] Japan Certification Services, Inc., Yokohama, Japan
[3] Tokyo Metropolitan University, Tokyo, Japan
[*] Corresponding author–E-mail: rnakashita@pure-foods.co.jp; Phone: +81-45-949-4664; Fax: +81-45-949-4621

Multi-stable isotope analyses have become an important tool to provide information on the provenance of foods [1]. In general, stable carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) of animal tissue reflect those of their diet. On the other hand, stable hydrogen and oxygen isotopic compositions (δD and $\delta^{18}O$) of animal materials (e.g. lipid, tissue water, hair and urine) reflect those of drinking water. Multi-stable isotope analysis would therefore be a useful tool to trace geographical origins of beef [2-4]. In this study, we determined bulk carbon, nitrogen, and oxygen isotope compositions of beef from Australia, Japan, USA, and New Zealand, in order to confirm the method as a potential tool for verifying geographical origin of beef commercially distributed in Japan. Each element of isotope ratios of beef reflects the environmental fattening condition (region and feed) than the breed. The beef from USA is characterized by higher carbon isotope ratio than that from other countries. Also, the beef from Australia is characterized by higher oxygen isotope ratio than that from other countries.

We also determined oxygen stable isotopic compositions ($\delta^{18}O$) and compound-specific hydrogen isotopic compositions (δD) of fatty acids (C16:0, C18:1 and C18:2) in Japanese beef to discriminate its geographical location within Japan. $\delta^{18}O$ and δD of fatty acids in Japanese beef were the highest in the beef from Okinawa, which was the most southerly area among the four regions. Such ratios became lower as the latitude became higher. Especially, a large variation in the δD values of fatty acids was observed between the northern (-251‰) and southern sites (-210‰) in Japan. These results suggest that stable isotope ratios are applicable as a potential tool to the discrimination of beef between not only different countries but also different regions within Japan.

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[2] Förstel, H., (2007) *Analytical and Bioanaytical Chemistry* 388:541–544.
[3] Heaton, K., et al., (2008) *Food Chemistry* 107: 506–515.
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Keywords: stable isotope, geographical origin

J-23

MS-BASED ELECTRONIC NOSE FOR DISCRIMINATION BETWEEN GOUDA CHEESES OF DIFFERENT MATURATION STAGE AND PRODUCER

Anna Bereźińska[1*], Sylwia Kacała[2], Mieczysław Obiedziński[3]

[1 2 3] Warsaw University of Life Science (SGGW), Faculty of Food Technology, Warsaw, Poland
[*] Corresponding author–E-mail: mieczyslaw_obiedzinski@sggw.pl; Phone: +48(22) 59 37 672

Quality of long ripening cheeses is mainly determined by maturation conditions. Differences observed between cheeses of the same variety originate from the technological operations applied by the producer. The usability of volatiles analysis for differentiation between cheeses of different age and variety has been proven. The so-called electronic noses have been applied to cheese analysis and some drawbacks of these instruments have been reported. An alternative seem to be instruments which use mass spectrometry (MS).

The aim of the current study was to develop MS-based electronic nose for differentiation between Gouda cheeses of different producers and maturation stage.

Cheese samples from three different dairies were purchased in a local supermarket. Half of the cheese samples were subjected to accelerated ripening (4 weeks at 14°C).

Volatiles of the cheeses studied were evaluated by means of headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography and mass spectrometry (GC/MS). A total of 79 volatile components, mainly acids, esters, alcohols, aldehydes, and ketones, were identified. Compounds' relative peaks areas were subjected to principal component analysis (PCA).

To develop the so-called MS-based electronic nose averaged mass spectra from the chromatograms obtained were analysed. Eventually, 9 ions of *m/z*: 42, 43, 45, 56, 57, 87, 88, 95, and 96 were selected as "sensors" and subjected to PCA. The first two principal components accounted for 84% of the total variance. Results showed that ions of *m/z* 42, 45, 87, and 88 were more intense in samples frozen immediately after purchase. Headspace composition of these cheeses were characterized by greater relative peaks areas of 3-hydroxy-2-butanone and 3-methyl-3-buten-1-ol as well as of some volatile acids which were typical to less ripen cheeses. The four ions listed characterize mass spectra of the compounds mentioned. More mature cheeses distinguished themselves by greater intensities of the other five ions. Ions of *m/z* 95 and 96 characterize mass spectrum of 2-furfural and the other three ions (*m/z* 43, 56, 57) dominate in mass spectra of various alcohols, ketones, aldehydes, and short-chain fatty acids identified in ripen cheeses. Stepwise discriminant analysis (sDA) was used to develop simple models for discrimination between cheeses of different age and dairy. 3-hydroxy-2-butanone, 3-methyl-3-buten-1-ol, 2-butanone, 2-butanol, and n-butanol were decided to be crucial for differentiation between cheeses studied. SPME-MS-based electronic nose was suggested to be a promising resolution for further cheese authenticity studied.

Keywords: MS nose, cheese, PCA, identification

J-24

DISCRIMINATION BETWEEN ROASTED COFFEES OF DIFFERENT GEOGRAPHICAL ORIGINS – USABIBLITY OF MS-BASED ELECTRONIC NOSE

Anna Bereźińska[1], Marcin Bryła[2], Beata Cieślak[3], Mieczysław Obiedziński[4*]

[1 2 3] Warsaw University of Life Science (SGGW), Faculty of Food Technology, Warsaw, Poland
[4] Warsaw Agricultural University, Faculty of Food Sciences, Division of Food Quality Evaluation
[*] Corresponding author–E-mail: mieczyslaw_obiedzinski@sggw.pl; Phone: +48(22) 59 37 672

Coffee flavor characteristics are believed to strongly depend on beans geographical origins and are associated with volatiles composition after roasting. Results of volatile fraction analysis have been used to discriminate between Arabica and Robusta coffees from plantations of different geographical location, although the approach seems to be a quite time-consuming one. Thus, it would be useful to develop a fast and effective tool based on headspace assessment for evaluation of roasted coffee beans geographical authenticity in order to meet customers' expectations as well as law requirements. Special reference should be made to products offered in small coffee-roasteries where risk of frauds seems to be more probable.

The goal of the current study was to assess usability of mass spectrometry (MS) based electronic nose for discrimination between roasted Arabica coffees of different geographical origins. Objects of the research were samples of Arabica coffees from Ethiopia, Guatemala, Brazil, Costa Rica and Columbia obtained from a small coffee-roastery.

Headspace composition of roasted coffee beans has been assessed by means of solid-phase microextraction (SPME) coupled with gas chromatography and mass spectrometry (GC/MS). A total of 56 volatile compounds were identified, mainly pyrazines, furans, and ketones. Ethiopian and Guatemalan coffees contained 39 volatiles each. Headspace fraction of Colombian, Costa Rican and Brazilian beans comprised of 30, 34, and 37 components, respectively. In order to illustrate qualitative and quantitative differences between coffees studied data were subjected to principal component analysis (PCA). The first two principal components (PCs) accounted for about 74% of the total variance. A tendency for coffee samples to group according to the region of origin was observed. Average-integrated mass spectra of chromatograms obtained (2,0–17,5 min) were calculated. 8 out of 92 relative ion intensities were chosen for further evaluation by means of PCA. Ions chosen were of *m/z*: 79, 80, 82, 94, 121, 122, 135, and 136. The first two PCs explained about 94% of the total variance and allowed clear grouping of coffee beans on the basis of their geographical origins. stepwise discriminant analysis (sDA) was used to develop simple discriminating model. Close inspection of volatiles mass spectra revealed that ions selected were typical to various pyrazines, furans, and pyridines present in all coffee varieties studied. It was concluded that the results obtained had reflected the overall beans' headspace composition. MS-based electronic nose could be a valuable tool for future coffee quality, especially authenticity, assessment.

Keywords: MS nose, coffee, PCA, discrimination

J-25
TRANS FATTY ACIDS IN INFANT FORMULAS AND FOLLOW-UP FORMULAS IN POLAND

Hanna Mojska[1*], Anna Ćwikowska[2], Katarzyna Stoś[3]

[1 2 3] National Food and Nutrition Institute, Warsaw, Poland
[*] Corresponding author–E-mail: hmojska@izz.waw.pl; Phone: 48 22 550 96 56; Fax: 48 22 842 11 28

Trans isomers of fatty acids may be formed during industrial fat and oil processing and naturally during biohydrogenation by rumen microorganisms in the ruminants. *Trans* fatty acids have an influence on development of coronary heart disease (CHD) and, what is important, on impairment of essential fatty acids` metabolism to their long-chain metabolites. These long-chain polyunsaturated fatty acids are of great physiological importance during prenatal and postnatal development. Therefore, according to European Union law regulation, *trans* fatty acid level in infant formulas and follow-up formulas cannot exceed 3% of total fat content. The aim of our study was to determine fatty acid level, particularly *trans* isomers of fatty acids in randomly selected 32 samples of infant formulas and follow-up formulas in 2009 in Poland. The fatty acid methyl esters (FAME) were analyzed by high-resolution capillary gas chromatography using MS detector (GC/MS). Results are expressed as percentage (%wt/wt) of all fatty acids detected with a chain length 8 and 24 carbon atoms and are presented as mean with standard deviation minimum and maximum levels. Mean values of fatty acids in infant formulas and follow-up formulas were as follow: 38.60% and 37,93% for saturated fatty acids (SFA), 43.29% and 43.24% for monounsaturated fatty acids (MUFA), 18.02% and 18.70% for polyunsaturated fatty acids (PUFA) and 0.25% (0.17–0.33%) and 0.28% (0.18–0.40%) for *trans* fatty acids (TFA). There was significant difference ($p<0.05$) between infant formulas and follow-up formulas in amount of SFA and PUFA. There was no difference in *trans* isomers content. In TFA group the main fatty acid was *trans* 18:2 making up over a half of all trans isomers in both of formulas and next *trans* 18:3 (over 30%). The high level of above-mentioned *trans* isomers is pointing out on presence, mainly, vegetable oils in fat fraction of infant nutrition formulas.

In conclusion, the *trans* fatty acid level in all analyzed products do not exceed 0.4% so we confirm that they are safe for infants, who are not breastfed.

Keywords: Trans fatty acids, infant formulae

J-26

CHARACTERIZATION OF SERBIAN MONOFLORAL HONEY ACCORDING TO THEIR MINERAL CONTENT USING ICP-OES

Kristina Lazarević[1*], Jovetić S. Milica[2], Anđelković Ivan[3], Milojković-Opsenica Dušanka[4]

[1 2 3] Center for Food Analysis, Belgrade, Serbia
[4] Faculty of Chemistry, University of Belgrade, Serbia
[*] Corresponding author–E-mail: kristina.lazarevic@cin.co.rs; Phone: +381 11 65 32 29 36

The determination of mineral content, such as Ca, Mg, Zn and Sr or Mn is widely used in food authenticity studies. Among other substances (carbohydrates, amino acids, proteins, enzymes, various organic and inorganic acids, vitamins) honey contains significant amount of minerals. Due to increasing international interest in honey characterization there are many studies in which authors made the classification of botanical and geographical origins of honey based on the estimation of its mineral content. In accordance to fact that the presence of individual elements in Serbian honey has not yet been investigated in detail, in this study mineral content of different monofloral honey samples, collected from different regions of Serbia is determined using optical emission spectrometry with inductively coupled plasma (ICP-OES). Nine minerals (K, Na, Ca, Mg, Fe, Cu Zn, Mn Ni) were quantified for each honey. Chemometric techniques were used to classify honey according to chemical data. It is shown that botanical origin of honey samples correlate with their mineral composition.

Keywords: honey, mineral content, ICP-OES

J-27

SPME-GC×GC-TOFMS ANALYSIS OF SLOVAKIAN TRADITIONAL ALCOHOLIC BEVERAGES

Antónia Janáčová[1], Ivan Špánik[2*]

[1] Institute of Analytical Chemistry FCHPT STU, Bratislava, Slovakia
[2] Institute of Analytical Chemistry, Bratislava, Slovakia
[*] Corresponding author–E-mail: ivan.spanik@stuba.sk; Phone: +421259325282

Authenticity of spirit drinks is a permanent problem of the market. Authentic alcoholic beverages have to have defined origin, quality and often they must originate from specific sources (e.g. brandy from grapes, Rum from sugar cane, Crème de Cassis from black currant, etc) and many of them must be produced only in certain countries or even in specific region of country (like Cognac, Champagne, Tokai, Ouzo etc.). On the contrary, there are significant effort of low-cost producers or sellers to reduce production costs, in other words, to use cheaper, frequently less-valuable materials, while they are offered to the consumers as „full-valuable" under the same name or brand.

This work was aimed on utilisation of comprehensive gas chromatography for characterization of the aromatic profile of several types of traditional Slovak alcoholic beverages using SPME as sample treatment procedure. VOC from studied samples has been extracted at 50°C using SPME fibre coated by PDMS/DVB phase. In the step of instrumental analysis orthogonal two dimensional gas chromatography coupled to time-of-flight mass spectrometry) had been used. The sample was injected onto following set of columns: in 1st dimension nonpolar BPX-5 (3 0m, i.d. 0.25 mm, film thickeness 0.25 μm) and in 2nd dimension polar BPX-50 (1.5 m, i.d. 0.1 mm, film thickeness 0.1 μm).

The composition of aroma profiles obtained for different brands of popular juniper flavoured alcoholic beverage BOROVICKA as well as their comparison with other aroma profiles obtained for fruit distillates will be also discussed within this poster.

Keywords: authenticity, GC×GC, alcoholic beverages

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J-28

CHARACTERIZATION OF JAPANESE POLISHED RICE BY STABLE HYDROGEN ISOTOPES OF FATTY ACIDS FOR TRACING THE REGIONAL ORIGIN

Yaeko Suzuki[1*], Rumiko Nakashita[2], Fumikazu Akamatsu[3], Takashi Korenaga[4]

[1 2] Japan Certification Services, Inc.
[3] Water Environment Research Group, Public Works Research Institute
[4] Tokyo Metropolitan University
[*] Corresponding author–E-mail: ysuzuki@pure-foods.co.jp; Phone: +81-45-949-4664; Fax: +81-45-949-4621

Recently, characterizing the isotopic compositions of food materials has been used to prevent illegal mislabeling and adulteration [1]. For example, the adulterated products of honey and juice and the geographical origin of meat, dairy products, wine and cereal crops can be traced by using natural differences of carbon, nitrogen and/or oxygen isotopic compositions. In our previous study, we determined carbon and nitrogen contents (C and N contents) and stable carbon, nitrogen, and oxygen isotopic compositions ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$) of polished rice cultivated in Australia (New South Wales), Japan (12 different cultivation areas), and USA (California), in order to discriminate geographical origin of the rice (Suzuki et al., 2008). We suggest that the comparison of C and N contents and $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values would potentially be useful for the discrimination of geographical origin of the polished rice.

Hydrogen isotopic compositions of organic materials, has been developed in the field of geochemistry and ecology, may be a useful tool for tracing the geographical origin because of the large isotope variation compared with other light elements such as carbon, nitrogen, and oxygen. Thus, we hypothesize that hydrogen isotopic compositions of total fatty acids in Japanese rice have a wide variation and trace its cultivation area within Japan. Unfortunately, hydrogen isotope analysis of organic materials is more complicated than other light element because –COOH, -OH and $-NH_2$ functional groups can easily exchange hydrogen with ambient water vapor.

In this study, we determined the stable hydrogen isotopic compositions of fatty acids of Japanese rice. The hydrogen isotope values of fatty acids can be corrected by isotopic mass balance for contribution of added hydrogen during esterification. The δD values of fatty acids from Japanese rice exhibit a wide variation ranging from –216‰ (Hokkaido) to –183‰ (Okinawa), which are well correlation with that of ambient water (R^2=0.72) and mean temperature (R^2=0.58). These results suggest that δD values of fatty acids reflect growth environments within Japan. Therefore, hydrogen isotope analyses of fatty acids would be probably useful for tracing regional origin of polished rice cultivated within Japan.

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Keywords: rice, δD, fatty acids

J-29
NMR-BASED QUALITY CONTROL OF FRUIT JUICES

Cristina Daolio[1*], Eberhard Humpfer[2], Hartmut Schäfer[3], Manfred Spraul[4]

[1 2 3 4] Bruker BioSpin, Rheinstetten, Germany
[*] Corresponding author–E-mail: cristina.daolio@bruker-biospin.de; Phone: (49) 72151616218

Nuclear Magnetic Resonance (NMR) has been used for structural analysis of pure compounds over decades. Opposite to this, the extraordinary potential of NMR has just started to be recognized and exploited in the analysis of mixtures in the context of food beverages. This became possible due to the availability of high throughput automation technology, the increase of sensitivity and modern water suppression NMR sequences. Even nowadays the quality control of fruit juices is still based on many parameters, which so far have to be assessed in multiple analytical and chemical tests. Here we introduce a high resolution NMR based method, allowing to quantify multiple relevant compounds and to draw statistical conclusions on a single NMR measurement. Preparation, measurement, evaluation and final reporting need less than 15 min a sample. This includes more than 24 quantitative parameters and the statistical evaluation. It is also shown, that an authentic fruit juice spectral database can be used reliably to assess the following juice properties by statistical means:

Type of fruit like orange, tangerine, blood-orange

Direct juice versus rediluted concentrate

Geographical Origin

Mixing of different fruit types

Fruit content

In addition relevant molecules for quality of the juices are quantified by the NMR-method using optimized 1-dimensional and a fast 2-dimensional experiment. Due to the statistical analysis on the acquired data, also frauds and problems can be detected, that the system is not trained for like the addition of whole fruit to the juice. In this context the statistics can be applied to characteristics signals indicating outliers and to establish the analysis of so far unknown signals by using a reference compound database. As an alternative tool, the hyphenation approach based on e.g. LC-NMR, LC-NMR-MS and LC-SPE-NMR-MS completes the repertoire of powerful possibilities of NMR-based analysis.

Keywords: NMR, Fruit Juice Screening, Fraud

J-30

USING MULTIELEMENTAL PROFILES AS TRACERS OF THE GEOGRAPHICAL ORIGIN OF EARLY POTATO FROM SOUTH ITALY

Mariavittoria Zampella[1*], Paola Adamo[2], Christophe Quétel[3], Fabio Terribile[4], Simona Vingiani[5]

[1 2 4 5] Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università di Napoli Federico II, Napoli, Italy
[3] EC–Joint Research Centre – Institute for Reference Materials and Measurements, Geel, Belgium
[*] Corresponding author–E-mail: mvzampel@unina.it; Phone: +3214571969

Early potato is a high quality agricultural product of south Italy, sold locally and also exported to other European countries (mainly UK and Germany). It is subject to frauds with lower quality products, mainly from Cyprus and North Africa, and needs to be protected. This presentation will introduce the first results of our investigations for finding ways to authenticate potatoes from six typical production areas in three separate regions (Sicily, Apulia and Campania). We are studying if the chemical composition of the tubers and of the associated soil samples can serve as markers of origin of these potatoes. Soil (0–30 cm) and different varieties of potatoes were sampled in triplicates, at the same sites, in the main production areas of the three Italian Regions. Soil was air dried and sieved to 2.0 mm. Potatoes were washed with ultrapure water and pealed; the central part of the tubers was sampled and freeze-dried. Soil and potato samples were acid digested and analysed by ICP-MS for 41 and 53 elements respectively. Samples of commercialised potatoes from Apulia and of the soil adhering to these products were also analysed. The main physical and chemical properties and the mineralogical composition of the soil samples were also investigated. It is possible to well discriminate the soils of the three Regions from the mineralogy and from the multivariate analysis (PCA) of their geochemical data. In most cases, the different production areas within the same region can also be distinguished. Potatoes coming from the same region can be grouped together on the basis of their chemical composition for the majority of samples. However, a partial overlap between Sicily and Apulia is observed and the multielement profiling of tubers alone does not seem powerful enough in this case. Different varieties from the same soil do not show significant differences in their multielement profiles. Early potatoes are usually not washed or brushed before commercialization and we are also investigating the soil adhering to tubers. It is characterised by a texture and a geochemical composition different than for the respective whole soil. Interpretation of the results is on-going taking into account the possibility of a contamination during the commercialization process.

Keywords: traceability, authenticity, multielement analysis, mineralogy

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J-31

THE USE OF DIRECT ANALYSIS IN REAL TIME (DART) IONIZATION–MASS SPECTROMETRY FOR AUTHENTICATION OF ANIMAL FATS

Vojtech Hrbek[1], Lukas Vaclavik[2], Bo-Anne Belkova[3], Petr Pipek[4], Jana Hajslova[5*]

[1 2 3 4 5] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: jana.hajslova@vscht.cz; Phone: +420220443185

The authenticity of foods is an important qualitative parameter. The present study deals with utilization of novel ionization technique, direct analysis in real time (DART), coupled to time-of-flight mass spectrometry (TOFMS) for rapid assessment of animal fats authenticity (lard, beef tallow and fish oil). Employing minimal sample preparation (dilution in toluene or extraction with methanol–water mixture), profiles of triacylglycerols (TAGs) and some minor compounds were obtained by DART–TOFMS analysis of samples. To explore the potential of DART–TOFMS for detection of lard adulteration with beef tallow, also model admixtures of selected samples were prepared and examined. The internal structure of obtained data (mass spectral fingerprints) was inspected with the use of principal component analysis (PCA). In the next step, linear discriminant analysis (LDA) was employed for sample classification. The best results were observed when profiles of TAGs were used as input data for LDA (both prediction and recognition ability were 100%), detection of beef tallow in lard at level as low as 5% (*v/v*) was achieved. Significantly worse results were obtained for profiles of methanol–water extracts. The second part of experiments was focused on profiling of fish oil. Besides of the characterization of TAGs and minor compounds present in examined samples, the capability of DART–TOFMS to detect the presence of vegetable oil in fish oil was successfully demonstrated.

Keywords: animal fats, DART, authenticity, LDA

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J-32
COMPREHENSIVE PROFILING FOR QUALITY ASSESSMENT OF DISTILLED ALCOHOLIC BEVERAGES

Monika Cerna[1], Katerina Riddellova[2], Tereza Valentova[3], Tomas Cajka[4], Jana Hajslova[5*], Vladimir Kocourek[6], Lukas Vaclavik[7]

[1 2 3 4 5 6 7] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: jana.hajslova@vscht.cz; Phone: +420220443185

Two approaches were applied to assess the volatile and semi-volatile fingerprints of Czech plum brandies (10 samples), Czech 'rum-like' spirits (10 samples) and genuine rum (3 samples; Barbados, Bahamas and Puerto Rico origin), with the aim to confirm the authenticity of the samples. The first approach employed a headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-time-of-flight mass spectrometry (GC-TruTOFMS), the second was based on the use of a direct analysis in real time source (DART) coupled to a high-resolution time-of-flight mass spectrometer (HR-TOFMS). The DART-HRTOFMS technique was also combined with direct immersion solid-phase microextraction (DI-SPME). Among all the analytes detected, 12 to 18 characteristic compounds were selected for further chemometric evaluation using Principal Component Analysis (PCA). Statistical evaluation enabled the classification of local 'rum-like' and real rum samples; within the group of plum brandies it was possible to distinguish pure plum distillates from the rest of the samples.

Keywords: alcoholic distillates, SPME, TOF, DART

J-33

AUTHENTICATION OF THE BOTANICAL ORIGIN OF HONEY USING ADVANCED MASS-SPECTROMETRIC TECHNIQUES AND MULTIVARIATE ANALYSIS

Tomas Cajka[1*], Katerina Riddellova[2], Jana Hajslova[3], Dalibor Titera[4]

[1 2 3] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[4] Bee Research Institute, Dol 94, 252 66, Libcice nad Vltavou, Czech Republic
[*] Corresponding author–E-mail: Tomas.Cajka@vscht.cz; Phone: +420220443142

Honey is one of the oldest known sweeteners composed primarily of sugars (fructose and glucose) and water. Honey also contains enzymes, vitamins, organic acids, amino acids, minerals, dyes, pollen, and volatile compounds. In order to determine botanic origin, pollen analysis is preferred, but in some cases (*e.g.* removing of pollen by filtration) this method is not reliable for the determination of floral origin.

For the authenticity assessment of honey samples, two different profiling strategies were tested:

(i) Head-space solid-phase microextraction–comprehensive two-dimensional gas chromatography–low-resolution time-of-flight mass spectrometry (HS-SPME–GC×GC–LRTOFMS)

(ii) Direct immersion solid-phase microextraction–direct analysis in real time ion source–high-resolution time-of-flight mass spectrometry (DI-SPME–DART–HRTOFMS)

While HS-SPME–GC×GC–LRTOFMS approach can be considered to be a “gold standard” for the profiling of volatiles (including those in honey samples), the latter approach (DART–HRTOFMS), introduced into the laboratory use only recently, represents a novel analytical strategy enabling rapid examination of product composition.

In this presentation, a comparison of these two approaches is discussed. Advanced chemometric strategies were employed for interpretation of acquired data sets.

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Keywords: Honey, GC–MS, SPME, DART

J-34

TRACING THE ORIGIN OF FOOD: AN OVERVIEW OF THE OUTCOMES FOR HONEY, OLIVE OIL, AND BEER

Tomas Cajka[1*], Katerina Riddellova[2], Monika Tomaniova[3], Jana Hajslova[4]

[1 2 3 4] Institute of Chemical Technology Prague, Department of Food Chemistry and Analysis, Technická 3, Prague 6, 166 28, Czech Republic
[*] Corresponding author–E-mail: Tomas.Cajka@vscht.cz; Phone: +420220443142

Food authenticity is of concern to the consumer, food processor, retailer, and regulatory bodies. One authenticity issue of emerging importance is geographic origin, which is the case for some products permitted to be marketed using a PDO (Protected Designation of Origin) label on the basis of their production area. However, given the price premia associated with such labels, economic fraud (*e.g.* labelling a non-PDO olive oil as a PDO product) is believed to be a significant problem [1].

The EU-funded TRACE project (www.trace.eu.org) aims at development of cost-effective fingerprinting methods, which provide consumers with added confidence in the authenticity of food at the European market.

Within the project, various fingerprinting methods were developed and applied as profiling tools for the authentication of honey, olive oil, and beer samples [2–5]. The presented methods are based mainly on the examination of volatiles isolated/extracted from these food matrices using solid-phase microextraction followed by gas chromatography–mass spectrometry (SPME–GC–MS). In the case of beer samples, also a novel strategy based on direct analysis in real time–time-of-flight mass spectrometry, allowing to obtain spectral fingerprints without previous chromatographic separation, was employed.

For the chemometric analysis, the principal component analysis (PCA) was used for a preliminary inspection of the data structure. In the next step, various classification methods such as linear discriminant analysis (LDA), partial least squares discriminant analysis (PLS-DA), or artificial neural networks (ANN) were the options used for data processing.

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Keywords: Authenticity, Food, GC–MS, SPME, DART

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J-35
DETECTION OF ANIMAL REMAINS IN AN ORGANIC CATTLE FEED

Peter Colwell[1*], Michael Walker[2], Kirstin Gray[3]

[1 2 3] LGC, Teddington, England
[*] Corresponding author–E-mail: pete.colwell@lgc.co.uk; Phone: +44 (0)20 8943 7443

Bovine spongiform encephalopathy (BSE) was first observed in UK in 1984 and specifically diagnosed in 1986. The epidemic in cattle peaked in 1992-93 at almost 1,000 cases per week. BSE is generally thought to be caused by a rogue prion in processed animal remains fed to cattle and is thought to give rise to an invariably fatal vCJD in humans. The ruminant feed ban introduced in July 1988 prevented cattle remains being fed to cattle and was a key measure in halting the epidemic and has been described as: "... a spectacularly successful control measure... one of the notable success stories of global disease control". [1]

In the UK the Government Chemist is required to act as the national focus of technical appeal in specified areas where there is an actual or potential dispute between food businesses and regulator. The specified areas are broadly drafted but in practice tend to focus on the results of chemical analysis or their interpretation in the agrifood sector.

This paper describes the analysis undertaken by the Government Chemist in a case were a cattle feed sample was alleged by an enforcement analysis to contain animal remains i.e. Processed Animal Protein (PAP) and/or Meat & Bone Meal (MBM) a finding disputed by a negative result from a defence analysis carried out in another Member State.

The presence of PAP and MBM in feed is currently controlled in the UK by the Transmissible Spongiform Encephalopathies Regulations 2008 and enforced by Annex VI of Commission Regulation (EC) No 152/2009 of 27th January 2009 laying down the methods of sampling and analysis for the official control of feed which prescribes a microscopy method with a limit of detection of 0.1% for PAP/MBM in feed. This method is focused on the presence and characteristics of bone fragments, and other structures e.g. muscle fibres, hairs etc. to provide evidence of the respective animal types. Recent developments are the identification of bone fragments at the level of classes (mammal versus bird versus fish), supported by image analysis of bone characteristics. Two other approaches, immunology and DNA based assays, were successfully applied to spiked controls in this case. However only the official microscopy method yielded useable results in the case when applied to the referee sample.

[1] The BSE Inquiry Volume 3

Keywords: Feed, Animal, MBM, PAP, Microscopy

J-36
QUALITY ASSESSEMENT OF HONEY MULTIELEMENT ANALYSIS USING BY ICPMS

Maria Chudzinska[1*], Danuta Baralkiewicz[2]

[1 2] Adam Mickiewicz University, Poznań, Poland
[*] Corresponding author–E-mail: maria.mm@amu.edu.pl; Phone: +48604423059

ICP-MS is becoming widely accepted in food analysis as a sensitive and accurate technique for the determination of major and trace elements at same sample injection. It offers simultaneous multielement measurement capability and very low detection limits in a large number of matrices. A method is described for the simultaneous ICP-MS determination of 14 elements in three type of honey from Poland. Traceability of measurements in honey analysis meet with difficulties by reason of absence of reliable reference standards. Among a CRM commercially available, it is hard to find equivalent of honey complex matrix with regard to high carbohydrate content. Hence achieving traceability of the analytical results have been established in a different ways. As there no readily available CRM the recoveries of the analytes were measured using spiked solution and used estimation of the accuracy of the method.

The aim of this study were 1) contribute to establish quality control of measurement in honey analysis by applying two CRM (corn flour-(INCT-CF-3) and apple leaves (NBS 1515)) 2) validate analytical method by characterizing qualitative parameters: linearity range, detection LOD and quantification limits LOQ, precision, recoveries, repeatability and within-laboratory reproducibility. The capability of the method as a routine analysis method was estimated through the determination of the detection limits of every elements studied. The results obtained for limit of detection (LOD) and limit of quantification (LOQ) in $mg \cdot kg^{-1}$ were respectively: Al (0.95 and 3); B (0.5 and 1.75); Ba (0.15 and 0.5); Ca (24 and 80); Cd (0.004 and 0.01); Cr (0.007 and 0.02); Cu (0.11 and 0.35); K (7.5 and 25); Mg (5 and 12.5); Mn (0.08 and 0.27); Na (5 and 17); Ni (0.025 and 0.125); Pb (0.02 and 0.075); Zn (0.225 and 0.75). While for the recoveries in% for CRM corn flour-(INCT-CF-3), SRM apple leaves (NBS 1515) were respectively: Al (94 and 105); B (102 and 100); Ba (100 and 89); Ca (97 and 100); Cd (89 and 103); Cr (94 and 106); Cu (103 both); K (99 both); Mg (93 and 99); Mn (98 and 91); Na (98 and 107); Ni (96 both); Pb (105 and 98); Zn (95 and 94). The precision was evaluated by measuring the repetatability of the method for all elements. As for the repeatability (CV%): Al 3.7; B 7; Ba 4; Ca 6; Cd 7.7; Cr 7; Cu 9; K 8; Mg 3.5; Mn 6; Na 3.5; Ni 4; Pb 4; Zn 3. The method can be applied as a routine analysis quality of the samples.

Keywords: honey, multielements, ICP-MS, quality control,

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J-37

DETERMINATION OF MELAMINE IN EGGS BY MICROWAVE-ASSISTED EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY

Liushui Yan[1*], Xin Jiang[2], Xubiao Luo[3]

[1 2 3] School of Environmental and Chemical Engineering, Nanchang Hangkong University, Nanchang, PR China
[*] Corresponding author–E-mail: yanliushui.1980@yahoo.com.cn; Phone: +86 791 3953371

March of 2007, pet food contaminated with melamine resulted in a major outbreak renal disease and associated deaths in cats and dogs in the USA. High melamine-containing pet food was fed to poultry, part of melamine will be metabolized in the body, resulting melamine were found in meat and eggs. The excessive melamine-containing food consumption has carcinogenic potential, so a rapid and sensitive analytical method for determination of melamine in eggs was essential.

For the determination of melamine in eggs, the extraction of melamine from sample plays an important role during the whole analytical process. Now ultrasonic-assisted extraction has been used to extract melamine from samples. However, this pretreatment method is usually used for extraction of melamine from samples in which melamine was added by unethical manufactures. it is not suitable for extraction from egg samples because melamine in eggs is not added directly, which formed after metabolism in organism.

Microwave-assisted extraction becomes one of the important techniques for extracting target compounds from a wide variety of sample matrices because it has obvious advantages in extraction time and solvent consumption compared with other extraction methods. The purpose of this study is to develop a simple and sensitive method for determination of melamine from eggs by microwave-assisted extraction and HPLC/MS quantification.

Melamine in egg samples was extracted in 2% trichloroacetic acid and methanol (8/2, *v/v*) at radiation temperature 100°C and under microwave radiation power 400 W for 10 min. Clean-up of extracts was performed with PCX solid-phase extraction cartridge using ammonia methanol as the elution solvent. The determination of melamine in the final extracts was carried out by high performance liquid chromatography/mass spectrometry. Isocratic HPLC separation was performed using C_{18} column with acetonitrile and 0.1% trichloroacetic acid (12/88, *v/v*) as the mobile phase. Melamine was detected by electrospray ionization in positive ion mode and multiple reaction monitoring. Quantitation of samples was performed using eight-point matrix calibration curve at concentrations between 30 and 2000 ng/mL of melamine. The method was validated by analysis of blank egg samples fortified with 25 ng/g, 50 ng/g and 100 ng/g of melamine. The recoveries were 84%, 100% and 103%, respectively, with relative standard deviation lower than 3.0%. The detection limit was 10.0 ng/g. The proposed method was successfully used for determination of melamine in egg samples.

Keywords: Melamine, eggs, microwave-assisted extraction, HPLC-MS

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J-38

DETERMINATION OF ETHYL-CARBAMATE FOR AUTHENTIFICATION PURPOSES OF HUNGARIAN CIDER SPIRITS BY HPLC-ESI-MS

Edit Deák[1*], Mihaly Dernovics[2]

[1 2] Corvinus University of Budapest, Budapest, Hungary
[*] Corresponding author–E-mail: edit.deak@uni-corvinus.hu; Phone: 0036702153871

Ethyl-carbamate (also known as urethane, $C_3H_7NO_2$) is a naturally occurring component in fermented foods and beverages. After 1984, several studies concluded that various alcoholic beverages including wines, beer, and straight spirits contained ethyl-carbamate even up to the concentration of 12 ppm. On the strength of the animal tests, ethyl-carbamate is considered genotoxic and carcinogenic and potential human carcinogen. The EFSA (European Food Safety Authority) concluded that the intake of ethyl-carbamate from fermented foods is not a serious medical hazard, but from spirits, particularly from stone fruits brandies and cider spirits the high quantity can be a real risk.

On the other hand, upcoming scientific publications have not reported high ethyl-carbamate concentration. Besides, as this compound is a naturally emergent component of the cider spirits, it is supposed that the quantity of ethyl-carbamate depends on the fruit base material and the fermentation procedure of the given spirit. Therefore, our goal was to set if the quantity of this compound can be an authenticity mark to a special spirit family, the Hungarian cider spirits, “pálinka”, that must entirely originate from fruit distillate. To achieve this, a new HPLC-ESI-MS method was developed based on a former HPLC-UV technique applying xanthydrol derivatisation of ethyl-carbamate. Taking over the advantages of the simple and high throughput sample preparation with xanthydrol derivatisation and the high selectivity of the developed MRM (multiple reaction monitoring) technique, both the limit of detection (LOD) and sensitivity of the method were significantly ameliorated, reaching the LOD at 0.1 ng as an absolute value.

Covering even the year-to-year variations of the products, samples from spirits of the following fruits were analysed: plum (*Prunus domestica* L.), sloe (*Prunus spinosa* L.), apricot (*Prunus armeniaca* L.), pear (*Pyrus communis* L.), apple (*Malus domestica* L.), sorb (*Sorbus domestica* L.), strawberry (*Fragaria X. Ananassa* L.), sour cherry (*Prunus cereasus* L.), peach (*Prunus persica* L.), and cornel (*Cornus mas* L.).

Based on the data of ethyl carbamate concentration (varying between 2.4 ppb and 190 ppb), the application of the results aiming at spirit authenticity purposes will be discussed.

Keywords: ethyl-carbamate, HPLC-ESI-MS, cider spirit

J-39
1H HRMAS-NMR SPECTROSCOPY TO ASSESS FOOD QUALITY

Sara Cozzolino[1*], Caterina Cafiero[2], Mena Ritota[3], Anna Taglienti[4], Paolo Sequi[5], Massimiliano Valentini[6]

[1 2 3 4 5 6] Agricultural Research Council-Research Centre for Plant-Soil System, Instrumental Centre of Tor Mancina, Strada della Neve-S.P. Pascolarese Km 1, 00016 Monterotondo, Roma (Italy)
[*] Corresponding author–E-mail: sara.cozzolino@entecra.it; Phone: +39-06-9061223; Fax: +39-06-9068309

HRMAS-NMR (High Resolution Magic Angle Spinning Nuclear Magnetic Resonance) is an innovative NMR tool that offers the opportunity of measuring gel-like and suspension samples without any chemical and/or physical preparation, and is capable of producing highly resolved NMR spectra. This approach is of particular interest in food science, since it obtains quickly the metabolic fingerprints of the investigated foodstuff. Moreover, the lack of sample treatments minimize any possible qualitative and quantitative compound modifications. The observed spectroscopic data must be investigated with appropriate data analysis procedures; the use of chemometric methods is a need and allows the detection of molecular markers. The latter are used predominantly for classification and discrimination purposes.[1–3]. In this work we present an overview of the HRMAS-NMR approach applied to different foodstuff: fresh vegetables, e.g. sweet pepper, fresh meat, e.g. beef and processed. For the investigated systems HRMAS-NMR data allowed the identification of metabolites correlated to specific quality properties.

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Keywords: HRMAS-NMR, metabolic profiling, fingerprints

J-40

OPTIMIZATION OF A MATRIX SOLID-PHASE DISPERSION METHOD FOR THE DETERMINATION OF SUDANS I-IV IN TOMATO SAUCE SAMPLES

L. Enríquez Gabeiras[1], R.M. Garcinuño Martínez[2*], P. Fernández Hernando[3], J.S. Durand Alegría[4]

[1 2 3 4] UNED, Madrid, Spain
* Corresponding author–E-mail: rmgarcinuno@ccia.uned.es; Phone: 0034913987366; Fax: 0034913988379

During the last few years there has been concern about the use of organic colorants in food and foods ingredients. Colorization or amending of the original color with Sudan dyes can make food unsafe because of the toxicity and carcinogen properties of such compounds. Sudans I-IV are phenyl-azoic derivatives widely used as food additives, although their use, at any level, is illegal in Japan, Europe and United States in food products destined for human consumption. The European Commission (EC) requires that all food products coming into any European Union Member State are certified to be free of Sudan dyes. Therefore, it is necessary the development of analytical methods to determine these analytes in food samples.

In the presented work, a simple, fast and effective extraction method based on matrix solid-phase dispersion (MSPD) has been developed to determine Sudans I–IV in several tomato and chilli sauces by high-performance liquid chromatography coupled to a photodiode array detector. Different parameters of the method were evaluated, such as type of solid-phase sorbent, (C_{18}, alumina, silica-gel and Florisil, sand), the amount of solid-phase and eluent (dichloromethane, acetone, ethyl acetate, acetonitrile, n-hexane and n-hexane:ethyl acetate), using different ratios. The best results were obtained using a silicagel-C_{18} mixture as dispersant sorbent, and acetonitrile saturated with n-hexane as eluting solvent. The method was validated using tomato and chilli sauce samples fortified with Sudans I-IV at different concentration levels (0.12–2.40 mg kg^{-1}). Average recoveries ranged from 75% to 114%, with relative standard deviations between 2% and 10%. The proposed method is specific and selective, allowing the analysis of over 15 samples per working day.

Keywords: Sudans I-V, MSPD, foods, HPLC

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J-41

PROFILING OF TOCOPHEROLS AND STEROLS OF RAPESEED OIL FOR CONFIRMATION OF CONTAMINATION OF RAPESEED BY SEEDS OF WEED PENNY-CRESS

Agnieszka Obiedzińska[1*], Dorota Ogrodowska[2], Julita Nawratil[3], Ryszard Zadernowski[4]

[1] Warsaw University of Live Sciences, Faculty of Human Nutrition and Consumer Sciences, Poland
[2 3 4] University of Warmia and Mazury, Olsztyn, Faculty of Food Sciences, Poland
[*] Corresponding author–E-mail: agnieszka_obiedzinska@sggw.pl; Phone: +48502354551

The purpose of this project was to determine the characteristics of the penny-cress` lipids in order to develop methods for detection of rapeseeds contamination by weed seeds of penny cress. Penny-cress is a weed that contaminates the cultivation of rape. During the process of producing of oil from double zero (zero erucic acid and zero glucosinolates) rape seeds, oil from penny-cress seeds might get to the rapeseed oil and contaminate it with erucic acid, which is presented in penny-creed seed oil up to 40% content of TAGs. For these purposes the oil from penny-creed seeds was characterized by means of GCMS in respect of profiles of sterols and tocopherols. The contents of sum of tocopherols was 99.15 mg/100 g in detail α-tocopherol–61.77 mg/100 g, β-tocopherol–0,78 mg/100 g, γ-tocopherol–35.24 mg/100 g, δ-tocopherol–1.35 mg/100g. The content of sum of sterols was–280 mg/100g including cholesterol–12.2 mg/100 g, brassicasterol–14.9 mg/100 g, campesterol–107.9 mg/100 g, sitosterol–124.7 mg/100 g, avenasterol–18.9 mg/100 g. Interesting is the squalane content which was 46.24 mg/100 g.

The relative ratios of sterols and tocopherols could be used as a confirmatory analysis for the contamination of rapeseeds by seeds of weed penny-cress.

Keywords: rapeseed, penny-cress, tocopherols, sterols

J-42

GAS CHROMATOGRAPHY METHODS FOR CHARACTERIZATION OF CONTAMINATION CONSEQUENCES OF RAPESEED BY WEEDS SEEDS OF PENNY-CRESS

Agnieszka Obiedzińska[1*], Dorota Ogrodowska[2], Julita Nawratil[3], Ryszard Zadernowski[4]

[1] Warsaw University of Live Sciences, Faculty of Human Nutrition and Consumer Sciences, Poland
[2 3 4] University of Warmia and Mazury, Olsztyn, Faculty of Food Sciences, Poland
[*] Corresponding author–E-mail: agnieszka_obiedzinska@sggw.pl; Phone: +48502354551

Penny-cress is a common weed that contaminates the cultivation of rape. It is especially important when herbicides treatment is not properly and/or in time performed. As a consequence the penny-cress seeds could enter into the process of producing of oil from rape seeds (double zero, low erucic acid <2% and low glucosinlates content < 25micromoles /g) and contaminate it by increasing level of erucic acid and glucosinolate content in rapeseed meal. The average profile of fatty acids of penny-cress seeds oil was analyzed by GC-FID/MS and the percentage content is presented in Table 1. The results are revealing penny-cress as a reach source of oil (38–42%) and of erucic acid, which could contaminate oil produced from double zero rapeseed.

The determinations of the level of contamination could be based on the comparison ratio of specific fatty acid of rapeseeds with ratios for different levels of contamination of rapeseed with penny-cress seeds in range 2–10%.

Table 1. Precentage profile of fatty acids of penny-cress seeds oil

Fatty acids	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:1}$	$C_{20:2}$	$C_{22:1}$	SUM SUFA	SUM MUFA	SUM PUFA
%	3.5	0.7	11.4	19.8	11.5	10.2	2.1	40.1	4.2	60.7	33.6

Keywords: rapeseed, penny-cress, FA, erucic acid

J-43
MEASURING FRUIT JUICE ADULTERATION BY CHANGES IN FLAVANOID CONTENT USING MEPS–HPLC

Hans-Jurgen Wirth[1], Naza Lahoutifard[2], Paul Wynne[3*]

[1 3] SGE Analytical Science Ltd, Ringwood, Australia
[2] SGE Europe, Courtaboeuf, France
[*] Corresponding author–E-mail: pwynne@sge.com; Phone: +61 3 98374230

Cranberry and blueberry juice are notable example of a functional foods that may be eroded in value by dilution or adulteration with lower value products. The cranberry is known as a source of polyphenolic antioxidants (including anthocyanidin flavonoids, cyanidin, peonidin and quercetin) and is the subject of investigation for potential anti-cancer properties and its effects on the cardiovascular and immune systems. The tannins are reputed to reduce urinary tract infections, exhibit anti-clotting properties and reduce gingivitis. Other fruits are also known or reputed to have functional characteristics and therefore of high value.

A rapid Micro-Extraction Packet Sorbent (MEPS) method is described for extracting and concentrating the phenolic components from a variety of commercial fruit juices. The juice was passed through a C_8 or C_{18} MEPS cartridge and the retained fraction eluted with methanol for direct injection into a HPLC and analysis on a Protecol C_{18} HQ105 column using a 0.1% *v/v* aqueous trifluoroacetic acid – methanol mobile phase. Detection of the phenolic fraction at 350 nm was used to generate a characteristic profile for each species of fruit.

The method allowed profiling of fruit juice and the detection of diluents or juice mixtures. Because the solid-phase step is flowrate dependant, the small sample and elution volumes of MEPS allow rapid sample extraction that may be completed in realtime with the HPLC analysis.

Keywords: cranberry, HPLC, MEPS, fruit-juice, profiling

J-44

NMR METHOD FOR IDENTIFYING THE BOTANICAL ORIGIN OF POLISH ORIGIN HONEYS

Izabela Jasicka-Misiak[1*], Aleksandra Kycia[2], Paweł Kafarski[3]

[1 2 3] Opole University, Faculty of Chemistry, Opole, Poland
[*] Corresponding author–E-mail: izajm@uni.opole.pl; Phone: +48 774527115; Fax: +48 774527115

Honey is produced by bees from plant nectars, plant secretions and excretions of plant sucking insects. It has been an excellent nutritional option for many generations due to its health benefits. From a nutritional point of view too, the consumer needs a guarantee about the botanical origin and quality of honey. For this reason the European Council Directive 2001/L10 states the need for methods for the verification of honey's botanical origin. Normally, the identification of honeys according to its botanical or geographical origin is performed through microscopical analysis of pollen and organoleptic evaluation and on the determination of total flavonoids, profiles of volatiles, amino acids, metals and nonmetal trace elements. There are very few studies on the characterization of specific structural markers of botanical origin, which are obviously the most reliable and consistent, especially if they are present in significant amounts. Nuclear magnetic resonance (NMR) techniques, which require minimal sample processing and only a few milligrams of sample, sound the most promising. The aim of this work was to establish a reliable and reproducible analytical procedure to obtain "fingerprints" of honey samples, as an alternative to conventional melissopalynological, organoleptic and instrumental methods. The procedure is based on the 1H nuclear magnetic resonance (NMR) profile analyses of crud honey extracts. Additionally after solid-phase extraction (SPE), were examined phenolic profiles of the honeys. Extracts of 5 Polish honeys (lime, heather, rape, golden rod and buckwheat) from 10 different geographical sources were analyzed. Obtained results, although obviously only preliminary, suggest that the 1H NMR profile can be used as a reference work for identifying the botanical and/or geographical origin of honey.

Keywords: NMR, honey, phenolic compounds

J-45
ILLEGAL FOOD DYES MULTIEXTRACTION AND MULTIDETERMINATION APPLYING QUECHERS AND LC-MS/MS

Rita Lorenzini[1*], Davide Garbini[2], Martino Barbanera[3]

[1] Department of Environmental Science and Technologies
[2 3] Coop Italia
[*] Corresponding author–E-mail: rita.lorenzini@unibo.it; Phone: 0039-333-6745562

Presence of illegal dyes in food is not at all a superseded problem in frauds control activity, as noticeable by consulting the RASFF weekly report (1). Sudan family molecules and other illegal dyes are still found in spices (chilly and red pepper powders and pastes, tumeric, curry, saffron, safflower, etc.), but also in fatty raw ingredients as palm oil, thus contaminating every food product which may contain them.

QuEChERS is a miniaturised dispersive solid-phase extraction method that has proven particularly advantageous for pesticide multiresidue analyses in food from vegetal origin, being its use quickly spreading among routine food control laboratories. Most of the illegal dyes have a quite non polar character, thus well fitting into the QuEChERS scope of action. In this study, we tested QuEChERS ability to extract from two different food matrixes (ketchup and potato flakes) eleven illegal dyes: Sudan I, II, III, and IV, Sudan Orange B, Sudan Red B, Sudan Red G, Sudan Red 7B, Para Red, Orange II, and Rhodamine B.

Extraction recoveries for tested matrixes spiked at levels from 400 to 1600 ppb ranged from 81% for Sudan I to 116% for Rhodamine B.

The reverse phase LC-MS/MS chromatographic run lasts 30 min and is divided into 6 different periods in order to emphasize MS sensitivity to the different analytes. Quantitation was performed setting the MRM mode on three daughter ions for each analyte, sometimes difficult choice due to some analytes being isomers, thus having same daughter ions. LODs were in the ppb fraction order for most molecules.

Analyses were then performed on different food samples and gave satisfactory recovery results, apart for completely fatty foods as vegetable oils, for which a more complex extraction method should be designed.

[1] RASFF Week 2009/10, 2009/11, 2009/14, 2009/15, 2009/17, 2009/18, 2009/21, and 2009/22.

Keywords: food dyes, QuEChERS, LC-MS/MS

J-46

CHARACTERIZATION OF WINES THROUGH CHROMATOGRAPHIC PROFILES OF FLUORESCENT POLYPHENOLIC COMPOUNDS USING CHEMOMETRICS FOR DATA ANALYSIS

Arantza Leon[1], Ana Sanfeliu[2], Ramón Oliver[3], Santiago Hernández-Cassou[4], Javier Saurina[5*]

[1 2 3] Polytechnic University of Catalonia, Barcelona, Spain
[4 5] University of Barcelona, Barcelona, Spain
[*] Corresponding author–E-mail: xavi.saurina@ub.edu; Phone: +34934039778; Fax: +34934021233

The characteristics and quality of wines depend on multiple parameters comprising climatic and geological factors of production regions, grape varieties and maturation, technological practices, etc. [1]. Apart form organoleptic tests by expert panelists, analytical methods are being increasingly applied to wine characterization. Several authors have proposed the use of polyphenolic compounds as potential deors of wine characteristics [2]. However, extracting feasible information from this type of data may result in a difficult task so that the application of exploratory chemometric tools may be required [3].

This communication describes a new method for wine characterization based on the analysis of the compositional profile of polyphenolic compounds obtained by HPLC with fluorescence detection. Analytes have been separated in a Synergy Hydro-RP C_{18} column using an elution gradient obtained from 9 mM H_3PO_4 aqueous solution and methanol as an organic modifier. Fluorescent detection is carried out at an excitation wavelength of 260 nm and an emission wavelength of 376 nm. The resulting chromatographic data have been advantageously exploited for extracting relevant information regarding wine features such as elaboration procedure, vintage or origin region.

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Keywords: wine, characterization, polyphenols, HPLC, fluorescence

J-47

CHARACTERIZATION OF WINES THROUGH THE COMPOSITIONAL PROFILES OF POLYPHENOLS AND BIOGENIC AMINES USING CHEMOMETRICS

Javier Saurina[1*]

[1] University of Barcelona, Barcelona, Spain
[*] Corresponding author–E-mail: xavi.saurina@ub.edu; Phone: +34934039778; Fax: +34934021233

Strategies for the characterization of wines relying on compositional profiles as a source of information are here discussed. Contents of low molecular organic acids, volatile species, polyphenols, amino acids, biogenic amines and inorganic species seem to be dependent on climatic, agricultural and winemaking issues. As a result, compositional profiles of these families of natural wine components can be exploited as potential deors of wine features as well as quality.

Many characterization studies are based on chemometrics as a way of facilitating the extraction of information about wine properties. For this purpose, cluster analysis, principal component analysis and related methods are currently used for classification, modeling and correlation purposes. The type of data handled in these studies is, in general, of multivariate nature. Discrete values of concentrations of selected substances, instrumental signals and other more complex combinations can be used as analytical data as follows:

(i) Concentrations of species: this option obviously requires a previous quantification task that may be complex and time-consuming. Besides, errors affecting to the quantification may lead to a misinterpretation of results.

(ii) Instrumental signals: complex instrumental data may result in characteristic fingerprints of the corresponding samples. Examples of instrumental responses utilized in wine characterization include chromatographic and electrophoretic profiles, and UV-Vis, near-infrared, fluorescence and mass spectra. Furthermore, the identification and full separation of all components occurring in the samples are not strictly necessary since the overall structure of instrumental profiles is dependent on the sample composition. This approach is gaining popularity for simplicity reasons due to those time-consuming steps devoted to separate and quantify analytes can be avoided.

Keywords: wine, characterization, polyphenols, chemometrics

J-48

TIPIPAPA: A NATIONAL PROJECT TO CHARACTERIZE AND TRACE EARLY POTATO PRODUCTION

Paola Adamo[1], Domenico Carputo[2*], Romana Bravi[3], Marilena Cipollaro[4], Nunziatina De Tommasi[5], Pasquale Lombardi[6], Bruno Parisi[7], Maria Antonietta Rao[8]

[1 2 8] Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples Federico II, Via Università 100, 80055 Portici, Napoli, Italy
[3] Ente Nazionale Sementi Elette, Via Ugo Bassi 8, Milano, Italy
[4] Dipartimento di Medicina Sperimentale, Seconda Università degli Studi di Napoli, Via De Crecchio 7, 80138 Napoli, Italy
[5] Dipartimento di Scienze Farmaceutiche, Via Ponte Don Melillo, 84084, Fisciano, Salerno, Italy
[6] Centro per la Formazione in Economia e Politica dello Sviluppo Rurale, Via Università 96, 80055 Portici, Napoli, Italy
[7] CRA–Centro di Ricerca per le Colture Industriali, Via di Corticella 133 Bologna, Italy
[*] Corresponding author–E-mail: paola.adamo@unina.it; Phone: +390812539172; Fax: +390812539186

TIPIPAPA (Tipicizzazione e caratterizzazione di varietà precoci di patata con l'impiego di tecniche molecolari e spettroscopiche) is a national research project funded by MiPAF. It was conceived with the idea of promoting research activities and developments related to the traceability, characterization and valorization of potatoes produced in southern Italy. A research main target is the genomic, proteomic, tranomic and metabolomic characterization of potato varieties normally grown off-season in southern Italy. In these areas early potatoes represent an essential element in the exportation of agricultural products. In addition, the mineral, multi-element and Sr-isotope ratio analyses on soil and potato samples are carried out to find soil-related indicators of potato's geographical origin. Potatoes are sampled in Campania, Apulia and Sicily regions each year, and lyophilized samples are distributed to the six research units for the analyses. Together with traceability and characterization, TIPIPAPA also includes the phytopathological screening of potato in the production areas, the definition of protocols ascertaining the presence of transgenes in tubers, and a program that evaluates economic aspects and costs-benefits related to the potato traceability pipeline. For more information www.pbglab.com.

Keywords: potatoes, molecular-fingerprinting, metabolites, multielement-analysis, cost-benefit-analysis

J-49

CLASSIFICATION OF ORGANIC CROP PRODUCTS BY MEASURING SECONDARY PLANT COMPOUNDS?

Johannes Kahl[1*], Marco Roose[2], Angelika Ploeger[3]

[1 2 3] University of Kassel, Witzenhausen, Germany
[*] Corresponding author–E-mail: kahl@uni-kassel.de; Phone: ++495542981715; Fax: ++495542981713

The concept of food quality can be defined in many different ways. Plants and food products contain different classes of secondary plant compounds like polyphenoles and carotinoides. The role of secondary plant compounds in relation to stress and resistance of plants is well known. Moreover these compounds may have a positive effect on human health and are discussed in organic research. Beside techniques with which the total sum of one class of compounds can be determined, HPLC-methods for the detection of single constituents have been developed and validated for each crop product and compound class. As a first step the methods are applied for the differentiation of products derived from different farming systems.

The content of the secondary plant compound classes polyphenols and carotenoids in plants is influenced by various environmental factors. Cultivation and fertilization are factors which are characteristic for the farming system organic or conventional. Within a German governmental funded project apples, carrot, maize and wheat samples from different farming systems are differentiated and classified using their polyphenolic and carotenoid profile. The farm pairs derived from geographically neighbouring locations. Each farm pair consisted of one farm producing according to organically land use (according to Council Regulation [EEC] No. 834/2007 of the European Union) and one farm producing conventionally. Factors of influence like plant cultivars and site were included. Moreover samples from defined field trials are measured. The coded samples were freeze dried and extracted with different solvents followed by HPLC and DAD-detection. For the classification multivariate statistical analysis is applied (Linear Discriminant Analyses; LDA).

The samples could be only partly classified by both, polyphenolic as well as carotenoid profiles. The sum parameters only can not be used for the differentiation and classification fo the samples. Factors like cultivar and site have a strong influence on the classification. Whereas the carotenoid profile is related to the development stage of e.g. the carrots (ripeness), the polyphenols are altered by the geographical site where the sample derived from.

Keywords: organic food, authenticity secondary compounds

J-50

DETERMINATION OF TRACE ELEMENTS IN INDIAN TEA SAMPLES USING INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY (ICP-AES) FOR GEOGRAPHICAL CLASSIFICATION

Rupali Lagad[1], Alamelu Devanathan[2], Kanchan Pande[3], Suresh Aggarwal[4*]

[1 2 4] FCD, BARC, Mumbai, India
[3] Earth SCiences, IIT, Mumbai, India
[*] Corresponding author–E-mail: skaggr2002@rediffmail.com; Phone: 91-22-25593740;

In view of the growing concern and awareness among the consumers regarding the quality of foodstuffs and herbal products, there is a need to know the regional identity in addition to purchasing the authentic product for the value paid. The identification of geographical origins of food materials is also of great importance due to rampant mislabeling done for getting a higher market price for the agricultural produce. Several researchers are working on trace elemental profiling with an objective to identify/classify the provenance of food samples using Chemometric Analysis techniques like Principal Component Analysis (PCA) and Discriminant analysis. Tea (Camellia Sinensis) is planted in various locations in India – such as Assam, Darjeeling (both in the north-eastern part of India), Nilgiris & Munnar (at the southern parts of India) and in Himachal Pradesh (North-western part of India). Since these locations are geographically distinct, there could be significant variations in the elemental profiling for these samples depending upon the geochemistry of the soil. Data on elemental profiling of tea samples available commercially as well as of some samples directly from the tea growing regions were obtained experimentally. A total of 45 tea samples were collected from market and different tea plantations in India. Accurately weighed amounts of tea samples were digested in duplicate in SuprapureTM (Merck) concentrated HNO_3 in Class-100 grade clean lab. The digested samples were analyzed using ULTIMA – IITM (M/s. Jobin Yvon, France) Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP-AES) instrument having a resolution of 0.005 nm. Calibration curves in the range of 0.1 mg/g to 10 mg/g were developed for 18 various elements using certified multielemental standard (Merck). The samples were analyzed after suitable dilution so as to obtain the concentrations within the range of the calibration curves. Average elemental concentrations of tea samples by ICP-AES are shown in Table1 along with spectral lines used for elemental profiling. Here principal component analysis was used to classify tea samples according to their origin in India. Assam samples appear to be separable from Munnar as well as Darjeeling samples. Sample from North Indian region also showed its distinct signatures compared to samples from other regions. The results obtained in the present work, the statistical analyses carried out and the data reported in literature would be discussed during this presentation. Table1. Results obtained on Indian Tea Leaves Samples by ICP-AES analysis (Average values) Sr. No. Elements Wavelength(nm) Concentration (n=6) Mean ± S.D (µg/g) 1 Al 309.2 801.4 ± 38.9 2 Ba 455.4 36 ± 3.2 3 Bi 223.0 N.D 4 Ca 317.9 4629.5 ± 205.0 5 Cd 228.8 N.D. 6 Co 228.6 N.D. 7 Cr 283.5 8.9 ± 0.7 8 Cu 324.75 14.6 ± 0.8 9 Fe 259.9 189.3 ± 16.2 10 Ga 294.3 N.D. 11 K 766.4 19844.3 ± 611.5 12 Mg 279.5 2014.5 ± 79.6 13 Mn 257.6 625.4 ± 18.3 14 Na 589.0 80.6 ± 11.4 15 Ni 221.6 16.6 ±2.7 16 Pb 220.3 N.D. 17 Sr 407.7 15.6 ± 1.4 18 Zn 213.9 28.9 ± 2.9

Keywords: Tea leaves, Trace elements, ICPAES

Allergens

(K-1 – K-16)

K-1
MONITORING OF DAILY GLIADIN INTAKE IN PATIENTS ON GLUTEN-FREE DIET

Dana Gabrovská[1*], Petr Kocna[2], Jana Rysová[3], Dana Borovská[4], Helena Tlaskalová-Hogenová[5]

[1 3] Food Research Institute Prague, Czech Republic
[2] Institute of Clinical Biochemistry and Laboratory Diagnostics, General Faculty Hospital and 1st Medical Faculty, Charles University, Prague, Czech Republic
[4 5] Department of Immunology and Gnotobiology, Institute of Microbiology, v.v.i, Czech Academy of Sciences, Prague, Czech Republic
[*] Corresponding author–E-mail: d.gabrovska@vupp.cz; Phone: +420 737 287 004; Fax: +420 272 701 983

The aim of the study was to monitor the adherence to gluten-free diet of patients suffering from celiac disease within the context of their routine everyday diet regimen and to quantify their daily intake of gliadin on the basis of gliadin determination in their current daily food.

The gluten-free diet was followed for 30 days. The patients for the monitoring were both the adults and children and they were chosen by the cooperation with coeliac society. Prior to the monitoring the patients were given PE bottles and grip-seal PE bags for sampling their food, beverages, and possibly also the medicines and food supplements ingested each day. Individual components of the daily menu, the producers or distributors of respective commercial foods (not strictly required in samples not suspected to contain gluten), and weight or volume of all consumed components of the menu were recorded using a standardized form. The content of gliadin was determined by the sandwich ELISA method. The daily gliadin intake was calculated on the base of the reported amount of meals ingested. Changes in the level of serum antibodies against transglutaminase and gliadin were monitored during the study as well.

1900 food samples were analyzed within the work of this study. Several contaminated commercial foods were found, nevertheless this fact did not influence the otherwise satisfactory overall picture of the daily gliadin intake by the patients followed. The results in 14 patients revealed a satisfactory adherence to the gluten-free diet. Daily gliadin intake was in the range 0.8 – 5.5 mg per day. These values falls below the lower limit of the theoretical tolerated dose, which is supposed to range between 5 and 15 mg gliadin per day.

It was proved that conscientiousness and enough awareness of celiac patients, or persons taking care of these, is of paramount importance for the choice of foods comprising the gluten-free diet.

Keywords: gluten-free diet, allergens, gliadin determination

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K-2

SENSITIVE ALLERGEN TESTING IN RED AND WHITE WINE USING RIDASCREEN® FAST CASEIN AND RIDASCREEN® FAST EI/EGG ELISA

Sigrid Haas-Lauterbach[1], Ulrike Immer[2], Markus Lacorn[3], Tina Katzenmeier[4], Christian Gößwein[5*]

[1 2 3 4 5] R-Biopharm AG, Darmstadt, Germany
[*] Corresponding author–E-mail: c.goesswein@r-biopharm.de; Phone: +49 6151 81 02 578

Traditionally, proteins from milk and egg have been used for selective tannin adsorption in wine production. In recent years, reasonable suspicion has emerged that residues of these proteins can remain in the wine and this could exert an allergic reaction in hypersensitized consumers. According to directive 2007/68/EC, all wines labelled after May 31st 2009 must declare if allergens including egg and milk proteins were used during production. Therefore, methods for the detection of proteins from egg and milk are needed. The detection limit should be as low as possible since no limit is given in the directive.

For sample preparation, the wine is diluted in extraction buffer, heated for 10 min at 60°C and centrifuged. The monoclonal antibodies used in the sandwich-ELISA RIDASCREEN®FAST Casein allow the specific detection of all caseins from cow`s milk, both in the native form and denatured e.g. by boiling. The measurement of several red and white wines revealed no matrix interferences and resulted in a limit of detection of 0.1 mg/L. Using a wine spiked at a level of 3.5 mg/L the intra-assay and inter-assay coefficients of variation were 4.7% and 7.5%, respectively. Spiking experiments with casein, skimmed milk powder and heat-treated milk revealed recoveries between 83% and 115%.

The polyclonal antibodies used in the sandwich-ELISA RIDASCREEN®FAST Ei/Egg detect the egg white proteins ovalbumin and ovomucoid in their natural concentration ratio, in the native and denatured form. There were no matrix effects and the detection limit was 0.08 mg egg white protein/L. For calibration, a reference material (NIST, RM 8445) was used. Spiking at a level of 2 mg/L gave an inter-assay coefficient of variation of 9.2%. Recoveries of an egg white extract in red and white wine were between 90% and 120%.

Both ELISA are therefore suitable for the simple and reliable detection of the allergens casein and egg white proteins in wine at the very low ppm-level.

Keywords: wine, allergen, ELISA, casein, egg

K-3
STANDARDIZATION IN ALLERGEN DETERMINATION

Sigrid Haas-Lauterbach[1], Ulrike Immer[2], Tina Katzenmeier[3], Christian Goesswein[4], Markus Lacorn[5], Stephanie Bader[6], Ute Schweiggert[7*]

[1 2 3 4 5] R-Biopharm AG, Darmstadt,Germany
[6 7] Fraunhofer Institute, Freising, Germany
[*] Corresponding author–E-mail: ute.schweiggert@ivv.fraunhofer.de; Phone: +49 8161 491431

Within the last decade, food allergies were recognized as a genuine risk for consumers, because already small traces of an allergen at the ppm level could exert an allergenic reaction. Since these low concentrations are often the result of a contamination during food processing, they are not apparent to consumers. As a consequence, legislative authorities ordered producers to declare possible allergens without giving a threshold level, since it is difficult to set a minimum dose where no allergic individual will react. This let to the development of methods to test for the content of an allergen in food. Besides chromatographic and DNA-based methods, antibody-based ELISA systems are routinely used and seem to be the most robust assays. As all quantitative analytical systems, an ELISA is characterized by a number of validated parameters like specificity, precision, recovery and sensitivity. In difference to chemical substances, food allergens are in most cases a mixture of proteins, which can vary in amount and composition of proteins. The amino acid sequence and tertiary structure of the proteins can differ dependent on e.g. regional origin, growing, harvesting, processing and storage. For practical reasons, the antibodies used in an ELISA are not raised against all possible variants of an allergen, so that various antibodies used in test kits of different producers give varying immune responses. In consequence, standardization plays a decisive role if results should be comparable. As a first step to standardize an allergen ELISA we would like to propose a standard for lupine determination. In the last few years, lupine is increasingly used in food products as e.g. an emulsifying substitute for egg and milk proteins. Seeds of *Lupinus angustifolius* L. were flaked, hexane de-oiled and pre-extracted under acidic conditions. Proteins were extracted under alkaline conditions, precipitated and spray-dried after neutralization on pilot-plant scale. The product contains more than 95% protein, less than 1% oil and can be applied for example in salad dressings, muffins and ice-cream. This lupine protein isolate could be delivered in kg-amounts and should be used by test kits suppliers as a standard preparation and by users to undertake own spiking experiments.

Keywords: Standardization; ELISA; Lupine, Allergen Determination

K-4
ELISA KIT FOR MUSTARD PROTEIN DETERMINATION – COLLABORATIVE STUDY

Dana Gabrovská[1*], Jana Rysová[2], Ingrid Malmheden Yman[3], František Štumr[4], Jan Plicka[5], Stefania Lametti[6], Jorge Martínez[7], Kristiina Takkinen[8], Martin Kubík[9], Miguel Angel Pardo[10]

[1 2] Food Research Institute Prague, Czech Republic
[3] National Food Administration, Uppsala, Sweden
[4] SEDIUM RD, Ltd., Pardubice, Czech Republic
[5] Immunotech a Beckman Coulter Company, Prague, Czech Republic
[6] Universitâ degli studi di Milano, DISMA, Milano, Italy
[7] University of the Basque Country, Faculty of Pharmacy, Vitoria-Gasteiz, Basque Country, Spain
[8] VTT Technical Research Centre of Finland, Espoo, Finland
[9] Czech Agriculture and Food Inspection Authority, Prague, Czech Republic
[10] AZTI Tecnalia, Technological Park of Biskay, Derio, Basque Country, Spain
[*] Corresponding author–E-mail: d.gabrovska@vupp.cz; Phone: 00420 737 287 004; Fax: 00420 272 701 983

A collaborative study in 11 laboratories was performed to prove the validation of ELISA method developed for quantitative mustard proteins determination in foods. The ELISA kit used for this study is based on rabbit polyclonal antibody. This kit does not produce any false positive results or cross-reactivity with broad range of food matrix with zero content of mustard proteins. All participants obtained Mustard ELISA kit with standard operational procedure, the list of the samples, samples and a protocol for test results recording.

The study included 15 food samples and 2 spiked samples.

Seven samples of food matrix with zero content of mustard and four samples with declared mustard as an ingredient showed mustard proteins content lower than the first standard (mustard proteins content 0.42 mg/kg). Four samples with declared mustard as an ingredient revealed mustard protein content higher than the highest standard (12.5 mg mustard protein/100 g). The statistical tests (Cochran, Dixon and Mandel) and analysis of variance (ANOVA) were used for the evaluation of collaborative study results. Repeatability and reproducibility limits as well as limit of quantification (LOQ, 0.15 mg mustard proteins/kg) and limit of detection (LOD, 0.06 mg mustard proteins/kg) for the kit were calculated.

Keywords: mustard proteins, ELISA

The work was supported by projects of Ministry of Agriculture of the Czech Republic, Research Plan VZ 00027022 and SAFEFOODERA, 08125 Detection of Traces of Allergens in Foods

K-5

DEVELOPMENT OF A NEW ANALYTICAL METHOD FOR THE DETERMINATION OF SULPHITES IN FRESH MEATS AND SHRIMPS BY ION EXCHANGE CHROMATOGRAPHY WITH CONDUCTIVITY DETECTION

Diego Centonze[1*], Marco Iammarino[2], Aurelia Di Taranto[3], Donatella Nardiello[4], Carmen Palermo[5]

[1 4 5] Dipartimento di Scienze Agro-Ambientali, Chimica e Difesa Vegetale and BIOAGROMED, Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy
[2 3] Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Via Manfredonia 20, 71100 Foggia, Italy
[*] Corresponding author–E-mail: centonze@unifg.it; Phone: +390881589104

Sulphites have been widely used for many years in a large variety of foodstuffs and beverages (fish, potatoes, wine, etc.) as preservatives and blanching agents, to prevent oxidation and bacterial growth. Nevertheless, since the ingestion of foods containing a high amount of sulphites was associated with asthmatic reactions and food intolerance symptoms, the occurrence of sulphite in foods can be considered dangerous to human health, and the presence should be strictly limited (Directives 1995/2/EC and 2006/52/EC). Hence, the sulphites determination in food products is essential for legislation purposes, nutrition and public health, and the development of sensitive, selective, fast and low-cost methods represents an important feature for food safety and quality control, in agreement with European legislation. In this work, an accurate and reliable analytical method, based on ion chromatography with suppressed conductivity detection, is described for the quantitative determination of sulphites in fresh meats and shrimps. The chromatographic separation was accomplished by using an anion exchange column eluted with an optimized step-change gradient, based on sodium carbonate and sodium hydroxide, which has guaranteed a very good selectivity towards endogenous interfering substances and an excellent retention time repeatability (1.1%, n = 6). The method validation, performed by an in-house model, according to Decision 657/2002/EC and Regulation 882/2004/EC, provided excellent results with respect to linearity (r = 0.9998), limits of detection and quantification (2.7 mg/kg and 8.2 mg/kg, respectively, expressed as SO_2), expanded measurement uncertainty (below 10%), recovery values (ranging from 85–92%) and repeatability (CV% below 8%), demonstrating the conformity of the proposed method with the European directives.

Keywords: sulphites, ion-chromatography, validation, meats, shrimps

K-6
PURIFICATION AND CHARACTERISATION OF POTENTIAL ALLERGENIC HAZELNUT PROTEINS

Parisa Ansari[1*], Judith Rudolf[2], Justyna Rechthaler[3], Christoph Hasenhindl[4], Sabine Baumgartner[5]

[1 2 4 5] University of Natural Resources and Applied Life Sciences, Vienna, Austria
[3] University of Applied Sciences Wiener Neustadt, Tulln, Austria
[*] Corresponding author–E-mail: parisa.ansari@boku.ac.at; Phone: + 43-2272-66280-456; Fax: + 43-2272-66280-403

In the past decade, research on cumulative risks has raised awareness for the control of health risks. Food consumers demand more rigorous food safety testing since finished food products may contain a wide range of contaminants from both natural and anthropogenic sources. Allergenic proteins are an example for naturally occurring contaminants which have to be avoided by the allergic consumer. With the absence of clear threshold levels and the risk associated with cross-contamination, a major focus of allergenic food contaminant analysis is the detection and characterisation of food allergens listed in Annex IIIa of the EC-Directive 2007/86/EC in different foodstuffs. In the case of non-commercially available proteins such as hazelnut proteins, the purification and characterisation of allergenic proteins is important. For this purpose, innovative sample extraction techniques were introduced. Furthermore, existing strategies and methods for efficient extraction of target allergenic proteins from foodstuffs such as using cooled acetone to defat the nuts were addressed. Also, isolation methods were further developed for allergen characterisation and identification both in the raw and refined state.

The major food allergens present in hazelnut (HN) include a luminal binding protein (Cor a 10), 11S (Cor a 9) and 7S (Cor a 11) seed storage globulins which have a molecular weight between 45-70 kDa. In the presented study the hazelnut proteins in this range were searched and purified. For extraction of hazelnut, the nuts were ground and defatted. After vacuum-filtration, the remaining sediment was dried overnight in a fume-hood. Extraction of the proteins was done with ammonium bicarbonate buffer. Due to the mild isolation conditions, denaturation of proteins during extraction was minimised. Furthermore, the extracts were dialysed to remove excessive salts for further electrophoresis.

Different chromatographic methods such as size exclusion, ion exchange, (immuno)affinity and reversed phase chromatography were used for the purification of crude HN extracts. To obtain the best results, the combination of these methods was tested.

All of the fractions were subjected to SDS-PAGE leading to the separation of the proteins according to their molecular size. The densitometric quantification of protein bands and the protein concentration assay with BCA allowed a fast control of the efficiency and the profile of extracted protein content. Furthermore the immunological studies of western blot have been used to confirm the immunoreactivity of the isolated HN proteins. Finally, the structural characterisations and molecular weight determination of the purified fractions were performed via Mass Spectrometry (HPLC-ESI/TOF/MS).

Keywords: Hazelnut, allergen, Purification, Characterisation, LC-ESI-TOF/MS

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K-7
DETECTION OF LUPINE (LUPINUS SPP.) DNA IN FOOD BY REAL-TIME PCR

Anja Demmel[1*], Christine Hupfer[2], Ulrich Busch[3], Karl-Heinz Engel[4]

[1] Chair of General Food Technology, Technische Universität München, Freising, Germany/Bavarian Health and Food Safety Authority, Oberschleissheim, Germany
[2 3] Bavarian Health and Food Safety Authority, Oberschleissheim, Germany
[4] Chair of General Food Technology, Technische Universität München, Freising, Germany
[*] Corresponding author–E-mail: anja.demmel@lgl.bayern.de; Phone: +498931560250

Lupine-based ingredients are increasingly being used in human nutrition. They can be employed as substitutes for soy in products that are to be produced without the use of genetically modified organisms and they show additional beneficial technological and nutritional properties. However, lupines can elicit allergic reactions in sensitive individuals. The Directive 2000/13/EC [1] lists allergenic ingredients that have to be declared on the label regardless of the amount of allergenic ingredient in the respective food product. Lupine was included in Annex IIIa of the Directive 2000/13/EC by directive 2006/142/EC [2]. Therefore, methods for the supervision of the compliance with labelling directives and for the protection of allergic consumers are needed. Due to cross-contact in the production chain not only products containing lupine as an ingredient, but also initially lupine-free foods might contain amounts of lupine that are relevant to food allergic consumers. For this reason, analytical methods applied in the field of food allergen testing should be capable of detecting low amounts of the ingredient under consideration. Currently there is no threshold specifying the amount of allergen below which labelling is not mandatory.

Methods for the detection of allergens in food include DNA-based systems als well as protein-based systems. Enzyme-linked immunosorbent assays (ELISAs) show lower dynamic ranges than polymerase chain reaction (PCR) methods and frequently cross-react with taxonomically related species. Therefore, a real-time PCR method for the detection of lupine DNA in foods has been developed, with particular emphasis on the parameters specificity and sensitivity [3]. The validation of the method included the determination of the limit of detection in raw foods as well as the examination of the influence of food processing and of various matrices on the detectability of lupine DNA. Additionally, an international ring trial was carried out. Currently, approaches to the quantification of lupine in foods using real-time PCR are being made.

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Keywords: food allergen, PCR, lupine

K-8
DETECTION OF TOXIC FRAGMENTS FROM GLUTEN USING A NEW MONOCLONAL ANTIBODY-BASED TEST

Elisabeth Halbmayr[1*], Michael Zheng[2], Donna Houchins[3]

[1] Romer Labs Division Holding GmbH, Tulln, Austria
[2] Romer Labs Singapore Pte., Singapore
[3] Romer Labs Inc., Union, Missouri, USA
[*] Corresponding author–E-mail: elisabeth.halbmayr@romerlabs.com; Phone: 00432272615330

Celiac disease (CD) is an immune-mediated enteropathy caused by the ingestion of gluten, a protein fraction found in certain cereals. Immunotoxic gluten peptides that are resistant to degradation of digestive enzymes appear to trigger celiac syndrome.

Celiac disease occurs in genetically predisposed persons and leads to the destruction of the microscopic finger-like projections of the small intestine, called villi. The disease is triggered by the ingestion of peptides from wheat, barley, rye, and in some cases oats. It currently affects roughly 1% of the world´s population, primarily adults.

This work is showing the results of a new monoclonal antibody that specifically recognises the pathogenic fragment of the gliadin protein present in gluten. This fragment is called 33-mer and triggers the auto-immune reaction in celiac patients. Homologues of this peptide were found in every food grain (except oats) that is toxic to CD patients, but were absent in all nontoxic food grains. The antibody was specially developed to determine the toxic fractions present in gluten. When food and drinks are hydrolysed or heat processed, the gliadin protein is degraded in small fragments which are difficult to detect with classic antibodies because their epitopes may be destroyed. The newly developed monoclonal antibody can reliably detect toxic fragments of gluten in hydrolysed food. The outstanding advantage of this new antibody is the possibility to detect the actual toxic fragment of gluten with a very high sensitivity.

Due to current Codex Alimentarius recommendations and Commission Regulation (EC) No 41/2009, food can be labelled gluten free when containing less than 20 mg/kg gluten.

A lateral flow test kit using this monoclonal antibody was developed to detect the toxic fractions of gluten from wheat and other cereals such as barley, rye and, with a much lower level of sensitivity, oats. The detection at a lower level of sensitivity in oats correlates with the lower degree of toxicity of oats to CD patients.

The semi-quantitative immunochromatographic strip test is based on sandwich format. The reagents for the test and control line are immobilized on a nitrocellulose membrane. Toxic gluten fragments in the sample extract react with anti-gliadin 33-mer monoclonal antibody, coupled to coloured microspheres, which is dried on the strip showing a visible line when binding to the anti-gliadin 33-mer monoclonal antibodies on the test line. The mix of conjugate moves through the membrane to the control line where anti-species specific antibodies used for verifying the correct test performance are sprayed.

Keywords: gluten, strip test, monoclonal antibody

K-9
EXAMINATIONS OF THE MAIN ALLERGENIC PROTEIN-CODING GENES IN SOME DOMESTIC APPLE VARIETIES

Erika E. Szabó[1*], Éva Gelencsér[2], Anna Jánosi[3], Erzsébet Kiss[4]

[1 2 3] Central Food Research Institute, Budapest, Hungary
[4] Gödöllő, Hungary
[*] Corresponding author–E-mail: ee.szabo@cfri.hu; Phone: 00-36-1-355-8244; Fax: 00-36-1-225-3342

The apple is one of the most important fruit grown and consumed all over the world. The fresh apple is recommended as the part of a healthy diet; at the same time it poses a risk to certain percent of the population suffering from allergic reaction. Apple allergy is common in Europe and affects about 2% of the population. The syntheses of allergenic proteins in apple are affected by genetically (variety) and growing factors, maturity and postharvest processing.

In apple four main classes of allergens (Mal d 1, Mal d 2, Mal d 3 and Mal d 4) have been identified by protein (ELISA, gel electrophoresis) and DNA (PCR) techniques. The allergenic reactions caused by above-mentioned allergens are different, and the most severe effect is evoked by Mal d 3.

In our research we studied the occurrence of the main apple allergen in 16 different and most preferably consumed apple varieties. After the DNA isolation by Wizard method the simple PCR reaction was used to examine the apple allergens. To determine the presence of the four allergenic protein-coding genes two primer pairs were chosen in each case. The presences of these allergens are confirmed in most apple varieties. According to our results two varieties–Jonathan and Granny Smith–were founded to contain the least amount of the coding genes of the apple allergenic proteins studied by us. Besides this, polymorph pattern were given by use of Mal d 1 primer, which may be able to use to determine apple varieties with small amount of Mal d 1 allergens.

The study of potential apple allergens by RNA and protein techniques is our plan in near future. In addition we would like to determine the association between the result of DNA measurement and the allergenic activity of apple sample.

Keywords: apple, allergens, DNA isolation, PCR

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K-10
PERSISTENCE OF PEANUT PROTEIN ON HANDS AND CLOTHING

Kirstin Gray[1*], Michael Walker[2], Indu Patel[3], Malvinder Singh[4], Peter Colwell[5]

[1 2 3 4 5] LGC, Teddington, England
[*] Corresponding author–E-mail: kirstin.gray@lgc.co.uk; Phone: +44 (0)20 8943 7309

Deliberate sabotage of food is dealt with by management with or without the involvement of police but generally with minimum media coverage. Objects used range from the innocuous (paper) to the highly offensive (contraceptives) and have included glass, needles, blades, staples and mercury.

Following a request from police the LGC Food Chemistry team tested a number of exhibits for evidence of peanut protein. The exhibits had been seized by police investigating the alleged contamination of a nut-free food factory. The case generated much publicity and was the first time, so far as we are aware, that a major food allergen was involved in deliberate sabotage. Peanut can and does cause fatal anaphylaxis when inadvertently eaten by a peanut allergic consumer. About 10 fatalities occur in the UK each year, with many more near misses, caused by the major allergens. Exhibits were examined and tested by Enzyme Linked Immuno-Sorbent Assay (ELISA) in a dedicated restricted access laboratory suite. Specific procedures were employed to prevent laboratory cross contamination e.g.: restricted swipe card access; step-over barrier; separate (colour coded) lab coats, maximum use of disposable equipment and care re analyst's own diet. Based on our findings (high concentrations of peanut protein in all the pockets of two garments belonging to the defendant) a criminal trial commenced in January 2009.

At the request of the defence, further tests were conducted on the potential for trace contact transfer of peanut protein. Results indicated that after brief contact with a peanut, peanut protein was readily detected and transferred to clothing. This persisted with casual hand washing but was attenuated when rigorous hand washing procedures were followed. In summary, handling a peanut for 10 seconds transferred sufficient peanut protein to the fingers for it to be picked up from fabric even after 10 successive finger/fabric contacts. Casual washing did not remove enough peanut protein to prevent subsequent transfer; however thorough hand washing to a food handling protocol did so (although peanut protein was still detectable on the fingers by direct swabbing).

LGC findings in relation to this case broke new ground in relation to the persistence of peanut protein on fingers after handling peanuts, even after hand washing. The work is of importance not only in forensic situations but also to food factories and potentially in relation to sensitisation by dermal contact.

Keywords: Peanut, Allergen, ELISA, Nut-free, Contamination

K-11

DETECTION OF PEANUT, MILK AND GLUTEN ALLERGENS BY LC-MS/MS: TOWARDS A MULTI-ALLERGEN ASSAY FOR MAJOR ALLERGENS IN FOOD

Stephen Lock[1*], Cathy Lane[2], Bert Popping[3], Donna Potts[4], Phil Jackson[5]

[1 2 4 5] Applied Biosystems, Warrington, UK
[3] Eurofins, Pocklington, UK
[*] Corresponding author–E-mail: stephen.lock@lifetech.com; Phone: +447720276948

Introduction:

Food allergy is a significant health issue in western countries. The prevalence of food allergies in the United States is estimated at 6% for children and 3.7% for adults and therefore there is a need for specific and sensitive methods to detect allergens at trace levels. Screening for allergens in food is traditionally performed using enzyme-linked immunosorbent assays. Qualitative and quantitative results generate regularly variable results and false-positive as well as false-negative results occur, constituting a severe limitation of this technique. Here we present a rapid LC-MS/MS method for detection of peanut, milk, egg and gluten allergens. The method utilizes scheduled multiple reaction monitoring (sMRM) and can easily be extended to incorporate detection of additional food allergens.

Preliminary data:

The first step in developing a LC-MS/MS method for allergen detection is the selection of the peptides to be monitored for each allergen protein(s). The peptides must be unique to the allergens of interest and must ionize efficiently and chromatograph in a stable and reproducible manner. Peptides were selected based on two approaches: a non-targeted approach, in which full scan linear ion trap mass spectra are used to trigger full scan MS/MS spectra on the most intense ions eluting from the LC (information dependent acquisition, IDA); and an approach in which target protein amino acid sequences are used to construct a set of MRM transitions. These MRMs are used as a 'survey' scan: a signal registered in an MRM channel triggers the acquisition of a full scan MS/MS spectrum on the parent peptide (MRM-initiated detection and sequencing, MIDAS). After selection of target peptides, full scan MS/MS spectra were used to choose the most suitable MRM fragment ions. Five to 7 peptides were chosen per allergen, with 2 to 4 MRM transitions per peptide.

A method was constructed using an l/min. Separation was achieved with an 11 min gradient.μHPLC flow rate of 300 Highly reproducible retention times allowed the use of sMRM, enabling multiplexing of MRM transitions. Detection levels of 0.2 ppm were observed, with excellent linearity over 3 orders of magnitude.

Keywords: Allergens, Peanut, LC-MS/MS

K-12

RAPID SIMULTANEOUS DETECTION AND QUANTIFICATION OF ALLERGENIC PROTEINS WITH AND WITHOUT POSTTRANSLATIONAL MODIFICATION IN DIETETIC BABY FOOD BY USING HIGH SENSITIVE TANDEM LCMSMS

Detlev Schleuder [1], Tomas Korba [2], Marco Euler Euler[3], Jianru Stahl-Zeng[4*], Marko Mank[5], Gilda Georgi[6], Bernd Stahl[7], Guenther Raffler[8]

[1 4] Applied Biosystems, Damstadt, UK
[2] Applied Biosystems, Warrington, UK
[3 5 6 7] Danone Research Centre for Specialised Nutrition, Friedrichsdorf, Germany
[8] GmbH, Friedrichsdorf, Germany
[*] Corresponding author–E-mail: stephen.lock@lifetech.com; Phone: +447720276948

Introduction: Reliable and exact methods for the detection of trace quantities of food allergens, usually proteins with or without post translational modification (PTM) in food matrices are needed. The standard method in food industry is immunological detection with specific antibodies (e.g. ELISA) for quantification of food allergen protein. Despite advantages these methods have some limitation: for example, they are strongly dependent on the type of antibody chosen, and each test is only suitable for a given protein. Detection of PTMs of proteins is also not commonly used. Mass spectrometry provides many possibilities in proteomics and LCMSMS in combination with advanced sample preparation methods is a powerful tool for rapid, reliable detection of both modified and unmodified food allergen protein.

Preliminary results: LCMSMS Linear Ion Trap technology provides the possibility to do Multiple Reaction Monitoring (MRM) scanning which is a sensitive and reliable method for quantifying small molecules and proteins in various matrices. 4000 Qtrap® Linear Ion trap instrument (ABI) and new 5500 Qtrap® (ABI) in combination with a RP chromatography can be used here for analyzing dietetic food allergen protein, e.g. gluten and oval albumin. LCMSMS together with modern quantification isotopic tags, such as iTRAQ™ was used to analyze and quantify non-enzymatic glycation of cow milk protein, e.g. beta-lactoglubulin.

Our data shows that simultaneous detection and quantification of food allergen protein, e.g. gluten from dietetic foods is possible. The Limit of Quantification (LOQ) of the MRM method is at 0.1 ppm level, ten times better than what can be observed by ELISA with 8% CV or better observed. At least 4 unique peptides monitored by at least two MRM transitions for each allergen were selected in an Information dependent Acquisition (IDA) method where extra MS/MS spectra were collected for simultaneous building a library for further confirmation and identification proteins.

Monitoring of unique peptides for each allergen did not show different results with different levels of PTM. On another hand, in terms of modified allergen protein, our data show that non-enzymatic posttranslational glycosylation of complex protein mixtures can be analyzed by using iTRAQ™ labeling. As an example the glycation of beta-lactoglubulin are quantitatively determined. The degree of glycation is based on the individual sequence of the respective proteins as well as on the processing parameters like temperature, pH-value and duration. The different amounts of glycation in connection with different physico-chemical processing conditions was determined by using iTRAQ™ labeling and LC-MS analysis.

Keywords: allergens, PTM, LC-MS/MS

K-13
A REAL-TIME PCR METHOD FOR THE DETECTION OF WHITE MUSTARD (SINAPIS ALBA)

Magdalena Fuchs[1*], Margit Cichna-Markl[2], Rupert Hochegger[3], Hermann Hoertner[4]

[1 3 4] Austrian Agency for Health and Food Safety, Vienna, Austria
[2] Department of Analytical and Food Chemistry, University of Vienna, Vienna, Austria
[*] Corresponding author–E-mail: magdalena.fuchs@ages.at; Phone: +43 50 555 32203

Food allergies pose a rising health problem. Allergenic food can cause mild symptoms affecting the skin, the gastrointestinal tract and the respiratory system, but it can also lead to life-threatening reactions like anaphylaxis. The only way for allergic persons to handle their allergy is the strict avoidance of the allergenic food. To facilitate the information concerning allergenic ingredients in food, 14 food allergens have to be declared in the European Union according to the Directive 2007/68/EC. Among those 14 food allergens is found mustard and products thereof.

For controlling the implementation of the regulation, sensitive analytical methods are necessary. There are two analytical ways to verify the right labelling of food allergens. The first one is detecting the allergenic protein by using an ELISA, the second one is detecting the allergenic species by amplifying DNA using PCR or real-time PCR. For the detection of mustard (*Sinapis alba*, *Brassica juncea, Brassica nigra*, all belonging to the plant familiy *Brassicaceae*) several ELISAs [1, 2], but only one real-time PCR method [3] have already been published. Due to cross-reactions with all *Brassica* species and radish, the real-time PCR method published so far is not able to specifically detect mustard.

This paper presents a real-time PCR method for the specific detection of white mustard. The gene coding for *Sinapis alba* MADS D was selected and found to be specific for white mustard among more than 60 different investigated food matrices. The limit of detection, PCR efficiency and reproducibility were determined in serially diluted mustard DNA extracts and spiked food samples.

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Keywords: Allergen detection, Mustard, Real-time PCR

K-14
ELISA KIT FOR DETERMINATION OF EGG WHITE PROTEINS – COLLABORATIVE STUDY

Petr Cuhra[1*], Petr Hanák[2], Květa Tomková[3], Pavla Dvorská[4], Hana Bulawová[5], Jorge Guisantes[6], Idoia Postigo[7], Marja-Leena Laukkanen[8], Ana Baranda [9], Iñigo Martínez de Marañon[10]

[1] Czech Agriculture and Food Inspection Authority, Prague, Czech Republic
[2] Food Research Institute, Prague, Czech Republic
[3 4] SEDIUM RD, Ltd., Pardubice, Czech Republic
[5] State Veterinary Institute, Jihlava, Czech Republic
[6 7] University of the Basque Country, Vitoria-Gasteiz, Spain
[8] VTT Technical Research Centre of Finland, Espoo, Finland
[9 10] AZTI Tecnalia–Technological Park of Biskay, Derio, Spain
[*] Corresponding author–E-mail: petr.cuhra@szpi.gov.cz; Phone: 257199540; Fax: 257199541

A collaborative study in 11 laboratories was performed to prove the validation of ELISA method developed for quantitative egg white proteins (EWP) determination in foods. The ELISA kit used for this study is based on sheep polyclonal antibody. The kit does not produce any false positive results or cross-reactivity with broad range of food matrix with zero content of egg white proteins. All participants obtained Egg ELISA native kit with the SOP, samples and results form.

The study included 10 food samples (rice, mixture for yoghurt cake, farmer soup, egg pasta, 2 samples of red wine, a mixture for potato dumplings, a mixture for bread roll dumplings, a mixture for pancake, a pizza mixture and 6 flour mixtures).

Four samples of blank food matrices showed EWP content lower than the first standard (EWP concentration 0.5 mg/kg). One sample with declaration of zero content of EWP revealed EWP content higher than standard 3 (15 mg/kg) caused by contamination. Five samples containing EWP as an ingredient in composition list were tested as positive. A sample with declaration of EWP content which was determined as negative will be discussed. The statistical tests (Cochran, Dixon and Mandel) and analysis of variance (ANOVA) were used for the evaluation of collaborative study results. Repeatability and reproducibility limits as well as limit of quantification (LOQ, 0.5 mg/kg) and limit of detection (LOD, 0.2 mg /kg) for the kit were calculated.

Keywords: egg-white proteins, allergy, ELISA

The work was supported by projects of Ministry of Agriculture of the Czech Republic, Research Plan VZ 00027022 and SAFEFOODERA, 08125 Detection of Traces of Allergens in Foods

K-15

DETERMINATION OF ALLERGENIC PROTEINS IN WHEAT FLOUR BASED FOOD MODEL SYSTEMS–A SCIENTIFIC COOPERATION WITHIN MONIQA NETWORK OF EXCELLENCE

Zsuzsanna Bugyi[1*], Sándor Tömösközi[2], Judit Nagy[3], Kitti Török[4], Lívia Hajas[5]

[1 2 3 4 5] Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science, Budapest, Hungary
[*] Corresponding author–E-mail: bugyi@mail.bme.hu; Phone: +36-1/463-1419; Fax: +36-1/463-3855

Food allergy and food intolerance are abnormal reactions to certain food proteins in human body. The statistical data shows that within the adult population the prevalence of food allergies is 3-4%, in case of children prevalence is 4-8%. The evident treatment of food allergy is the total avoidance of the allergenic proteins. In the EU, for ensuring the safety of allergic people, 14 allergenic food components must be labelled.

The regulation can't be effective without appropriate monitoring and/or measuring tools. Therefore the proper quantification of allergens is necessary which requires reliable and validated analytical methods. The validation of analytical methods for allergen determination is a hot topic of the activity of the EU funded Network of Excellence, MoniQA (Monitoring and Quality Assurance in the Food Supply Chain). During the investigation of the present situation on this area some critical points were identified by the Allergen Working Group of this NoE. For instance the lack of confirmatory method and standard materials and also the not satisfactory knowledge about the effects of food processing on the allergenic components can mean barriers for evaluating validation protocols and fulfilling the requirements of laboratory accreditation.

As the Hungarian member of this cooperation the aim of our work was to study the effects of processing (mainly heat treatment) on allergenic proteins in such processed food matrices which contain the allergenic components in dedicated amount.

During our experiments firstly a processed food matrix was produced, namely wheat flour based cookies. The examined allergen components were milk and egg proteins. For investigating the effects of processing an experimental design was made which contained the analytical examination of every step of the production of the model samples: the mixture of dry components, the raw dough and the cookies. The analytical tools for studying these samples were different ELISA methods. According to the results a large-scale decrease in protein amount after baking was measured. This phenomenon can be explained in different ways: a total or partial heat denaturation which can cause lower extractability, minor modifications of the protein structure which can cause less detectability during the ELISA method or interactions between the allergenic protein and other food components.

Studying the background of these assumptions and applying our findings for a future validation protocol are the main goals of our future work. Besides these results can mean possible contribution for proper method development and refinement of legislation as well.

Keywords: allergy, validation, effects of processing

This is work is carried out with the financial support of MoniQA project and the fellowship for PhD students awarded by the government of Hungary. The ELISA measurements were implemented with the professional support of Hungarian Central Food Research Institute and Eurofins Analytik GmbH in Hamburg, Germany.

K-16

RAPID AND RELIABLE METHODS FOR QUALITATIVE AND QUANTITATIVE DETERMINATION OF SOYBEANS PROTEINS AND GLUTEN IN MEAT PRODUCTS

Vesna Matekalo-Sverak[1*], Vesna Jankovic[2], Lazar Turubatovic[3], Slobodan Lilic[4], Danijela Vranic[5], Jelena Babic[6]

[1 2 3 4 5 6] Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia
[*] Corresponding author–E-mail: vesnams@inmesbgd.com; Phone: +381-11-2650-722; Fax: +381-11-2651-825

Regardless of many health benefits attributed to soybeans products, which is also scientifically proved, utilization of soybeans proteins in foodstuffs, including meat products, has to be clearly stated on product declaration since soybeans is one of the most significant food allergen. Main cause of soybeans allergies are proteins glycinin and three units of beta-conglycinin, proteins that are carriers of functional properties and therefore present in all soybeans products used as functional additives in meat products manufacture. Also, significant ingredient in production of meat products is gluten, wheat protein, which shows good emulsifying and stabilizing properties and has positive influence on product texture. Used in prescribed quantities, gluten has no influence on taste, odour and colour of meat products which makes it hard to identify by sensory analysis. Gluten is also a well-known allergen and its declaration on the product is mandatory. However, gluten, as well as soybeans preparations are often used in mixtures intended for emulsifying and stabilizing of meat products. These mixtures are sold under various commercial names and can be used in manufacturing of meat products while their ingredients are not clearly stated. This poses significant health risk for allergic or food intolerant consumers.

Within the Strategy for consumers' protection, it was necessary to develop rapid and reliable analytical methods for determination of soybeans proteins and gluten in meat products.

Traditional analytical methods for identification of soybeans proteins are time-consuming. Therefore, in our research on identification of these proteins in meat products we tested the reliability of ELISA method, specific for determination of soybeans proteins. Time needed for analysis completition was 2–3 hours. Obtained results show that soybeans proteins added to meat products can be detected by this analytical method. Detection limit was 0.4% and quantification limit was set to 0.7% of soybean proteins.

We also investigated gluten content in meat products by the same analytical technique. Analysis is based on direct „sandwich" immunoenzyme reaction, protein binding to specific antibodies. Calibration curve was made in order to accurately quantify gluten content. The method was proved to be reliable and simple. Analysis time is two hours. The method allows reliable determination od added gluten in various meat products. Detection limit is 0.01% while limit of quantification was 0.1%. This practically allows determination of gluten added to meat products as stabilizer trough commercial mixtures

Keywords: food allergens, gluten, soybean proteins

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FLAVOURS AND ODOURS

(M-1 – M-25)

M-1
ELECTRONIC NOSE AS A RAPID AND INNOVATIVE TOOL FOR THE DIAGNOSIS OF GRAPEVINE CROWN GALL

Sonia Blasioli[1*], Enrico Biondi[2], Ilaria Braschi[3], Umberto Mazzucchi[4], Carlo Bazzi[5], Carlo Gessa[6]

[1 2 3 4 5 6] Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, Bologna, Italy
[*] Corresponding author–E-mail: sonia.blasioli@unibo.it; Phone: +39-051-2096207

The electronic nose (E-nose) is an innovative device developed for microbiological, food/drink and environmental safety, and medical applications. E-nose comprises appropriate sensor arrays, able to detect and discriminate several flavours and odours from different sources. In the last decade, E-nose is being applied to recognize plant diseases caused from different quarantine bacterial pathogens such as Erwinia amylovora, Ralstonia solanacearum and Clavibacter michiganensis subsp. sepedonicus. In grapevines, tumourigenic strains of Agrobacterium vitis cause crown gall disease which may give serious problems in nurseries and vineyards. For the first time, a portable E-nose (PEN3) was used to discriminate between galled and healthy vines (controls), experimentally inoculated with two different strains of A. vitis and water, respectively. E-nose data were related to the increase of inoculation site diameters measured 9 months after inoculation with the pathogen. Preliminary gas chromatography-mass spectrometry (GC-MS) analysis were performed to identify volatile species from grapevine samples and to optimize experimental conditions for sensorial analysis. Solid phase microextraction (SPME) CAR/PDMS fibers were used to concentrate trace level vapour emissions. Spectra from tumoured vines and controls had almost identical volatile compositions, except the key compound styrene detected in infected samples. In plants, styrene formation is compatible with decarboxylation of cinnamic acid involved in secondary metabolism of plants. All data (E-nose sensors responses and diameter increases) from samples were processed with Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). PCA application allowed a discrete separation of the samples into two groups, according to the presence or absence of disease symptoms. Moreover, the LDA model allowed the correct sample classification between healthy and galled vines (83.3%, cross validation). Tumours caused by the two strains of the pathogen were not discriminated according to the volatile compound composition identified by GC-MS. Although a larger number of grapevine samples should be analysed to create a more robust model, our results give interesting clues to go further with research on the diagnostic potential of this innovative system associated with multi-dimensional chemometric techniques.

Keywords: E-nose, Agrobacterium vitis, GC-MS

M-2
APPLICATION OF POLYURETHANE FOAMS TO CHARACTERIZE AROMA COMPOUNDS FROM COFFEE BLENDS

Carla Rodrigues[1*], Fátima C.M. Portugal[2], J.M.F. Nogueira[3]

[1] University of Lisbon, Faculty of Sciences, Centre for Environmental Biology, Stable Isotopes and Instrumental Analysis Facility (SIIAF), Ed. ICAT, Campo Grande, 1749-016 Lisbon, Portugal
[2 3] University of Lisbon, Faculty of Sciences, Chemistry and Biochemistry Department and Centre of Chemistry and Biochemistry, Campo Grande, Ed. C8, 1749-016 Lisbon, Portugal
[*] Corresponding author–E-mail: cirodrigues@fc.ul.pt; Phone: +351960082532; Fax: +351217500172

Commercial available roasted coffees derive from two species, *i.e. Coffea Arabica* and *Coffea canephora* var. *robusta* that are cultivated in all continents except Europe. Usually, consumers buy coffee blends composed by a mixing of Arabica and Robusta. Different compositions originate different blends whose aroma and flavour can be quite distinct and exquisite. Nevertheless, if the type of coffee is an important parameter determining blend quality, other characteristics such as origin and roasting conditions may also affect the final product quality. To obtain a good cup of coffee, the step of roasting is, therefore, a very important task in order to develop specific organoleptic properties, *i.e.* flavour, aroma and colour. Nowadays, headspace solid-phase microextraction (HS-SPME) is one of the most used analytical approaches for sampling aroma compounds from coffee matrices, as already reported in literature. Nonetheless, searching new polymeric phases, other than the commercial polydimethylsiloxane (PDMS) or mixtures of polymeric coatings, commonly used in SPME fibbers, is always very important in order to improve the sensitivity and the selectivity of new aroma compounds with impact. In recent years, new polymeric materials such as polyurethane (PU) foams have gained importance since they present very interesting properties. These PU foams have been used in a wide range of applications, such as coatings for stir bar sorptive extraction (SBSE), with excellent results that include higher selectivity for compounds with higher polarity. In this work, we propose the use of these PU foams in headspace sorptive extraction (HSSE) of roasted coffee obtained through distinct roasting conditions and from different commercial coffee blends, in order to characterize the aroma compounds in their headspace. The data obtained showed that the recover efficiency obtained by HSSE (PU) was considerably much higher when compared with HS-SPME, using gas chromatography coupled to mass spectrometry (GC-MS) analysis. Moreover, the HSSE (PU)/GC-MS methodology combined with multivariate data analysis showed to be a very powerful tool to discriminate different degrees of roasting in between commercial coffee blends.

Keywords: SPME, Polyurethane foams, coffee, aroma

M-3
FLAVOUR&FRAGRANCE ANALYSIS: EASY HEART CUT MDGC WITH MASS SPECTROMETRIC DETECTION IN 1ST AND 2ND DIMENSION

Hans-Ulrich Baier[1*]

[1] Shimadzu Euopa, Duisburg, Germany
[*] Corresponding author–E-mail: hub@shimadzu.de; Phone: 00492037687464

In flavour and fragrance analysis co-elutions are often observed in one-dimensional gas chromatography. In order to separate those regions are transferred into a second column using heart cut multidimensional gas chromatography GC×GCMS [1]. A Carbowax column with 30 m, 0.25 mm i.D. and 0.25 µm film was coupled to a chiral RTβ DEX sm 30m, 0.25 mm, 0.25 µm in the second dimension in order to separate chiral compounds.

To have also identification of the peaks in the first dimension an FID/MS splitting was realised. This was created by a capillary split connection to feed the effluent at the FID (1st dimension) partly into MS simultaneously (1/15 relativ to FID). For this a desactivated fused silica tube (1 m, 0.175 mm ID) was lead via the interface from the GC of the first dimension into the second dimensional GCMS. Both columns the split connection and the second dimensional column are mounted into the MS detector by using a special connector [3]. While the FID chromatogram can be used for an area normalisation report the MS full scan data can be used for identification. Chiral compounds can be then transferred to the second column in a subsequent run. In cut runs the FID/MS splitting transfer line has to be blocked to prevent co-elutions of cut peaks from the second dimension with first dimensional analytes. This is achieved by a pressure increase of an auxiliary pressure unit which reverses the flow in the splitting line. Several cuts were done on commercial flavours. Below major peaks of for example linalool and terpineol the two enatiomers of each are resolved after transfer to the second dimension. The identification was done using the library FFNSC 1.3 dedicated to Flavour and Fragrance compounds with linear retention indices.

As a conclusion this multidimensional GC×GCMS configuration offers easy and reliable analysis of flavour and fragrance samples.

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[3] Shimadzu Application note 74

Keywords: Flavour characterisation, heart cut MDGC

M-4

THE INFLUENCE OF MICROOXYGENATION ON THE DEVELOPMENT OF VOLATILE AROMA COMPOUNDS OF RED WINES. AROMAPROFILING ANALYSIS AS A TOOL TO STUDY TECHNOLOGICAL VARIANTS

Hans-Georg Schmarr[1*], Alexander Stephan[2], Dominik Durner[3], Ulrich Fischer[4], Jörg Bernhardt[5]

[1] Dienstleistungszentrum Ländlicher Raum (DLR) Rheinpfalz, Abteilung Weinbau & Oenologie, Neustadt an der Weinstraße, Germany
[2 3 4] Dienstleistungszentrum Ländlicher Raum (DLR) Rheinpfalz, Abteilung Weinbau & Oenologie, Neustadt an der Weinstraße, GermanyLändlicher Raum (DLR) Rheinpfalz, Neustadt an der Weinstraße, Germany
[5] Ernst-Moritz-Arndt-University Greifswald, Institut für Mikrobiologie, Greifswald, Germany
[*] Corresponding author–E-mail: hans-georg.schmarr@dlr.rlp.de; Phone: +49-6321-671289; Fax: +49-6321-671375

Due to modern processing technologies and storage of wine in large-scale stainless steel containers the oxygen uptake during vinification and ageing is reduced considerably. However, insufficient exposure to oxygen is thought to be disadvantageous for the sensory qualities of red wine. The controlled supply of oxygen (microoxygenation) during vinification and ripening is used as a technique to deliver beneficial amounts of oxygen to red wine and elaborate its sensory characteristics (*1*). Other than the influences of oxygen on the tannin structure and color of red wines (*2-4*), changes in aromatic profile are not investigated very well. Our objective was to examine the impact of microoxygenation with different oxygen dosages during alcoholic and after malolactic fermentation on the volatile composition of different red single variety wines. The volatile components of the red wines were analyzed using headspace-solid phase microextraction (HS-SPME) coupled to comprehensive two-dimensional gas chromatography-quadrupol mass spectrometry (GC×GC-qMS). A profiling analysis of the aroma compounds analyzed by GC×GC was achieved using a software package from the proteomics field, working on the two-dimensional images (2D images) of the GC×GC chromatograms (*5*). The approach described here allowed a clear differentiation (clustering) of the oxygenation treatments investigated. Image processing of the GC×GC data also involves statistical methods, allowing the identification of the relevant zones in the 2D images which are responsible for the differentiation. Furthermore, based on the underlying mass spectrometric information, it has been possible to identify the underlying chemical substances (aroma compounds), which is an important pre-requisite for the future explanation of the different aromatic profiles encountered during production of microoxygenated wines.

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Keywords: Microoxygenation, Aroma, Profiling, GC×GC

M-5
THE EXITING FLAVOR WORLD OF PROCESSED ONIONS

Michael Granvogl[1*], Peter Schieberle[2]

[1] Technical University of Munich, Garching, Germany
[2] Technical University of Munich and German Research Center for Food Chemistry, Garching, Germany
[*] Corresponding author–E-mail: michael.granvogl@lrz.tum.de; Phone: +49 89 289 13264; Fax: +49 89 289 14183

It is well accepted that sulfur compounds play an important role in the complex flavor chemistry of onions. On a molecular level, the degradation of sulfur containing precursors and a lot of redox reactions occur in the thermoconversions responsible for the differences in the aroma of different heat-processed onions. Numerous compounds have already been identified in onions, but in particular their contribution to the overall aroma is not well understood. Furthermore, many substances have only tentatively been identified only based on their mass spectra without a structural confirmation.

Supported by application of concepts of molecular sensory science (gc/olfactometry, aroma extract dilution analysis, stable isotope dilution analysis (SIDA), odor activity value), the key aroma compounds for cooked as well as for deep-fried onions were elucidated.

The data showed that in deep-fried onions predominantly alk(en)yl sulfides contributed to the characteristic aroma, while in the smell of cooked onions, cyclic sulfur compounds and thiols were additional odor-active key constituents. On the basis of syntheses and NMR-measurements several new onion odorants in the overall set of aroma compounds were unequivocally identified for the first time, e.g. tetrathianes and pentathiepanes. These flavor compounds were quantified by means of various newly developed stable isotope dilution analyses using different synthesized isotopologues as internal standards revealing the differences in the key odorants caused by different processing conditions.

Further, the importance of the use of appropriate analytical methods with respect to artifact formation, e.g. excessive generation of volatiles from precursors during the work-up procedure (high vacuum distillation, simultaneous distillation-extraction, stir bar sorptive extraction) could be impressively shown resulting in significant concentration differences for some substances, e.g. for 3-mercapto-2-methylpentan-1-ol by a factor of 250.

Keywords: onion, flavor, structure elucidation, SIDA

M-6
EVOLUTION OF AROMATIC AND PHENOLIC COMPOUNDS OF SUPERIOR SEEDLESS GRAPES DURING RIPENING

Fenoll Jose[1*], Manso Angela[2], Hellin Pilar[3], Flores Pilar[4]

[1 2 3 4] IMIDA, MURCIA, SPAIN
* Corresponding author–E-mail: jose.fenoll@carm.es; Phone: 34 968366798; Fax: 34 968366792

The evolution of aromatic and phenolic compounds in *Vitis vinifera* cv. Superior Seedless grapes was studied during two years. Monoterpenes and other compounds such as eugenol, benzyl alcohol and 2-phenylethanol (free and glycosidically linked) were determined by using gas chromatography-flame ionization detection and gas chromatography-mass spectrometry after solid phase extraction (SPE) during ripening. Thirteen compounds were identified and quantified in the free and bound fractions. The majority free compounds detected were citral, geraniol and benzyl alcohol. In general, concentrations of the main free terpenes alcohols responsible for Sugraone aroma decreased during grape development. In the glycosidically bound fraction, the majority free compounds detected were geraniol, citral, nerol, citronellol, 3,7-dimethyl-1,5-octadien-3,7-diol (diendiol I), trans-furan linalool oxide (linaloloxide I), cis-furan linalool oxide (linaloloxide II), benzyl alcohol and 2-phenylethanol. At grape maturity, the results showed a higher content of bound compounds than free forms. Calculation of odour activity values showed that geraniol was the most active odorant. Other monoterpenes contributing to Sugraone aroma were citral and nerol. As regard as phenolic compounds are concerned, total polyphenolics and flavonols contents were determined with a UV/VIS spectrophotometer and expressed as gallic acid and (+)catechin, respectively. Flavan 3-ols and stilbenes were analyzed by high-performance liquid chromatography with photodiode array detection and quantified as quercetin and *trans*-resveratrol, respectively. Flavan 3-ols and flavonols contents highly increased until veraison followed by a decrease until the end of maturation. Total phenolics increased throughout all the maturation period while stilbenes behaved in the opposite way.

Keywords: aroma, phenolic compounds, terpenes, glycosides

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M-7
CHANGES IN THE AROMATIC COMPOSITION OF MOSCATUEL AND RUBY SEEDLESS GRAPES DURING RIPENING

José Fenoll[1*], María Martínez[2], María V. Molina[3], Juana Cava[4], Pilar Hellín[5], Pilar Flores[6]

[1 2 3 4 5 6] IDepartamento de Calidad y Garantía Alimentaria, IMIDA
[*] Corresponding author–E-mail: jose.fenoll@carm.es; Phone: 34 968366798; Fax: 34 968366792

Varietal aroma is one of the important factors for determining grape quality and is characteristic for every grape variety. This parameter has been widely studied, mainly in Muscat grapes varieties where numerous terpenes have been identified as responsible of the varietal flavour. Other aromatic compounds of great importance such hydrocarbons, norisoprenoids and some alcohols have been also found. These aromatic compounds are distributed between the pulp and skin of the berry, with the highest concentration in the latter. The aromatic components in grape are present in partly as free volatile forms, which may contribute directly to the aroma of the grape and partly as non-volatile, sugar-bound conjugates (mainly, glycosides), precursors. However, the glycosides can be transformed in free volatile compounds by hydrolysis increasing grape aromatic characteristic. These substances are synthesized during berry maturation and are qualitatively and quantitatively influenced by environmental and agricultural factors. A one-year study was carried out to determine the evolution of free and bound aromatic compounds of two cultivars of *Vitis vinifera:* Moscatuel (muscat aroma) and Ruby Seedless (neutral aroma) during ripening. The study of changes in aromatic and potentially aromatic compounds during maturation provide valuable information for evaluating aroma potential and the period of time to reach the maximum potential. For this reason, in this study, changes in both free and bound compounds of these varieties were monitored during maturation aiming to identify the compounds responsible for its varietal aroma, and to understand the evolution of these compounds. Analysis of aromatic compounds were performed by solid phase extraction (C_{18} cartridges), followed by enzymatic hydrolysis (for bound compounds), the subsequent determination by GC-FID and confirmation by GC-MSD. The most abundant compounds detected in Moscatuel grape were linalool, geraniol, citronellol, nerol, citral, α-terpineol, trans-furan linalool oxide (linaloloxide I), cis-furan linalool oxide (linaloloxide II), benzyl alcohol and 2-phenylethanol. In general, concentrations of the main terpenes compounds increased during grape development, except geraniol. However, the most abundant compounds detected in Ruby Seedless grapes were benzyl alcohol and 2-phenylethanol. At grape maturity in both varieties, results showed a higher content of bound compounds than of free forms.

Keywords: Aroma, grape, terpenes, glycosides

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M-8
ULTRASONIC ASSISTED MICROENCAPSULATION OF CARDAMOM ESSENTIAL OIL

Masoud Najaf Najafi[1*], Rassoul Kadkhodaee[2], Seyed Ali Mortazavi[3]

[1 3] Department of Food Science and Technology, Ferdowsi University of Mashad
[2] Department of Food Technology, Khorasan Research Institute for Food Science and Technology
[*] Corresponding author–E-mail: Masoudnajafi@yahoo.com; Phone: +98-511-8795619; Fax: +98-511-8717142

Essential oils, despite of having many advantages over ground spices, have limited applications in the food industry for being sensitive to light, heat and oxygen. One approach to overcome this is microencapsulation. The present work reports on ultra-Turrax (UT) and ultrasonic (US) emulsification of cardamom oil followed by its microencapsulation by freeze-drying, using different types of modified starch including Hi-cap100 and Capsule as wall materials. The microcapsules were evaluated for their content of entrapped 1,8 cineole and its stability during storage for six weeks. Ultrasound was demonstrated to be a useful tool in the emulsification and encapsulation of food components. Hi-cap100 offered greater protection towards cardamom oil than other starch varieties.

Keywords: Cardamom essential oil, Hi-cap, Encapsulation

M-9

DEVELOPMENT OF A STIR BAR SORPTIVE EXTRACTION METHOD COUPLED TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR THE ANALYSIS OF VOLATILE COMPOUNDS IN SHERRY BRANDY

Raul Delgado[1], Enrique Duran[2*], Remedios Castro[3], Ramon Natera[4], Carmelo G. Barroso[5]

[1 2 3 4 5] University of Cadiz, Puerto Real, Spain
[*] Corresponding author–E-mail: enrique.duranguerrero@uca.es; Phone: +34956016360

Sherry brandy (Jerez, SW Spain) is a high quality distilled beverage derived from Sherry wine. Its quality is very influenced by hundreds of flavour compounds. These compounds mainly come from the raw material, the wood ageing, and the several operations after this ageing such as addition of caramel colours and macerations and infusions of natural products. The analysis of the aromatic profile is normally carried out using gas chromatography, but a previous extraction step is needed in order to concentrate the compounds, improving the analytical signal, and to eliminate different interferences derived from the matrix. This step has not been successfully resolved yet. A Stir Bar Sorptive Extraction (SBSE) method coupled to gas chromatography-mass spectrometry has been developed for the analysis of volatile compounds in Sherry brandy. The optimization of the extraction procedure has been carried out by means of a statistical approach, using a factorial design. The best overall analytical conditions obtained were the followings: 35 ml of sample diluted 1:1 with water and extraction at 1100 rpm for 100 minutes. The further validation of the method has also been successfully carried out. Several performance characteristics such as calibration, linearity, precision (repeatability and reproducibility), detection and quantitation limits and recovery were studied. The obtained results revealed SBSE as a very appropriate technique for the analysis of volatile compounds in brandies.

Keywords: SBSE, Brandy, GC-MS, flavour, optimization

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M-10
DEVELOPMENT OF AN ELECTRONIC NOSE TO DISCRIMINATE CHEESE VARIETY

Vânia F. Pais[1], João António B. P. Oliveira[2], Maria Teresa S. R. Gomes[3*]

[1 2 3] CESAM & Dep of Chemistry, University of Aveiro, Aveiro, Portugal
[*] Corresponding author–E-mail: mtgomes@ua.pt; Phone: +351 968661256; Fax: +351 234370084

The strong flavour of some cheeses is widely recognized, while subtle differences in the cheese bouquet between some other cheese varieties can only be perceived by trained experts. An electronic nose built with eleven sensors based on coated piezoelectric quartz crystals was assembled in order to discriminate cheese aromas and to identify cheese variety. The sensors have been selected by their sensitivity to compounds known to be present on cheese bouquet. Each crystal was driven by an oscillator and the output frequencies were monitored with a Counter/timer device NI PXI-1033 and stored simultaneously on a PC. The volatile compounds of each cheese sample were extracted by static headspace method with a fibre coated with 75 mm carboxen-polydimethylsiloxane (CAR-PDMS). Solid phase microextraction (SPME) allows the extraction of many volatile compounds without the use of any solvent. Headspace SPME experimental conditions, such as extraction temperature and extraction time were carefully controlled. Experiments showed that extractions made at 30ºC for 30 minutes were adequate for the analysis. Compounds were later thermally desorbed and carried by a nitrogen flow to the sensors. The different interaction between desorbed compounds and the coatings of each quartz crystal, as well as the amount of each compound absorbed by the fibre resulted in compounds being detected by each sensor with different sensitivities. Therefore, each sample produced frequency decreases in each sensor of the array with magnitudes related to sample composition and sensor characteristics. By combining the outputs from several sensors it is possible to record a fingerprint, which is specific for a cheese variety. Analysed cheeses were selected among the world best known varieties, and included, soft cheeses, like Camembert and Brie, fresh cheeses, semi-hard and hard cheeses, like, Gruyére, Grana Padano, Gouda, and Manchego, among many others. Cheeses of the same variety produced in different places were included in the sample set. The minimum number of sensors in the array, as well as the best sensors to discriminate cheese varieties will be presented.

Keywords: cheese, electronic nose, acoustic sensor

FCT, POCTI and FEDER

M-11

CHARACTERIZATION AND DIFFERENTIATION OF SHERRY BRANDIES WITH DIFFERENT PERIODS OF AGEING IN WOOD

Enrique Duran[1*], Raul Delgado[2], Ramon Natera[3], Remedios Castro[4], Carmelo G. Barroso[5]

[1 2 3 4 5] University of Cadiz, Puerto Real, Spain
[*] Corresponding author–E-mail: enrique.duranguerrero@uca.es; Phone: +34956016360

Sherry brandy is an oenological distilled product from Sherry wine. For its manufacturing, alcohol derived from wine is stored in oak casks for a determined period of time. During this period, the typical “solera” ageing system is carried out. In this system, the oldest barrel in the “solera” (ground) is tapped for part of its content, which is bottled. Then that barrel is refilled from the next oldest cask, and that one in succession from the second-oldest, down to the youngest cask, which is refilled with new product. The transferred product mixes with the older product in the next barrel. Depending on the length of time the brandy is submitted to ageing in wood, three different categories of commercial product can be found in the market: Solera Brandy (from 6 months to 1 year), Solera Reserva Brandy (from 1 to 3 years) and Solera Gran Reserva Brandy (more than 3 years). According to this, brandy complex flavour profile is directly influenced by wood aging length, among others parameters. In this study, the characterization of the aroma profile of different commercial Sherry brandies, from the three categories earlier mentioned, has been carried out using a previously developed SBSE-GC-MS method. Data obtained were submitted to different statistical techniques. Most of the samples have been successfully differentiated according to their aromatic profile.

Keywords: SBSE, Brandy, differentiation, flavour, ageing

This study was supported by the Junta de Andalucia, (P05-AGR-00767)

M-12

LC-MS/MS STUDIES ON THE INFLUENCE OF THE PH VALUE ON THE FORMATION OF NOVEL ISO-A-ACID DEGRADATION PRODUCTS IN BEER

Annika Brock[1*], Andreas Stephan[2], Daniel Intelmann[3], Thomas Hofmann[4], Georg Stettner[5]

[1 2 5] Bitburger Braugruppe GmbH, Bitburg, Germany
[3 4] Lehrstuhl für Lebensmittelchemie und molekulare Sensorik, Freising, Germany
[*] Corresponding author–E-mail: annika.brock@bitburger-braugruppe.de; Phone: 00496561142286

The flavour of beer is heavily dependent on time of storage. The typical bitterness of fresh beer is well-known to slightly decrease in intensity and to change in quality with increasing age of the beverage. Non-volatile bitter compounds of beer have been investigated in the last decades, and it is agreed that the typical beer bitterness is caused by adding hop products during wort boiling. A number of isomerization processes during the wort boiling process have been reported to be of major importance for bitter taste development in the final beer product. Moreover, the iso-α-acids have been identified as the major bitter contributors in beer and were demonstrated to be generated upon a re-arrangement reaction of their hop-derived precursors, namely the α-acids. Already De Cooman et al. 2000 pointed out that particularly the trans-iso-α-acids are prone to degradation. In contradiction to previous findings, Intelmann and Hofmann revealed an acid-catalytic decomposition pathway for trans-iso-α-acids to tri- and tetra-cyclic degradation products. In the present study, we investigated the influence of the pH value of beer on the formation of these degradation products by means of quantitative HPLC-MS/MS experiments.

Beer was adjusted to various pH values and the profiles of iso-α-acids and their corresponding degradation products in fresh and aged beer were monitored by quantitative LC-MS/MS analysis using the multiple reaction monitoring (MRM) mode.

The results exhibited a negative correlation between the pH value, the instability of iso-α-acids, and the formation of their tri- and tetra-cyclic degradation products.

The newly discovered degradation products were demonstrated to be the major beer aging indicators explaining almost all of the loss of trans-iso-α-acids in aged beer samples. Due to the new findings, the brewing process is to reconsider with respect to pH modification since already a slight raise of pH shows an effect of flavour stability of aged beer.

This investigation clearly demonstrates that, besides aroma-active volatiles, also the generation of nonvolatile degradation products originating from bitter-tasting iso-α-acids contribute to the flavour instability of beer and underlines the complex nature of the deterioration of beer products. The industrial importance of this new finding was confirmed by a quantitative monitoring of the novel degradation products in real beer samples taken from a brewery production line.

Keywords: beer deterioration, pH dependency

M-13
MICRO-OXYGENATION AND AMERICAN OAK NON-TOASTED CHIPS ADDITION EFFECTS ON PETIT VERDOT RED WINES

María Jesús Cejudo-Bastante[1*], Isidro Hermosín-Gutiérrez[2], María Soledad Pérez-Coello[3]

[1] Centro Andaluz de Investigaciones Vitivinícolas (CAIV), Cádiz, Spain
[2] E.U. Ingeniería Técnica Agrícola, Ciudad Real, Spain
[3] Facultad Ciencias Químicas, Ciudad Real, Spain
[*] Corresponding author–E-mail: mariajesus.cejudo@uca.es; Phone: 0034956016363

Micro-oxygenation technique consists in the controlled addition of oxygen to wine using a micro-diffusion system which distributes the gas as very little bubbles that allow the oxygen dissolution in wine. The aforementioned technique is used to reproduce, or even accelerate, the stabilization processes of the red wine pigments which take place during red wine ageing in wood barrels. Anthocyanin-type pigments, chromatic, aromatic and sensorial profiles had been studied in Petit Verdot red wines submitted to micro-oxygenation. The aim of this work was to evaluate the changes induced by this treatment, compared with a control wine. The micro-oxygenation technique was carried out before the malolactic fermentation, with an oxygen addition of 45 mL/L/month during 20 days. After that, the addition of American oak non-toasted chips was made (7 g/L of chips during 25 days). The oxygen addition affected to the anthocyanin-type pigment fraction of Petit Verdot red wines, leading to an increase of the concentrations of both B-type vitisins and anthocyanin-ethyl-flavan-3-ol pigments, which was parallel to a decrease in the anthocyanin monomers concentration. This tendency was kept after American oak chips treatment, with the exception of a decrease of the concentration of the anthocyanin-ethyl-flavan-3-ol pigments. Consequently, micro-oxygenated red wines had a lower red colour intensity (lower values of a*) and a higher yellow colour intensity (higher values of b*). The micro-oxygenation treatment hardly affected to the Petit Verdot aromatic fraction after malolactic fermentation. The acetaldehyde concentration had not been significantly varied by the micro-oxygenation treatment, despite the anthocyanin pigments related variation (anthocyanin-ethyl-flavan-3-ol). Nevertheless, the addition of American oak non-toasted chips increased the concentrations of eugenol, 3-oxo-α-ionol and 4-vinilguaiacol; in contrast, the concentrations of 2-phenylethyl acetate, succinate derivatives and several alcohols decreased with chips treatment. Terpenes fraction was hardly affected by both the micro-oxygenation treatment and the addition of oak chips. Micro-oxygenated red wines showed higher scores for red fruits and plum attributes as compared to control wines; moreover, nutty and smoke attributes appeared as a result of the micro-oxygenation treatment. As a consequence of the aforementioned results, micro-oxygenated red wines reached higher scores for global quality than those of control wines. Finally, the addition of American oak non-toasted chips produced a significantly decrease in wood, vanillin and spicy attribute scores.

Keywords: wine, micro-oxygenation, polyphenols, flavour, sensorial

M-14

NATURAL MILK LIPASE: INFLUENCE OF ITS ACTIVITY ON THE FREE FATTY ACIDS AND VOLATILE COMPOUNDS PROFILES OF CHEESES DURING RIPENING

María Cristina Perotti[1*], María Ayelén Vélez[2], Irma Verónica Wolf[3], Mario César Candioti[4], Erica Ruth Hynes[5], Carlos Antonio Zalazar[6]

[1] Instituto de Lactología Industrial (UNL/CONICET), Santa Fe, Argentina
[2 3 4 5 6] Instituto de Lactología Industrial (UNL/CONICET), Santa Fe, Argentina
* Corresponding author–E-mail: cperotti@fiq.unl.edu.ar; Phone: 0054 342 4530302

Moderated lipolysis is a desirable event during ripening of hard cooked cheeses, as it increases piquant taste and genuine flavour. Milk possesses an indigenous lypolitic enzyme, the lipoprotein lipase, which is associated to casein micelle. However, its activity on triglycerides is limited because milk fat is protected by the milk fat globule membrane (MFGM).

This work was aimed at increasing fat hydrolysis and flavour compounds production in hard cheeses by increasing LPL activity and improving its access to milk triglycerides. For this purpose, hard cheeses (Reggianito type) were produced in pilot plant and ripened during 90 days. The effect of influence of two different factors was studied: the method of milk sanitization and the accessibility enzyme-triglycerides. These factors were studied by comparing milk with native and damaged MFGM, and pasteurized milk and non thermally-sanitized milk.

The free fatty acids (FFA) were investigated by gas chromatography (GC-FID) as ethyl esteres and quantified with the internal standard method at the begining and the end of the ripening. The volatiles compounds were isolated by microextraction in solid phase (SPME) and analysed by GC-FID/MS. The areas were quantified in arbitrary units.

For all cheeses, it was observed that the degree of lipolysis increased during ripening being similar for all the treatments. However, the profiles of FFA showed differences in the relative proportions of the FFA groups. In particular, the percentage of short chain fatty acids (SCFA) was higher in the cheeses made with no-thermally treated milk than in those made with pasteurized milk. This fact suggests that the LPL played a role in lipolysis, as it has a positional specificity to the sn-3 position of the triglyceride, where SCFA are esterified. Similar results were obtained for most of the volatile compounds that constitute the groups of ketones, alcohols, esthers (derivated of FFA catabolism) and the group of acids (derivated of lipolysis). Overall, the greatest area values were obtained for the cheeses made with no pasteurized milk.

In cheeses made with not heated milk and with native fat, the percentage of SCFA and the levels of the volatile compounds were in general higher than those found in cheeses made with milk in which the MFGM was damaged. However, the opposite behavior was found in cheeses made with pasteurized milk.

In the conditions of the study, results suggest that thermal treatment had a higher impact on cheese lipolysis and volatile compounds production than the destabilization of the fat emulsion.

Keywords: flavour, lipolysis, cheese ripening

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M-15

INFLUENCE OF AN ESTERASE ADDITION ON THE FATTY ACIDS AND VOLATILE COMPOUND PROFILES OF SEMI HARD CHEESES

María Cristina Perotti[1*], Irma Verónica Wolf[2], Luigi Mandrich[3], Giuseppe Manco[4], Carlos Antonio Zalazar[5], Carlos Alberto Meinardi[6]

[1 2 5 6] Instituto de Lactología Industrial (INLAIN-UNL/CONICET). Santa Fe, Argentina
[3 4] Istituto di Biochimica delle Proteine, CNR. Nápoles. Italia
[*] Corresponding author–E-mail: cperotti@fiq.unl.edu.ar; Phone: 0054 342 4530302

Incorporation of exogenous lipases in cheesemaking is usually used as a resource to develop the production of volatile compounds or to accelerate ripening process.

The aim of this work was to evaluate the effect of the addition of an esterase on the lipolysis and volatile compounds profiles of semi-hard cheeses. EST2 is a thermophilic pure esterase isolated from *Alicyclobacillus acidocaldarius* with moderated esterase and thiosterase activities.

Semi-hard cheeses (like Pategras variety) were made at pilot plant scale according to a standard process, using four levels of enzymes, 2, 5, 10 and 20 mg/L of milk (experimental cheeses, EI-EIV) and without enzyme (control cheese, C). The cheeses were ripened at 12ºC during 60 days.

Lipolysis and volatile compounds profile were analysed at the end of ripening. Free fatty acids (C4:0 to C18:2) were extracted from fat by solvent and quantified by GC-FID as ethyl esters, and concentrations were expressed in mg/kg of cheese. Analysis of the volatile fraction was performed by SPME-GC-FID/MS obtaining semiquantative data (peak areas in arbitrary units) by GC-FID. Global composition and degree of ripening were assessed according to IDF methods and the protein profiles were investigated employing the ProteinChip SELDI system. Sensory analysis was performed by a triangle test.

The degree of lipolysis (sums of 10 FFA concentrations) for experimental cheese with highest level of enzyme (EIV) was 1.5 fold higher than C cheese. Relative increases of 54% and 33% in short- (C4:0–C8:0) and medium- (C10:0–C12:0) chain fatty acids, were observed for EIV cheese in relation to control cheese. This fact suggests the preferential release of short chain fatty acids by EST2.

Twenty seven and thirty one volatile compounds were identified respectively in control and experimental cheeses. They belonged to different chemical families such as ketones, alcohols, esters and acids. Experimental cheeses showed higher areas values than control cheeses for almost all volatile compounds. In particular, it was interesting to notice that the EIII cheese showed the greatest values in spite it had not the highest enzyme level. In sensorial analysis significant differences among cheeses made with and without enzymes were observed.

Global composition and degradation of protein did not reveled significant differences between experimental and control cheeses, indicating that the enzyme had not a proteolytic activity.

Taking into account that the use of EST2 in cheese-making intensified the production of flavour compounds, specially those related with lipolysis and FFA catabolism, this esterase could be an interesting technological strategy to improve the sensory quality or accelerate the ripening process of cheeses.

Keywords: exogenous lipase, cheeses, flavour, lipolysis

M-16

PORTABLE, VERSATILE AND EASY-TO-USE HOME-MADE PROTOTYPE FOR THE GENERATION, ENTRAPMENT AND CONTROLLED DESORPTION OF AROMAS AND ODOURS

Alberto de Diego [1*], Josu Trebolazabala[2], Irantzu Martinez-Arkarazo[3], Eneko Atxa[4], Juan Manuel Madariaga[5]

[1 2 3 5] Department of Analytical Chemistry, University of the Basque Country, P. O. Box 644; 48080 Bilbao, Basque Country
[4] Azurmendi jatetxea, Legina auzoa, 48195 Larrabetzu, Basque Country
[*] Corresponding author–E-mail: alberto.dediego@ehu.es; Phone: 0034946015550

Nowadays, terms like fast, feasible and efficient are synonymous of success and quality of life. They are aggressive terms which encourage competition and underestimate others like quality, health and environment. As in any other professional field, cooking is also in continuous evolution. Since the expansion of the term *nouvelle cousine* all over the world, combination of concepts like quality of the product, respect for the natural product, health and multisensorial perception is mandatory to land on the terrain of the so-called *slow food* movement.

To stimulate consumer's taste and sight has been a challenge for any cook throughout cooking history. In fact, both senses have been largely used by cooks in their creations. Smell, however, has not received so much attention. Only in the last decade, top chefs have realized the powerful potentiality of an intelligent combination of smell with other more classically used senses.

In this work we present a home-made prototype able to generate, transport and entrap odours and aromas, and later desorb them in selected ambients, such as an eating room of a restaurant or an inverted cup which will cover a creation dish. The prototype consists on an air pump, filters, several interconnected closed recipients, connectors and switching valves, a column filled with a solid adsorbent, a cryogenic trapping system, a thermal desorption unit and an outlet port for organoleptic analysis, altogether connected with silicone tubing. It is inspired in the Flow Injection Analysis (FIA) concept often used in chemistry to create miniaturised systems of analysis. It is light and compact, portable, easy to use and, due to its FIA like characteristics, very versatile and susceptible of automation.

Different combination of substrates (soil, wet soil, spices, fruits, aromatic plants, wood,…) were used to generate and combine aromas, which were trapped in different liquid solvents (water, oil, vinegar, milk,…) or solid adsorbents (tenax, carbotrap, starch, tapioca,…) at the temperature of liquid nitrogen (cryotrapping) or at ambient temperature, and later thermally desorbed using an independent flow of hot water or ultraviolet light. A summary of the results obtained including several applications in the creation and final presentation of high quality dishes will be presented.

Keywords: Flavour entrapment, nouvelle cousine, perception

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M-17
MODERN ANALYTICAL METHODS FOR THE ANALYSIS OF SULPHUR FLAVORS IN MALT AND BEER

Zdeněk Svoboda[1*], Renata Mikulíková[2], Sylvie Běláková[3], Karolína Benešová[4]

[1 2 3 4] Research Institute of Brewing and Malting, Malting Institute Brno, Czech Republic
[*] Corresponding author–E-mail: svoboda@brno.beerresearch.cz; Phone: 545214110/34

Heterocyclic and sulphur compounds, some of them with high sensorial activity even in extremely low concentrations, belong to sensorially active substances principally affecting beer quality. Trace amounts of these compounds commonly detectable in foods contribute to their flavor, therefore this effect can generally be assessed as favorable. In malt and beer, however, this is true to a very limited extend only and the presence of heterocyclic and sulphur substances is evaluated rather negatively. Most of the sulphur compounds present in barley, malt and beer are non-volatile substances (amino acids, proteins, inorganic sulphates). These substances do not directly account for unfavorable beer flavors and odors but under certain conditions they may be important precursors of sensorially active substances. The produced substances are mostly volatile and their amount is usually less than 1% of the total amount of the sulphur containing substances in beer, i.e. actual amounts of substances responsible for sulphur odours are extremely low.

Direct analysis of sulphur sensorially active substances is not easily applicable regarding their very low concentrations (μg/kg,l–ng/kg,l) in the analyzed matrixes (malt, beer). Prior to the analysis, the analytes must be extracted from the matrix and concentrated. For extraction and concentration of sensorially active sulphur substances, following extraction methods were compared: thermal desorption (Tenax TA and Carbotrap sorbets) and HS-SPME (PDMS, CAR/PDMS, PEG and DVB/Carboxen/PDMS fibres) and HS-SPDE (needle PDMS). Sensorially active sulphur substances were determined with the method of gas chromatography with flame photometric detector, the GC/MS method was used for their identification. Suitability of different extraction techniques for the selected sensorially active sulphur substances and the obtained results of the analyses were discussed.

Keywords: sulphur, beer, SPME, SPDE, TDAS

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M-18

FURAN AND ITS DERIVATIVES IN POLISH TRADITIONAL BREAD "RAZOWY" WITH HONEY

Beata Cieślak[1*], Mieczysław Obiedziński[2]

[1 2] Department of Food Biotechnology, Microbiology and Evaluation, Warsaw University of Life Sciences, Warsaw, Poland
[*] Corresponding author–E-mail: beatacieslak@o2.pl; Phone: 48 22- 593 7486

Furan and its derivatives as 2-Furancarboxaldehyde,5-methyl-, are formed as products and/or intermediates of Maillard reaction. The formation of furan and its derivatives in food products is not clearly understood. USFDA's program of furan analysis in food was developed by exploring thermal processed products.

According to physical and chemical properties furan and its derivatives are a part of desired volatile compound fraction of bread and many other food products.

The aim of the study was to investigate formation of furan and its derivatives during baking process.

Material and methods: In experiment we baked traditional, wholemeal bread called "Wholemeal – Razowy" processed with rye flour with addition of honey.

Experimental baking of bread loafs were performed in different condition of temperature and time.

Tab. 1: Bread baking condition

Temperature Time	220ºC	230ºC	240ºC
30 minutes	x	x	x
40 minutes	x	x	x
50 minutes	x	x	x

Internal and external parts (crust) of bread loafs were examined on presence of furan and derivatives. Solid phase micro extraction (SMPE) was applied for sampling and analysis of furan by means gas chromatography- mass spectrometry (HS-SPME-GC/MS).

Quantitative determination were made using internal standard addition of 1,2-dichlorobenzen and simultaneous scanning mode (mass range 38–200 *m/z*) and selected ion monitoring (SIM) of ions (*m/z*) 39 and 68 for furan and respectively m/z 146 for IS.

Results:

All the results measured during analysis were quantified according to IS.

In of crust layer were identified: Furan; Furan, 2-pentyl (except 230ºC/50 min and 240ºC/50 min), 2-Furancarboxaldehyde, 2-Furancarboxaldehyde 5-methyl-, 2-Furanmethanol, Ethanone, 1-(2-furanyl)- (except 240ºC/30 min).

Smaller number and quantities of furan derivatives were determined in internal bread soft part: Furan

(in 0 samples); Furan, 2-pentyl (except 230ºC/40 min), 2-Furancarboxaldehyde (except 220ºC/30 min), 2-Furancarboxaldehyde, 5-methyl- (in 3 samples).

Tab. 2: Presence of furan and its derivatives (related to IS)

Name of compound	Average of three experimental baking			
	crust layer		internal part	
	temp/time	quantity	temp/time	quantity
1,2 dichlorobenzen–IS		1		1
Furan	240ºC -50	0.7		0
Furan, 2-pentyl-	240ºC -40	1.06	220ºC -30	0.79
2-Furancarboxaldehyde	220ºC -50	29.63	220ºC -50	5.22
2-Furancarboxaldehyde,5-methyl-	220ºC -50	1.77	220ºC -50	0.59
2-Furanmethanol	220ºC -50	1.45	230ºC -30	0.83
Ethanone, 1-(2-furanyl)-	240ºC -50	0.61	230ºC -50	0.33
Furan, 2-methyl-	240ºC -50	1.69	0	0

Discusion:
1. During baking process under experimental conditions there are evident differences in formation and concentration of examined substances in internal and external bread layer.
2. There is no linear relationship between time and/or temperatures of baking and formation of investigated substances.

Keywords: furan, furan drivatives, bread, SPME-GC/MS

M-19
FURAN IN POLISH TRADITIONAL BREAD "RAZOWY" WITH HONEY

Beata Cieślak[1*], Mieczysław Obiedziński[2]

[1 2] Department of Food Biotechnology, Microbiology and Evaluation, Warsaw University of Life Sciences, Warsaw, Poland
[*] Corresponding author–E-mail: beatacieslak@o2.pl; Phone: 48 22- 593 7486

Furan is a five-member ring colorless, cyclic ether with: low molecular weight, high volatility and low boiling point (aprox. 32ºC).

Furan is formed during thermal food processing, in large scale – in food industry products as well as in home cooking dishes.

According to its physical and chemical properties furan and its derivatives are a part of volatile compound fraction of polish bread and many other food products.

The aim of the study was to indicate measure furan appearing during baking process.

Material and methods: In our experiment we baked wholemeal bread called "Wholemeal–Razowy with rye flour with addition of honey.

Experimental baking of bread loafs process were performed in different condition of time and temperature.

Tab. 1: Changing condition of bread baking

Temperature Time	220ºC	230ºC	240ºC
30 minut	x	x	x
40 minut	x	x	x
50 minu	x	x	x

Internal leyer of bread were measured separately from crust layer.

Due to high volatility of furan solid phase micro extraction (SMPE) was applied for sampling and further analysis by means gas chromatography- mass spectrometry (HS-SPME-GC/MS).

Quantitative determination were made using calibration curve. Qualitative determination was performed using simultaneous scanning mode (mass range 38–200 *m/z*) and selected ion monitoring (SIM) of ions (*m/z*) 39 and 68 for furan.

Addition of internal standard furan D4 were performed for observation of furan.

Results

All the results measured during analysis were quantified according calibration curve.

Tab. 2 Furan amounts (ppb)

	30 minut			**40 minut**			**50 minu**		
	220ºC	230ºC	240ºC	220ºC	230ºC	240ºC	220ºC	230ºC	240ºC
internal layer	0	0	0	0	0	0	0	< 5	0
crust layer	0	17	0	0	0	0	< 5	12	37
	220ºC			**230ºC**			**240ºC**		
	30 min	40 min	50 min	30 min	40 min	50 min	30 min	40 min	50 min
internal layer	0	0	0	0	0	< 5	0	0	0
crust layer	0	0	<5	17	0	12		0	37

Discusion

1. Those results show, that the highest lewer of furan was observed in crust layer especially in crust layer in bread baking 50 min in 240ºC–37 ppb.
2. There is no furan in internal layer.
3. The difference in furan amount between crust and internal layer might be caused by different water content in matrix.
4. The linear relationship between time and/ or temperature apperar only for crust layer of bread baked 50 min. in 3 different temperatures.

Keywords: furan, SPME-GC/MS, polish bread

M-20

GREEN COFFEE QUALITY: OPTIMIZATION OF AN ANALYTICAL SBSE-GC-MS METHOD FOR DEFECT DETECTION

Silvia Colomban[1*], Valentina Lonzarich[2], Diego Rivetti[3], Luciano Navarini[4]

[1 2 3] Aromalab illycaffè S.p.A. – Area di Ricerca, Padriciano 99, 34149 Trieste, Italy
[4] illycaffè S.p.A. – via Flavia 110, 34147 Trieste, Italy
[*] Corresponding author–E-mail: silvia.colomban@illy.it; Phone: 0039 040 3755440

The coffee quality control is nowadays mainly performed by sensory analysis. This procedure, however, is affected by some limitations such as number of samples, availability of trained panellists, repeatability, time and costs. For these reasons, analytical methods are often developed and applied for this kind of practical purposes.

The aim of the present work is the optimization of an analytical method to detect off-flavor in raw coffee. The method must be simple but sensitive enough to detect the responsible compounds at low ppb levels, and it has to be able to match chemical and sensorial data.

A selection of 23 compounds, known from the literature as off-flavors in green coffee, has been used for defective samples recognition. The method has been optimised using stir bar sorptive extraction (SBSE) and thermal desorption coupled to capillary gas chromatography–mass spectrometry (GC–MS).

The parameters of the solid extraction step were optimized; being time and temperature of sampling, rotation speed and NaCl addition the more important variables of SBSE. Differences between headspace extraction and liquid extraction were also evaluated. The GC-MS analyses were optimized for detecting compounds in trace level, so single ion monitoring mode was preferred.

The matching between sensorial and chemical analysis was particularly good for fermented or “stinker” defective coffee, correlated with a high content of ethyl 2-methylbutanoate and ethyl 3-methylbutanoate. Not only was the correlation good between these components and defect recognition, but also the peak areas were proportional to intensity of defect perceived in cup.

By coupling SBSE with GC-MS analysis it is possible to obtain a powerful tool for quality control, particularly effective with fermented and over-fermented coffee. Moreover, it requires low amount of sample and offers good sensitivity and reliable results.

Keywords: greencoffee, quality, SBSE, GC-MS, defect

M-21
AROMATIC PROFILE OF ARGENTINEAN RED WINES PRODUCED FROM DIFFERENT YEAST

Daniel Wunderlin[1*], María Paula Fabani[2], Mario Ravera[3]

[1] Universidad Nacional de Córdoba, Argentina
[2] Universidad Nacional de San Juan, Argentina
[3] CEPROCOR, Argentina
[*] Corresponding author–E-mail: dwunder@fcq.unc.edu.ar; Phone: 0054 351 4333193 ext 141; Fax: 0054 351 4333194

Wine aroma is very complex due to its origin, which causes high number of volatile organic compounds (VOCs). *S. cerevisiae* is the prevailing yeast during alcoholic fermentation, being volatile esters ("fruity-floral" aroma), arising from yeast metabolism, an important contribution to wine aroma. Also lactic bacterium and no-*Saccharomyces* yeast contribute to wine aroma because they produce aromatic compounds. The aromatic profile of young wines is typical of a wine variety; however, most of them are formed during the fermentation process. So, we propose the study the VOCs from wines elaborated with different yeast to determine if wine aroma is mainly influenced by the grape variety or by fermentative process.

We studied VOCs profile in red wines from the Valley of Tulum (Province of San Juan-Argentina), aiming to assess if VOCs profile could allows to differentiate between different wines produced with different yeast (autochthonous *S. cerevisiae*: Yeast1, Yeast2; commercial *S. cerevisiae*: Yeast3, Yeast4) and fermentation type (inoculated or spontaneous). So, we studied 100% pure wine varieties: SyrahYeast1 (n=2), SyrahSpontaneous (n=2), MalbecYeast2 (n=2), MalbecSpontaneous (n=2), MalbecYeast3 (n=2), BonardaSpontaneous (n=2) and BonardaYeast4 (n=2), which were provided by the cellar Augusto Pulenta.

VOCs were determined by HS-SPME, coupled to GC-MS, using a DVB-Carboxen-PDMS fiber. Each sample was analyzed by triplicate. First, we did a qualitative analysis of VOCs present in different samples. Then we applied linear discriminant analysis (LDA) to select seven compounds, which were the most significant to differentiate among wine varieties. These components were identified and quantified using pure standards. The application of forward stepwise LDA on quantitative data matrix allows 98% right discrimination among wine studied, af

fording seven deors: Diethyl succinate (fruits), Isopentyl acetate (banana), Ethyl hexanoate (green apple), 1-Hexanol (herbaceous-vegetal), Ethyl octanoate (ripe fruits-pear-sweet) and Benzyl alcohol (flowery-sweet). We observed that VOCs profile is mainly affected by grape variety and to a less extent by the yeast used during fermentation, although relative amounts of VOCs are influenced by the inoculated yeast and fermentation process.

Keywords: wine, aroma, VOCs, SPME

CONICET and TRACE consortia UE-FP6

M-22
COMPARATIVE ESSENTIAL OIL COMPOSITION OF LAVENDULA SPECIES FROM INDIA

Archana Raina[1*], K. S. Negi[2]

[1] NBPGR, ICAR, New Delhi, India
[2] NBPGR, ICAR, Bhowali, India
[*] Corresponding author–E-mail: aprraina@yahoo.co.in; Phone: 91-9811198033

The chemical composition of the essential oils of two species of *Lavendula viz; Lavendula stoechas* and *Lavendula angustifolia* growing in India was investigated by capillary GC and GC/MS. The oil isolated from the aerial parts of the plant (leaves and flowers) by hydrodistillation showed an oil yield of 0.86% in *L. stoechas* and 1.53% in *L. angustifolia*. Twenty five components amounting to *ca.* 96.97% of the oil were identified in *L. stoechas* L while thirty one compounds were identified in *L. angustifolia,* representing 91.53%. The major compounds present in the oil of *L. stoechas* were camphor (52.12%), fenchone (11.96%), 1,8-cineole (9.72%), bornyl acetate (6.18%), camphene(3.26%), terpinene-4-ol (1.64%) and α-pinene (1.09%) whereas those in the oil of *L. angustifolia* were linalool (21.64%), linalyl acetate (35.77%), 1,8-cineole (1.54%), lavendulyl acetate (4.85%), β-caryophyllene (1.85%), terpinene-4-ol (2.01%), camphor(1.36%), borneol (1.44%) and α-terpinole (6.28%). Our results show that the oil of these two *Lavendula species* consists mainly of oxygenated monoterpenes ranging 86.64% *in L. stoechas* and 82.49% in *L. angustifolia*. A comparison of these *Lavendula* oils from other countries reported in literature shows qualitative and quantitative differences.

Keywords: linalool, camphor, fenchone

Director, NBPGR

M-23
ESSENTIAL OIL COMPOSITION OF ORIGANUM MAJORANA AND ORIGANUM VULGARE SSP. HIRTUM GROWING IN INDIA

Archana Raina[1*], K. S. Negi[2], S. K. Mishra[3]

[1 3] NBPGR, ICAR, New Delhi, India
[2] NBPGR, ICAR, Bhowali, India
[*] Corresponding author–E-mail: aprraina@yahoo.co.in; Phone: 91-9811198033

The chemical composition of the essential oils isolated by hydrodistillation of the aerial parts of *Origanum majorana L.* and *O. vulgare L.* from India were analyzed by gas chromatography and gas chromatography-mass spectrometry. A total of 34 and 28 components were identified accounting for 90.85% and 94.64% of the oils of both *O.majorana* and *O.vulgare*, respectively. The oil of *O. majorana* contained cis-sabinene hydrate (15.76%), terpinene-4-ol (31.15%), sabinene (6.91%), p-cymene (6.83%) and trans- sabinene hydrate (3.86%) as the major constituents. Major components of the *O. vulgare* oil were identified as thymol (33.92%), ρ-cymene (11.99%), γ-terpinene (17.67%), carvacrol (6.90%), myrcene (3.40%), β-caryophyllene (2.06%), linalool (1.04%). It was observed that thymol was the dominant constituent of *O. vulgare* where as oil of *O. majorana* contained cis-sabinene hydrate and terpinene-4-ol as the major constituents.

Keywords: essential oil, gas chromatography

M-24

CHEMOMETRICAL CORRELATIONS OF UMAMI TASTE COMPOUNDS AND MOLECULAR PROPERTIES FOR VARIOUS VEGETABLES OF ROMANIAN ORIGIN

Mirela Praisler[1], Gabriela Iordachescu[2*], Oana Mihaela Niculae[3], Camelia Bonciu[4]

[1] Department of Physics, Faculty of Sciences, "Dunarea de Jos" University, Galati, Romania
[2 4] Department of Food Bioengineering, Faculty of Food Science and Engineering, "Dunarea de Jos" University, Galati, Romania
[3] Institute of Food Bioresources, Bucuresti, Romania
[*] Corresponding author–E-mail: mirela.praisler@ugal.ro; Phone: 0040769292382; Fax: 004 -0236460165

The fifth basic taste UMAMI has characteristic qualities that differentiate it from other tastes, it contributes to savory, as well as palatability and offers a prolonged after taste. The main UMAMI taste compounds, i.e. monosodium glutamate, 5'-ribonucleotides (5'-inosinmonophosphate and 5'-guanosinmonophosphate), as well as proteins, lipids, carbohydrates and ash content were determined for a series of vegetables: dill (Anethum graveolens), lovage (Levisticum officinale), thyme (Thymus vulgaris), pea (Pisum sativum) and two types of mushrooms (Agaricus bisporus and Pleurotus ostreatus). Monosodium glutamate content was determined using an enzyme linked assay (ELISA), and the rest of the UMAMI taste compounds were determined using capillary electrophoresis. The molecular properties were determined by UV-VIS spectroscopy. An exploratory analysis was performed using Principal Component Analysis (PCA) in order to identify the clustering of the products showing similar chemical and molecular properties. The detected multivariate correlations were explained in relation with their meaning for the sensorial analysis. The effect of the geographical origin (climate and soil properties) on the analyzed properties and correlations for dill and lovage ingathering from various locations of the lower Danube region was analyzed.

Keywords: umami, MSG, ribonucleotides, PCA

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M-25

MULTIVARIATE AND SENSORIAL ANALYSIS OF TASTE COMPOUNDS AND SPECTROSCOPIC PROPERTIES FOR VARIOUS CHEESE ASSORTMENTS

Gabriela Iordachescu[1*], Mirela Praisler[2], Camelia Bonciu[3], Oana Mihaela Niculae[4]

[1 3] Department of Food Bioengineering, Faculty of Food Science and Engineering, "Dunarea de Jos" University, Galati, Romania
[2] Department of Physics, Faculty of Sciences, "Dunarea de Jos" University, Galati, Romania
[4] Institute of Food Bioresources, Bucuresti, Galati, Romania
[*] Corresponding author–E-mail: mirela.praisler@ugal.ro; Phone: 0040769292382; Fax: 004 -0236460165

One of the richest UMAMI taste food is Parmesan cheese. UMAMI taste compounds represented by monosodium glutamate, 5'-ribonucleotides (5'-inosinmonophosphate and 5'-guanosinmonophosphate) were determined for 4 types of cheese: Parmigiano reggiano, Emmenthal Svizzera, Pecorino, Caciotta con peperoncino produced in Italy and Parmezan Napolact, Emmenthal Dalia, Gouda Gold Natural and Gouda Gold Picant produced in Romania. Monosodium glutamate content was determined using an enzyme linked assay (ELISA). The other UMAMI taste compounds were determined using capillary electrophoresis. The content of proteins, lipids, carbohydrates and ash were also determined using AOAC methods. An exploratory analysis of the hybrid database was performed using Principal Component Analysis (PCA) in order to identify the similarities among the analyzed products from the point of view of their chemical, molecular and sensorial properties. The variables identified as being the main contributors to the formation of these clusters are discussed. The variables contributing to the cluster discrimination are also related to the origin (raw material) of the assortments and to the characteristic features of technological process. The conclusions of this study aim to stress the most important ways in which the results may be used for the optimization of the taste of the final product.

Keywords: umami, Parmesan cheese, MSG, PCA

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BIOLOGICALLY ACTIVE, HEALTH PROMOTING FOOD COMPONENTS

(N-1 – N-39)

N-1
PROTEIN FORTIFICATION OF CHEESE WITH WHEY PROTEINS

Soumya Prakash[1*]

[1] University of Wales Institute Cardiff
[*] Corresponding author–E-mail: soumyaharish07@gmail.com; Phone: 07725206926

Whey protein being one of the richest source of high quality protein, many attempts made by the researchers over three decades have shown that the production of cheese with whey proteins have difficulties associated with poor physical properties, deterioration in the taste and mouthfeel of the product. There remains a need for a method to incorporate whey protein into cheese without significantly reducing the organoleptic properties of the end product. The aim of this study was to develop a process that provides a method to incorporate the right proportion of whey proteins into cheese without significantly altering the physical and sensory properties. The whey proteins were partially hydrolysed using an enzyme (flavour-pro whey) to release bioactive peptides, thus to increase the nutritional value of cheese. This required dissolving of whey protein isolate powder in water and this whey hydrolysate was subjected to incubation at 37°C for 24 hours to provide functionally enhanced hydrolysed whey proteins. The Degree of Hydrolysis was determined using OPA and UV method. Three different concentrations, 5%, 10% and 15% of hydrolysed whey protein were added to mild cheddar cheese and processed. The resulting end products were subjected to the analysis of physical and sensory characteristics and they were compared with those of the control, mild cheddar cheese from the market. Sensory analysis revealed no flavour defects evident in the cheese with 5% concentration of hydrolysed whey protein blend, but a slight evidence of bitter flavour in the cheese with 10% & 15% concentration of hydrolysed whey protein blend. Texture analysis was conducted using Fracture wedge set (A/WEG)and showed that the cheese retained acceptable firmness with 5% hydrolysed whey proteins but with the increased levels of hydrolysed whey proteins (10% and 15%) the cheese failed to retain acceptable firmness. The cheese being a common ingredient in our daily diet, an attempt was made in this study to enhance the nutrional value by fortification of whey proteins. In this study, it could be concluded that by adding 5% hydrolysed whey protein into mild cheddar cheese we can obtain nutritionally enhanced cheese which retains all the organoleptic properties. This study could be further developed on enhancing the nutrional value of other popular cheese varieties available in the market. Sensory evaluation could be done involving subjects to rate the end product. Tests on other physical properties of cheese such as cohesiveness, adhesiveness, springiness, gumminess, chewiness etc could be carried out.

Keywords: Functional food, Bioactive Peptide

Dr. Ara Kanekanian, The Volac Comapany

N-2
NOVEL 20-HYDROXYECDYSONE CONTAINING PLANTS

Nilufar Mamadalieva[1*], Dilfuza Egamberdieva[2]

[1] Institute of the Chemistry of Plant Substances AS RUz, Tashkent, Uzbekistan
[2] National University of Uzbekistan, Tashkent, Uzbekistan
[*] Corresponding author–E-mail: mnilufar76@yahoo.com; Phone: +998973345272

Phytoecdysteroids are structural analogs of the insect molting hormone ecdysone. Interest to phytoecdysteroids has been stipulated for their big scientific and practical value. 20-Hydroxyecdysone (20E) has adaptogenic, anabolic, immune modulating activities and normalized the carbohydrate and lipids exchanges in the vertebrates which had experimental diabetes. The phytoecdysteroids relate to the low toxic substances–LD_{50} for 20E is 6.4 g/kg (per os) and 9.0 g/kg (preoral) in administration of medicine.

The application of phytoecdysteroids is a promising alternative to the use of anabolic-androgenic steroids because of the apparent lack of adverse effects. The prospective use of 20E may extend to treatments of pathological conditions where anabolic steroids are routinely applied. 20E do not binds to the cytosolic steroid receptors, instead, it is likely to influence signal transduction pathways, like the anabolic steroids, possibly via membrane bound receptors. One of the most cited aspects of phytoecdysteroid application is the increase of muscle size [*www.ecdysbase.org*].

The plants *Rhaponticum carthamoides, Pfaffia irisinodes, Serratula coronata* are used for production of the tonic and anabolic preparation *Ecdystene* (20E). But confined natural resources of these herbal raw plants, relatively low contents of phytoecdysteroide–20E, causes the high cost of the preparation, disturbs the production and its application in medicine. In general, the difference in concentration levels of 20E differs in region 2×10^{-5}–3% from dried weight. Usually the content of 20E in plants is very small – about 0.001 and 0.01%.

It is established that the promising 20E containing species are plants of genus *Silene L*. and it is necessary to find novel plants among this genus. In this study we screened content of 20E from aerial parts 5 *Silene* species plants: *Silene guntensis, S. linicola, S. praemixta, S. pseudotites, S. viridiflora* and *S. wallichiana.* Our studies have shown all *Silene* plants contain 20E, but in different concentration. Studies have shown that *S. praemixta* and *S. viridiflora* are rich phytoecdysteroid containing plants and the yields of 20E are 2.0% and 1.6% respectively (from weight of air dried aerial parts). The results of investigation in other species of *Silene*: *S. guntensis, S. linicola, S. pseudotites* and *S. wallichiana* showed that the yields of 20E of these plants are 0.8, 0.067, 0.5 and 0.8% respectively. Determination of structures and content of isolated 20E was established on basis of data of physical-chemical methods: HPLC-, ^{1}H and ^{13}C NMR spectroscopy.

Thus, the conducted screening results confirm the content of 20E in the *Silene* plants of Uzbekistan. The plants *S. praemixta* and *S. viridiflora* may be used for production of the tonic and anabolic preparation *Ecdystene*.

Keywords: Plant, phytoecdysteroids, biological activity

N-3
CHITOSAN THAT DISSOLVED IN HARD WATER SUPPRESS MUTAGENICITY

Kentaro Yoshikawa[1*]

[1] KINKI University, Nara, Japan
[*] Corresponding author–E-mail: ken_yoshikawa@nara.kindai.ac.jp; Phone: +81-742-43-8293; Fax: +81-742-43-8293

Chitosan is derived by deacetylation of chitin, a major component of the shells of crustacea such as crabs and shrimps. Chitosan has been reported to have antitumor and anticancer activities although those details have not been clarified yet. In this experiment using the Ames test, there was antimutagenic effect by the water-soluble chitosan that dissolved in hard water (WSC). For this study, Chitosan Food Industry, Ltd supplied WSC. The Ames test was performed using Salmonella typhimurium TA98 and TA100. For the antimutagenic test, and the desmutagenic and bioantimutagenic tests, Trp-P-2, 2AA and AF-2 were used as mutagens. Tests for spontaneous mutation rate without mutagens were performed using S. typhimurium TA98 and TA100. When several concentrations of WSC were directly added to Minimal Glucose Plates, there were no changes in the number of His+ revertants of TA98 and TA100. In desmutagenic and bioantimutagenic tests, the number of His+ revertants of TA98 and TA100 decreased. In the test for spontaneous mutation rate, the number of His+ revertants of TA98 and TA100 decreased slightly. When a concentration of WSC was added to examine for antimutagenic effects, there was no change in the viable cell count of TA98 and TA100. From these results, AF-2 that is a direct mutagen may combine with WSC, and that may also be carrying out the inactivation. WSC possibly acts directly on the DNA by decreasing the rate at which mutation is caused to the strain's promutagens Trp-P-2 and 2AA. This is evident from the decreased spontaneous mutation rate without a decreased viable cell count.

Keywords: chitosan, antimutagen, desmutagen, bio-antimutagen

N-4
DEVELOPMENT OF AN ALL-INCLUSIVE METHOD FOR THE MEASUREMENT OF DIETARY FIBRE.

Anna Draga[1*], Ida Lazewska[2], Barry McCleary[3]

[1 2 3] Megazyme International Ireland Limited, Bray, County Wicklow, Ireland.
[*] Corresponding author–E-mail: anna@megazyme.com; Phone: +(353 1) 2861220; Fax: +(353 1) 2861264

A procedure for the measurement of dietary fiber, that includes measurement of resistant starch (RS) and non-digestible oligosaccharides (NDO), has been developed. This procedure is consistent with the CODEX proposed definition of dietary fibre. The procedure involves the incubation of sample with pancreatic alpha-amylase plus amyloglucosidase under conditions similar to those used in the measurement of resistant starch. The pH is adjusted to 8, and the sample is heated to ~ 100°C to denature protein, and then incubated with protease. Adjustment of pH to 8 before heating to 100°C is performed to prevent depolymerisation of resistant starch that is solubilised during the heating step. The pH is re-adjustment to approx. 4 and high molecular weight dietary fiber is precipitated from solution and then recovered, along with insoluble dietary fibre, by filtration. The residue is dried, weighed and duplicate samples are analysed for protein and ash. The ethanolic filtrate is concentrated, desalted and analysed for non-digestible oligosaccharides. This method is currently the subject of an AOAC International/AACC International interlaboratory evaluation. In this presentation, the method will be described in detail and some of the problems experienced in running the method will be discussed.

Keywords: TDF, Total Dietary Fibre

N-5

CARBOHYDRATE SCREENING PLATFORM FOR FRACTIONATION, IDENTIFICATION AND QUANTIFICATION OF BIO-ACTIVE OLIGOSACCHARIDES IN COMPLEX FOOD MIXTURES

Leon Coulier[1*], Richard Bas[2], Johan Timmermans[3], Bart Keijser[4], Ted Slaghek[5], William van Dongen[6]

[1 2 3 4 5 6] TNO Quality of Life, Zeist, the Netherlands
[*] Corresponding author–E-mail: leon.coulier@tno.nl; Phone: +31 30 694 48 88; Fax: +31 30 694 48 94

Carbohydrate mixtures from biological sources such as human milk and plant, or produced via biosynthesis are by definition very complex. The analysis of these complex mixtures of oligosaccharides is still a challenge in the field of analytical chemistry. Various analytical techniques have been applied to complex mixtures of oligosaccharides in literature, the choice of analytical technique depending on the required level of detail, the type of carbohydrate product and perhaps availability. Due to the nature of carbohydrates and the characteristics of the synthesis of oligosaccharides, mixtures of oligosaccharides may contain hundreds of individual compounds including many isomers. These compounds might differ in the type of monomers, the number of monomers, the order of monomers in the chain and the linkages between monomers. We developed a screening platform that is capable of identifying and quantifying individual oligosaccharides in complex mixtures by a combination of analytical techniques. One of the techniques developed and applied is the direct coupling of high-performance anion-exchange chromatography (HPAEC) to mass spectrometry (MS) which combines the high separation power for (oligo)saccharides of HPAEC with the highly sensitivity and selectivity of MS. It will be shown that HPAEC-MS is an ideal technique for fingerprinting and quantifying/identifying individual compounds in complex mixtures of oligosaccharides.

It is known that oligosaccharides present in these complex mixtures can promote human health by stimulating health-beneficial bacteria in the colon or stimulate the immune system. The screening platform is also capable of isolating (bioactive) fractions that can be tested in an in vitro digestive system to study the bioavailability and to screen for prebiotic activity using advanced micro-array techniques. With HPAEC-MS the fractions before and after microbial screening can be analyzed to pinpoint specific compounds in the fractions that exhibit beneficial properties.

We describe the experimental set-up as well as application of the screening platform to complex mixtures of oligosaccharides, like galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS) and human milk oligosaccharides (HMO).

Keywords: prebiotics, oligosaccharides, bioactivity, analysis, HPAEC-MS

N-6

FATTY ACID CONTENT AND COMPOSITION OF INTRAMUSCULAR FAT IN "MINHOTA" BREED CALVES: COMPARISON BETWEEN MALE AND FEMALE FROM TWO DIFFERENT GROUPS OF ANIMALS, VEAL AND BEEF

P. Pires[1*], J. P. Araújo[2], M. J. O. Barros[3]

[1 3] Instituto Politécnico de Viana do Castelo, Escola Superior de Tecnologia e Gestão, Av. do Atlântico, 4900-348 Viana do Castelo, Portugal
[2] Instituto Politécnico de Viana do Castelo, Escola Superior Agrária, Escola Superior Agrária - Refóios do Lima, 4990-706 Ponte de Lima, Portugal
[*] Corresponding author–E-mail: ppires@estg.ipvc.pt; Phone: 258 819777; Fax: 258 827636

Fatty acid content and composition of intramuscular fat in "Minhota" breed calves: Comparison between male and female from two different groups of animals, veal and beef Minhota breed is an autochthonous bovine raised in between Douro and Minho (Norwest of Portugal), in which the meat production is the main aptitude. The fatty acid distribution in Longissimus thoracis muscle from forty one Minhota breed young animals of two groups, veal or "vitela" (22 animals, 14 males and 8 females) and beef or "vitelão" (19 animals, 11 males and 8 females) was measured and compared. Both groups of animals were reared in an traditional production system of unweaned young calves with indoor management, maternal suckling, and complementary concentrate-based diet, and slaughtered at six month of age for veal group (245-285 kg live weight) and at nine month of age for beef group (300–400 kg live weight). The veal group had lower ($p < 0.001$) weight and age than beef group. Samples of the Longissimus thoracis muscle with no visible adhering subcutaneous or intramuscular adipose tissue was analysed for fat content. The fat composition of the 10th rib was: 1.57% vs. 2.09% for the male veal and beef and 2.38% vs. 2.32% for the female veal and beef, respectively. The fatty acid (FA) composition of muscle was determined by gas liquid chromatography (GLC) after derivatization to the FAMEs. Comparing the veal and beef males, there is a decrease for all n-3 FA and for n-6 arachidonic acid, from 3.96 and 1.23 to 2.24 and 0.918 g FA/100g total FA, respectively. This corresponds to a reduction of 43% in total n-3 fatty acids and a reduction of 52% in n-6 arachidonic fatty acid in older animals when compared to younger males. In the female animals, it was verified that age does not influence the fatty acid profile (veal and beef have the same fat composition) and the percentage fat of the Longissimus thoracis muscle, with an exception for the lignoceric acid.

Keywords: FA, Meat quality, Healthy nutrition

N-7

SELECTIVE PRODUCTION OF CONJUGATED LINOLEIC ACID ISOMER CIS-9,TRANS-11 FROM LINOLEIC ACID BY BIFIDOBACTERIUM BREVE CECT 4839

Cristina García-Marzo[1*], Félix Amárita[2], Josune Ayo[3]

[1 2 3] AZTI-Tecnalia, Food Research Division, Derio (Bizkaia) Spain
* Corresponding author–E-mail: cgarcia@azti.es; Phone: +34 946574000; Fax: +34 946572555

Conjugated linoleic acid (CLA), a mixture of isomers of octadecadienoic acids with conjugated double bonds, has attracted much attention as a novel type of biologically beneficial functional lipid. Some isomers have been reported to reduce body fat, carcinogenesis and atherosclerosis, i.e. *cis*-9,*trans*-11 (c9,t11) 18:2 and *trans*-10,*cis*-12 (t10,c12) 18:2. There are different methods to produce these isomers such as the microbiological transformation of linoleic acid into CLA. This method has the advantage of being isomer-selective, unlike chemical synthesis which produces a mixture of CLA isomers, c9,t11 and t10,c12 at the same ratio. Several lactic acid bacteria have been described as CLA producers. Therefore, the objective of the present study was to investigate the ability of *Bifidobacterium breve* CECT 4839 to convert linoleic acid into CLA.

B. breve was cultured in MRS with 0.05% cystein, 2% tween 80 and 0.5 mg/mL of free linoleic acid and incubated under O_2-limited conditions for 0, 24, 48, 72, 96 and 168 h. *B. breve* was also cultured in the same medium without linoleic acid as a control. After each incubation time, fatty acids were extracted, quantified by GC-MS chromatography and viable count was determined by plating on MRS cystein agar. The assay was replicated. Each experiment was subjected to One-way ANOVA and LSD test was applied at a significance level of 0.05.

The isomer produced was identified as c9,t11 18:2. There was no production of other CLA isomers. The c9,t11 18:2 content increased ($p<0.05$) with incubation time from 0 to 72 h. While CLA increased, linoleic acid decreased significantly until 72 h, suggesting that linoleic acid was the substrate for CLA production. These results were closely related to the growth of *B. breve.* The maximum conversion rate of linoleic acid to c9,t11 18:2 was obtained after 72 h of incubation (31.3%). However, the optimum CLA production time was 48 h (maximum productivity – 2.1 µg/mL/h), during the exponential growth phase of *B. breve.* After 72 h, there were no changes ($p>0.05$) in the isomer and linoleic acid concentration, probably due to the bacterial low growth rate in the stationary phase.

It can be concluded that *B. breve* CECT 4839 is an interesting microorganism capable of selectively converting linoleic acid to the CLA isomer c9,t11 18:2.

Keywords: Conjugated linoleic acid (CLA)

The authors thank Natalia Gutierrez and Elisabette Bilbao for their technical assistance.

N-8
EVALUATION OF SOLUBILITY AND STABILITY OF LUTEIN IN FOOD GRADE MICROEMULSIONS

Zoran Rodić[1*], Breda Simonovska[2], Irena Vovk[3]

[1 2 3] National Institute of Chemistry, Ljubljana, Slovenia
[*] Corresponding author–E-mail: zoran.rodic@ki.si; Phone: +38614760432; Fax: +38614760300 (subject: Rodic Zoran)

Microemulsions are transparent, thermodynamically stable, isotropic mixtures of oil phase, aqueous phase and surfactant. Microemulsions are used in pharmacy as drug delivery vehicles for various drug agents such as poorly soluble drugs, therapeutical proteins etc., but they also have a promising potencial in the field of food technology since trends in food industry lead to the production of functional foods enriched with biologically active compounds beneficial to human health, which includes for instance vitamins, phytosterols, carotenoids, flavonoids, anthocyanins, fibers etc.

Carotenoids are a diverse group of lipophilic compounds that contribute to the yellow to red colours of many foods. Carotenoids are polyenes consisting of 3 to 13 conjugated double bonds and they have been proposed to exhibit several potential health benefits due to their antioxidant activity. Lutein belongs to this diverse group of compounds and has an important role in human vision. Lutein is concentrated in the yellow spot (*Macula lutea*), where it helps to protect from oxidative stress and acts as a blue-light filter and thus decreases age-related macular degeneration and cataracts. In human diet lutein occurs in green leafy vegetables such as spinach and kale. Endogenous carotenoids in foods are generally stable, however, as food additives carotenoids are relatively unstable in food systems, because they are susceptible to light, oxygen and autooxidation. Consequently, dispersion of carotenoids into ingredient systems can result in their rapid degradation by reactions that cause the loss of double bonds, scission of the molecule or isomerization.

Our goal was to prepare vehicles based on food grade ingredients for delivery of nutraceuticals and to determine solubilization capacity and stability of lutein in this systems. The impact of the food grade microemulsions, prepared according to Garti et al. [1], on solubility and stability of lutein was studied. Lutein content was determined by UV-VIS spectrophotometry and high performance liquid chromatography on C30 column, which is capable to separate the geometric isomers of lutein.

[1] N. Garti, I. Yuli-Amar, Micro- and nano-emulsions for delivery of functional food ingredients, In: Delivery and controlled release of bioactives in foods and nutraceuticals, N. Garti Ed., Cambridge: Woodhead Publishing Limited, 2008, pp. 149-183.

Keywords: lutein, microemulsions, solubility, stability

This study was carried out with financial support from the Slovenian Research Agency.

N-9

THE CONTENT OF SELENIUM AS WELL AS OTHER TEN BIOLOGICAL ACTIVE METALS IN BOLETUS EDULIS FROM TWO AREAS IN BULGARIA AND INHIBITION OF BLOOD LIPIDS PEROXIDATION BY MUSHROOMS

Georgi Bekyarov[1*], P. Paraskova [2], M. Kakalova[3], A. Iliev[1], Tsv. Bekyarova[4], G.V. Donchenko[5], O.M. Palyvoda[6], O.V. Viduk[7], O. Kuzmenko[8]

[1 2 3 4] Canning Research Institute, 154 V. Aprilov Blvd., Plovdiv, 4000, Bulgaria
[5 6 7 8] Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, 9 Leontovich St, Kiev, 01601, Ukraine
* Corresponding author–E-mail: gbekyarov@abv.bg; Phone: +359 32 951351; Fax: +359 32 952 286

The goal of this study was to determine the content of such biological active metals as Se, Hg, Al, Cu, Zn, As, Cd, Pb, Mg, Ca and Fe in *Boletus Edulis* mushrooms and to study the effect of mushrooms as inhibitors of blood serum copper-initiated lipid peroxidation. The metals content was determined by ICP–OES technique and blood lipids peroxidation *in vitro* was assessed by thiobarbituric acid-reactive substances measurement. The dependency between quality and content of the determined biological active metals has been traced. Samples were analyzed of wild growing mushrooms *Boletus Edulis* from two mountain regions in Bulgaria. On the average the content of Se in *Boletus Edulis* was found to be 25 mg/kg dried mushroom, this content being higher in tubules than in fleshy part. We found that *Boletus Edulis* mushrooms inhibited lipid peroxidation in the concentration dependent manner. The lipid peroxidation inhibition is starting at the *Boletus Edulis* concentration of 0.5 mG/ml and approaching maximum at the concentration of 1.0 and 2.5 mG/ml. The similar inhibition of lipid peroxidation by *Cantharellus Cibarius* occurs only at the concentration of 2.5 mG/ml. The effective concentration of *Boletus Edulis* is in 5 times lower compare to the concentration of *Cantharellus Cibarius* resulting in similar lipid peroxidation inhibition. This effect can be explained by 56 times higher content of Se and by 1.5 and 3 times lower content of such initiators of lipid peroxidation as Cu and Fe in *Boletus Edulis* compare to *Cantharellus Cibarius.*

A system with a source of infrared radiation heating, developed by authors, was used for the mushroom mineralization. We conclude that *Boletus Edulis* is an effective inhibitor of blood lipid peroxidation and in 5 times more stronger rather than *Cantharellus Cibarius.*

This work is supported by the Bulgarian National Science Funds of the Ministry of Education and Science of Bulgaria and the Ministry of Education and Science of Ukraine through the bilateral Grant to Dr. Bekyarov and Dr. Kuzmenko.

Keywords: Selenium Boletus Edulis antioxidants

This work is supported by the Bulgarian National Science Funds of the Ministry of Education and Science of Bulgaria and the Ministry of Education and Science of Ukraine through the bilateral Grant to Dr. Bekyarov and Dr. Kuzmenko.

N-10
EVALUATION OF ISOFLAVONOID CONTENT IN PHYTOESTROGEN FOOD SUPPLEMENTS

Radka Koblovská[1*], Lenka Pučelíková[2], Oldřich Lapčík[3]

[1 2 3] ICT Prague
[*] Corresponding author–E-mail: radka.koblovska@vscht.cz; Phone: +420-220443240

A large number of phytoestrogen food supplements indicated for their positive health effects in menopause are available on the market. Most often, they are based on isoflavones from legumes, i.e. soy (*Glycine max*) and red clover (*Trifolium pratense*) or on extracts from *Cimicifuga racemosa*, a non-legume. In previous studies, a lack of standardization of these products has been repeatedly reported.

The aim of this study was to evaluate how well the isoflavone content data supplied by the producers correspond to our analysis results. Thirteen different products purchased in Czech Republic were analyzed using high performance liquid chromatography with electrospray ionization-mass spectrometry (HPLC-ESI-MS) to determine total amount of isoflavones and identify the different forms of the isoflavone conjugates. Five products were based on clover extracts, six were from soy, one product was from combination of both legumes and one product was from *Cimicifuga racemosa*.

With one exception, the content of isoflavones measured in soy- and clover- based supplemets was always lower than declared on the label, varying from 8.4% to 104% of the nominative amount. The total isoflavonoid contents found in two different lots of the same product differed markedly (more than 20%) in four of five cases. In accordance with phytochemical data, higher ratio of 4´-methoxyisoflavones were found in clover-based products. No isoflavones were recorded in the *Cimicifuga racemosa*-based supplement.

Keywords: phytoestrogen, food supplement, HPLC-MS, isoflavone

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N-11
ISOFLAVONOIDS IN HOPS

Radka Koblovská[1*], Pavla Balátová[2], Petra Lanková[3], Pavel Dostálek[4], Oldřich Lapčík[5]

[1 2 3 5] Department of Chemistry of Natural Compounds, Institute of Chemical Technology, Technická 5, 166 28 Praha 6, Czech Republic
[4] Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology, Technická 5, 166 28 Praha 6, Czech Republic
[*] Corresponding author–E-mail: radka.koblovska@vscht.cz; Phone: +420-220443240

Isoflavones (3-phenyl chromones) are plant secondary metabolites displaying numerous biological activities in vertebrates, among which the estrogen-like activity is of major concern. They are abundant in the Fabaceae family (about 1000 known structures) and several others, e.g. Iridaceae, Moraceae, Rosaceae, Asteraceae. Previously, small amounts of isoflavonoids have been found in beer, but their origin in this foodstuff was unclear. Mazur detected daidzein and genistein in several samples of barley[1].

In recent study we have tested eight cultivars of hops (*Humulus lupulus*) of Czech and German origin (namely: Premiant, Sládek, Rubín, Žatecký poloraný červeňák, Bor, Harmonie, Hersbrucker, Tettnang) for the presence of twelve metabolites synthesized at the early steps of the isoflavonoid biosynthetic pathway. Seven compounds of interest were aglycones (i.e. daidzein, genistein, glycitein, formononetin, isoformononetin, biochanin A, prunetin) and five were glycosides (i.e. daidzin, genistin, glycitin, ononin, sissotrin).

Dry hops were pulverized and extracted with a mixture methanol/water. The extracts were analyzed by HPLC-ESI-MS and by specific ELISAs, either directly or after a SPE clean-up. These approaches revealed a spectrum of isoflavonoids, aglycones as well as glycosides, in all cultivars. The concentrations of individual compounds ranged from units up to tens of milligrams per kg (dry weight). Methoxy isoflavones prevailed to non-methylated ones. The highest content of isoflavones has been found in the Sládek cultivar.

Our data show, that hops represent the additional source of isoflavonoids in beer. However, when compared to legumes, the amount of bioactive isoflavonoids in hops is by several orders of magnitude lower.

Keywords: Isoflavone, hops, HPLC-MS, ELISA, phytoestrogen

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N-12

PROFILES OF BETALAINS IN RED BEET ROOT (BETA VULGARIS L.) EXTRACTS ENRICHED BY A NEW METHOD

Aneta Spórna[1], Paweł Stalica[2], Zbigniew Pietrzkowski [3], Boris Nemzer[4], Wayne Tresher [5], Slawomir Wybraniec [6*]

[1 2 6] Department of Analytical Chemistry, Institute C-1, Cracow University of Technology, Cracow, Poland
[3 4 5] Department of Bio-Research, FutureCeuticals, Inc., Momence, USA
[*] Corresponding author–E-mail: swybran@chemia.pk.edu.pl; Phone: +48-12-628-3074; Fax: +48-12-628-2036

Red beet (*Beta vulgaris* L.) roots have been the most exploited source of betalains. Betalains, primarily used as food colorants, have strong antioxidant properties. Red beet roots contain two groups of betalain pigments: red-violet betacyanins and yellow betaxanthins. The main betacyanin present in the roots is betanin. Red beet colorants are available as concentrates produced by evaporating beet juice under vacuum, or as a powder made by spray-drying the concentrate. The powdered products are more stabile because of lower degradation rate of the pigments. The degradation of the pigments depends on temperature, heat duration, pH and water activity of a product [1].

In spite of efforts to optimize the spray-drying process, the concentration of betalains in the resulting products is still limited. New methods that will make possible the production of high-pigmented formulations for food or medicinal use are searched.

Recently, a new proprietary method of a large-scale chromatographic purification of red beet root extract has been discovered and developed that allows for production of more concentrated betalain formulations. In order to trace the betalainic compositions of the new products in comparison to the currently in-use spray-dried extracts, a chromatographic study on betalains and their degradation derivatives analyzed by LC-DAD-ESI-MS was performed [1].

Besides the most prominent betanin/isobetanin, elevated levels of neobetanin, which had been frequently detected in *Beta vulgaris* L. roots, were also observed in the new products. The presence of excessive amounts of neobetanin had been attributed many times to the degradation of betanin during processing of the samples [1], however, in this study, the pre-concentration factor was similar to that of betanin/isobetanin suggesting that the presence of neobetanin was rather a result of its enrichment than dehydrogenation of betanin. Interestingly, the concentration of both diastereomers of betanin/isobetanin was similar, indicating a possible epimerization during the whole process of extract enrichment. The principal betanin/isobetanin pigments were mostly responsible for the total betalain content (24.6%) measured spectrophotometrically. This is the highest concentration of betalains reported in industrial products to date. In addition, the presence of other decarboxylated and dehydrogenated betanin derivatives [1] in the new products was discussed.

[1] Wybraniec S. 2005. Formation of decarboxylated betacyanins in heated purified betacyanin fractions from red beet root (*Beta vulgaris* L.) monitored by LC-MS/MS. *J Agric Food Chem* 59: 3483-3487.

Keywords: betanin; neobetanin; betalains; Beta vulgaris

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N-13
BUCKWHEAT AND WHEAT FLOUR: ANTIOXIDANT COMPONENTS AND ACTIVITY

Ivana Sedej[1*], Anamarija Mandić[2], Aleksandra Mišan[3], Marijana Sakač[4]

[1 2 3 4] Institute for Food Technology, University of Novi Sad, Novi Sad, Serbia
[*] Corresponding author–E-mail: ivana.sedej@fins.uns.ac.rs; Phone: +381 21 485 3812; Fax: +381 21 450 725

The cultivation of pseudocereal buckwheat (Fagopyrum esculentum Moench) has gained raising attention, due to many positive physiological effects. Therefore, presence of plant phenolics and tocopherols in two types of buckwheat flour (light and wholegrain) in comparison to wheat flour (light and wholegrain) were investigated. Plant phenolics and tocopherols (α, γ and δ) were identified and quantified by fast reverse phase HPLC method, with DAD detection. Obtained results of plant phenolics were compared to those obtained by Folin–Cioacalteu method for total phenolics determination. Since phenolics may significantly contribute to overall antioxidant activity, in addition, antioxidant activities of buckwheat and wheat flour were tested using 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$)-scavenging activity. Rutin and quercetin were quantified in both buckwheat flour. Rutin content was 0.08 in light and 0.19 mg/g in wholegrain flour, respectively. Quercetin content was 0.05 mg/g in both buckwheat flour. In wholegrain wheat flour ferulic acid amount was 0.018 mg/g. α, γ and δ tocopherols were identified and quantified in all four flour, with exception of light wheat flour where δ tocopherol was not detected. Total phenolics content (expressed as gallic acid equivalent, GAE) in wheat flour varied between 37.1 and 137.2 μg GAE/g extract, while its content in buckwheat flour ranged between 476.3 and 618.9 μg GAE/g extract. DPPH• activities were higher in buckwheat than in wheat flour, indicated by lower IC_{50} values, as the consequence of higher phenolic content in buckwheat flour. Those values were 34.24 and 31.26 mg/mL, and 1.87 and 1.49 mg/mL for wheat and buckwheat flours, respectively. Significant positive correlation between the results of total phenolics and antioxidant activity, expressed by IC50 values, was obtained (-0.98, $p<0.05$). Although highly positive, the correlation between total phenolics and total tocopherols (0.94, $p<0.05$) was not statistically significant. The data suggest the possibility to improve the antioxidant properties of wheat-based food products through addition of buckwheat flour or complete substitution of wheat flour in order to create functional foods

Keywords: buckwheat, wheat, phenolics, tocopherol

N-14

HPLC DETERMINATION OF MEDICINAL PLANT PHENOLICS IN CRUDE EXTRACTS

Aleksandra Mišan[1*], Anamarija Mandić[2], Neda Mimica-Dukić[3], Marijana Sakač[4], Ivana Sedej[5], Ivan Milovanović[6]

[1 2 4 5 6] University of Novi Sad, Institute for Food Technology Novi Sad, Bul. cara Lazara 1, 21000 Novi Sad, Serbia
[3] University of Novi Sad, Faculty of Sciences, Department of Chemistry, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia
[*] Corresponding author–E-mail: aleksandra.misan@fins.uns.ac.rs; Phone: +381 21 485 3829; Fax: +381 21 450 725

HPLC/DAD method, on an Agilent, Eclipse XDB-C_{18}, 1.8 μm, 4.6 × 50 mm column, was developed to enable a rapid separation, of a mixture of 17 compounds, which consisted of hydroxybenzoic acids, hydroxycinnamic acids, flavones, flavonols, flavanone, flavonol-glycoside and antraquinone, in a single run. The method was successfully applied to analyse the phenolic components in crude ethanolic extracts of parsley (*Petroselini fructus*), buckthorn (*Frangulae cortex*), mint (*Mentha piperitae folium*), and caraway (*Carvi fructus*) and birch (*Betulae folium*). Thus, the separation and quantification of gallic acid, vanillic acid, syringic acid, protocatechuic acid, trans-cinnamic acid, ferulic acid, caffeic acid, rosmarinic acid, chlorogenic acid, luteolin, apigenin, myricetin, kaempferol, quercetin, naringenin, rutin and aloe-emodin in the samples was achieved. In order to overcome the disability to quantify all the phenolic compounds present in the samples, caused by lack of external standards, the total phenolic content of the samples was calculated as the sum of all integrated areas at 280 nm and expressed as gallic acid equivalent, and also as the sum of all integrated areas at 350 nm, expressed as rutin equivalent. These results were compared with the total phenolics determined by Folin–Cioacalteu method, and total flavonoids measured according to the Markham method. Obtained values of the total phenolic content determined according to Folin–Cioacalteu method were of the same order of magnitude as those obtained by HPLC method expressed as gallic acid equivalents.

Keywords: HPLC, plant phenolics, medicinal plant

N-15

EFFECT OF DIFFERENT PARAMETERS ON THE PRODUCTION OF CONJUGATED LINOLEIC ACIDS FROM LINOLEIC ACID BIOCONVERSION BY LACTIC ACID BACTERIA

Maurizio Quinto[1*], Giuseppina Spadaccino[2], Giuseppe Gambacorta[3], Michele Faccia[4], Rosanna Tofalo [5], Maria Schirone[6], Sara Valmorri[7],Aldo Corsetti[8]

[1 2] Department of Agri-Environmental Sciences, Chemistry and Plant Protection, University of Foggia, via Napoli 25, 71100 Foggia, Italy
[3] Department of Food Sciences, University of Foggia, via Napoli 25, 71100 Foggia, Italy
[4] Department of Animal Products, University of Bari, via Amendola 165/A, 70126 Bari, Italy
[5 6 7 8] Departmet of Food Science, University of Teramo, 64100 Teramo, Italy
[*] Corresponding author–E-mail: m.quinto@unifg.it; Phone: +39 0881 589102; Fax: +39 0881 740211

In the last years, with relation to the numerous benefits attributable to CLA (conjugated linoleic acids), the interest is increasing about the synthesis of such compounds in order to prepare healthy foods or ingredients. To date just a few studies have dealt with the possibility to use lactic acid bacteria to increase the CLA content of foods. Above all the 56 possible CLAs, two of them have been found particularly active for their anti-carcinogenic properties (1); 9-cis,11-trans 18:2, and 10-trans,12-cis 18:2. CLAs are commonly found in foods, in particular in ruminant milk and meat, because these isomers are formed during biohydrogenation of linoleic acid in the rumen and/or through conversion of vaccenic acid in the mammary gland. It is well known that several strains of Lactobacillus, Propionibacterium, Bifidobacterium and Enterococcus, usually present during dairy manufacturing, are also able to form CLAs from linoleic acid (2). Their presence during cheese production, together with processing conditions, can then influence the concentration of CLAs in the final product. Based on this preliminary statements, our studies evaluated the capability of 22 strains of Lactobacillus casei isolated from cheeses to produce, in vitro, bioactive CLA isomers (especially cis-9,trans-11 and trans-10,cis-12 C 18:2) through linoleic acid (LA) bioconversion. Four CLA isomers were separated and quantified (respect to total lipid), by using silver-ion-HPLC. LA concentration in the culture media, the effect of strain pre-cultivation and adaptation in medium added with LA, time and temperature of fermentation as well as other parameters were evaluated in order to produce CLA and/or CLA enriched cells through cultivation in bioreactor. Preliminary results showed that CLA bioconversion was strain dependent and greatly related to the conditions applied to cultivate selected strains.

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Keywords: CLA, Functional food, silver-ion chromatography

N-16

VOLTAMMETRIC AND SPECTRAL CHARACTERIZATION OF ASCORBIGEN AND ITS DETERMINATION IN SAUERKRAUTS FROM WHITE CABBAGE BY HPLC WITH ELECTROCHEMICAL AND UV DETECTION

Danuta Zielińska[1*], Juanna Frias[2], Elena Penas[3], Serafin Valverde[4], Conception Vidal-Valverde[5]

[1] University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
[2 3 5] Instituto de Fermentaciones CSIC, Madrid, Spain
[4] Instituto de Quimica CSIC, Madrid, Spain
[*] Corresponding author–E-mail: dziel@uwm.edu.pl; Phone: +48 089 523 39 35; Fax: +48 089 523 48 01

Ascorbigen (ABG), 2-C-(indol-3-yl)methyl-α-L-xylo-hex-3-ulofuranosono-1,4-lactone (an indole derivative) is derived from alkaloid glucobrassicin in plants belonging to the Cruciferae family and naturally occurs mainly in Brassica vegetables. Glucobrassicin is enzymatically hydrolyzed by the endogenous enzyme myrosinase (β–thioglucosid glucohydrolase; EC 3.2.3.1.) to indole-3-carbinol, which in turn reacts with cyclized L-ascorbic acid to ABG. Ascorbigen is a dietary supplement under provisions of US Dietary Supplement Health and Education Act of 1994 and it has now been recognized as a useful pharmacological substance with a variety of actions both in vitro and in vivo. Ascorbigen is the most stable of commercially available dietary indols. It requires neither refrigeration nor protection from light. Degradation occurs at temperatures in excess of 50°C. In the present study, ABG was synthesized by the reaction of ascorbic acid and 3-hydroxymethyl indol. The confirmation of its structure and determination of its purity was carried out by means of 1H NMR and ^{13}C NMR spectra, and HPLC, respectively. The electrochemical behaviour of ASG was studied by cyclic voltammetry (CV) method for pH within the range of 3.0–7.5 and further characterization was performed by UV-VIS spectroscopy. Voltammetric studies of ABG at glassy carbon electrode showed one irreversible oxidation peak (centred at Ep = 0.952 V for pH 5.0) whilst that one originated from the oxidation of L-ascorbic acid occurred at Ep = 0.376 V for the same pH. The peak-current potential related to the irreversible oxidation of ABG was shifted towards more positive potentials with decreasing pH values. The linear response between concentration of ABG within the range of 0.08–0.75 mM and recorded current was observed. The UV-VIS spectral characteristic of ABG showed strong absorption band centred at 280 nm. Electrochemical and spectral behaviour of ABG was applied for the determination of natural ABG in sauerkrauts from white cabbage by HPLC with coulometric array and a variable-wavelength UV detectors. Both used detection methods were applicable for determination of ABG in sauerkrauts by means of HPLC technique.

Keywords: ascorbigen, voltammetry, HPLC, sauerkrauts

N-17
WALNUT PROTEINS AS SOURCE OF BIOACTIVE PEPTIDES

Maria del Mar Caja[1], Mercedes Ramos[2], Jose Angel Gómez-Ruiz[3*]

[1 2 3] Instituto de Fermentaciones Industriales (IFI), CSIC, Madrid, SPAIN
[*] Corresponding author–E-mail: Jagomez-ruiz@ifi.csic.es; Phone: 0034637747297; Fax: 0034915644853

In recent years, walnuts have gained a well-deserved recognition thank to their content in omega-6 and omega-3 polyunsaturated fatty acids (PUFA) and the positive health effects associated to the consumption of foodstuff rich in these components. Unlike other edible nuts, walnuts present an elevated content in omega-3 PUFA and their inclusion in a healthy diet has been reported to lower serum cholesterol and triglyceride concentrations protecting against coronary heart disease (CDH) [1].

In contrast to the lipid fraction, the protein fraction of walnut has received little attention. Different studies show that this fraction represents around the 15–20% of the edible part of the walnut [2,3]. Therefore, walnut proteins may be a potential vehicle of bioactive peptides. In order to investigate the bioactivity of the protein fraction, we have isolated the walnut proteins by sequential extraction using different solvents. Subsequently, the major fraction (glutelin) has been hydrolyzed with different enzymes from microbial and plant origin. Three different bioactivities have been assessed in this study. On the one hand, the potential role of walnut hydrolysates as hypocholesterolemic agents has been studied by different methods including the evaluation of the ability of walnut peptides to inhibit the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), a key enzyme in the biosynthesis of cholesterol [4].

On the other hand, the antioxidant activity has been evaluated by the oxygen radical absorbance capacity assay (ORAC) [5], while the antihypertensive activity has been studied assessing the capacity of walnut hydrolysates to inhibit the Angiotensing Converting Enzyme (ACE), enzyme implicated in the blood pressure regulation [6].

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Keywords: walnuts, proteins, bioactive peptides, health

N-18
ARTEFACTS IN THE ANALYSIS OF STEROL(-ESTER)S IN CHOLESTEROL-LOWERING FOOD PRODUCTS

Hans-Gerd Janssen[1*], Raymond Baris[2], Herrald Steenbergen[3]

[1 2 3] Unilever Research and Development Vlaardingen, P.O. Box 114, 3130 AC Vlaardingen, the Netherlands
[1] University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, the Netherlands
[*] Corresponding author–E-mail: hans-gerd.janssen@unilever.com; Phone: +31 10 460 5496

Coronary hearth diseases are amongst the most important causes of death in the Western world. High blood pressures and high blood cholesterol levels are two important risk factors for such diseases. It is for this reason the food industry tries to develop food products that promote blood pressure- and cholesterol lowering. Especially the range of sterol-enriched foods with cholesterol-lowering properties has been very successful. Such food products are now marketed by a number of food companies.

The active ingredients of cholesterol-lowering foods are the class of naturally occurring phytosterols/stanols, either as the free sterols/stanols or as their fatty acid esters. Numerous studies have proven that consumption of 2 gram of these sterols a day can reduce LDL cholesterol levels by 10%. For quality control as well as to protect customers against fraud it is extremely important that good methods are available for measuring the sterol levels and sterol-profiles of sterol-enriched foods. A number of methods has been published. Classically these methods are based on gas chromatography (GC), although other methods based on liquid chromatography or enzymatic principles are now also being described.

At first sight the analysis of sterol(-esters) in food products by GC appears to be straightforward. The levels are not very low, sterols are highly stable, both chemically and thermally, and the most important sterols are commercially available for use as (internal or external) standards. When actually performing the analysis, however, a large number of experimental difficulties can be encountered. This includes incomplete saponification of the sterol-esters to sterols, dehydration of sterols to steradienes, incomplete extraction of sterols after saponification, rapid column contamination due to fatty material in the food matrix, thermal degradation of sterol-esters in GC, problems in obtaining base-line resolution for all individual sterols, difficulties in obtaining a homogeneous sample, etc.

In this contribution we will present our practical experiences with sterol(-ester) analysis in cholesterol-lowering food products. Methods for the analysis of different food formats will be described. We will also describe practical hints for detecting problems such as incomplete recoveries, incomplete saponification or thermal degradation. Finally, recommendations will be given on how to avoid errors in the quantitative analysis.

Keywords: cholesterol-lowering, phytosterols, sterols, sterol analysis

N-19

CHARACTERIZATION OF ISOFLAVONE COMPOSITION IN SOY-BASED NUTRITIONAL SUPPLEMENTS VIA ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC™)

Gregor Fiechter[1*], Bernd Raba[2], Helmut K. Mayer[3]

[1 2 3] BOKU – University of Natural Resources and Applied Life Sciences, Department of Food Science and Technology, Food Chemistry Division, Vienna, Austria
* Corresponding author–E-mail: gregor.fiechter@boku.ac.at; Phone: +43 1 47654-6116; Fax: +43 1 478 91 14

Phytoestrogenic isoflavones, namely *daidzein*, *glycitein* and *genistein* as well as their respective glucoside-, malonyl- and acetyl derivatives, all inherent in soybean, are though to be related with health protecting effects especially in context with cancer prevention. Hence, due to their benefits, a multitude of nutritional soy- or red clover-based supplements are commercially available. However, prevalent nutrition facts only label total isoflavone concentration, thus specific isoflavone spectra often remain unknown.

Major objective of this study was to characterize the isoflavone composition in commercial soy-based nutritional supplements utilizing both, the native conjugated isoflavone derivatives, derived from direct solvent extraction, as well as the reduced aglycone spectra implemented through the enzymatic de-conjugation via *Helix pomatia*. Moreover, an UPLC™-UV technique was established, efficient of baseline-separating of all 12 soy intrinsic isoflavone derivatives within 10 minutes.

Isoflavone profiles, dedicated to solvent extraction, featured primarily the three glucoside derivatives *daidzin*, *glycitin* and *genistin*, indicating these as the predominate isoflavonic constituents throughout the analyzed supplements, whereas the detected trace amounts of aglycones suggested minor degradation processes due to supplement production or sample preparation. Concerning the possible instabilities as well as inter-conversion of some isoflavone derivatives during the extraction procedure, implicating variable profiles, an enzymatic de-conjugation towards stable isoflavone aglycones ensures a more accurate quantification. An overnight incubation of the extracts with *β-glucuronidase,* inherent in *Helix pomatia*, in consideration of enough organic solvent concentration to maintain isoflavone solubility without inhibiting enzyme activity, yielded in a complete de-conjugation, indicating exclusive aglycone residues with no traces of glucoside- or esterified derivatives. Hereby obtained profiles exhibited total aglycone amounts of 62 mg per supplement capsule, featuring *daidzein* and *genistein* in approximate equal shares of 27 mg. Moreover, in due consideration of glucoside dominated spectra and molecular weight ratios, the calculated total isoflavone amounts stated acceptable accordance with the labeled specifications.

Thus, the implemented methodology, incorporating optimized samples preparation and enzymatics in conjunction with a rapid, highly resolving UPLC™-UV separation, emphasizes an accurate verification of isoflavone composition in the vast variety of soy-based foods and supplements.

Keywords: Isoflavones, Soy, UPLC, Helix pomatia

N-20

COMPARISON OF POSITIVE AND NEGATIVE ELECTROSPRAY IONIZATION FOR DESULFOGLUCOSINOLATES' IDENTIFICATION BY LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY

Ewa Sosińska[1*], Mieczysław Obiedziński[2]

[1 2] Warsaw University of Life Sciences, Faculty of Food Science, Poland
[*] Corresponding author–E-mail: ewa_sosinska@sggw.pl; Phone: +48507344341

Plants from Brassicaceae family are known from their relatively high content of sulphur glucosides – glucosinolates. Those phytochemicals undergo enzymatic degradation which results in forming variety of hydrolysis products. Certain glucosinolates' degradation products have ability for multidirectional anticarcinogenic actions. Taking into consideration that there is more than 120 glucosinolates known, only several are commercially available, moreover their UV spectra are similar there is needed to apply suitable technique for their identification.

The method of desulfoglucosinolates identification by means of liquid chromatography coupled with mass spectrometry and positive as well as negative electrospray ionization (LC-ESI/MS) was developed. Glucosinolates were isolated from different plants form Brassicaceae family like broccoli, cauliflower, red cabbage, radish, rapeseed and turnip rape according to the international standard ISO 9167-1, with some modifications. Glucosinolates' extraction with methanol was followed by purification on ion exchange columns and subsequently desulfation. Desulfogluocosinolates were separated by reversed phase liquid chromatography with water and acetonitrile as mobile phases in gradient elution. There was detection by photodiode array detector (DAD) conducted, moreover mass spectrometer with single quadrupole mass analyser with positive and negative electrospray ionisation (ESI) interface was used.

The individual desulfoglucosinolates present in selected cruciferous plants were detected and identified using liquid chromatography coupled with mass spectrometry and electrospray ionisation applied (LC-ESI/MS). There were different conditions tested to ensure desulfoglucosinolates efficient ionisation via electrospray in both positive and negative modes. Nevertheless, in both positive and negatives modes the identification of desulfoglucosinolates was possible, better results were assigned when positive ions where detected. Desulfoglucosinolates were detected as sodium $[M+Na]^+$ and potassium $[M+K]^+$ adducts in the postivie mode ESI(+) and double ammonium adducts $[M+(NH_4)_2\text{-}H]^-$ in the negative mode ESI(-).

Keywords: glucosinolates, electrospray ionisation, LC/MS

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N-21
LUTEIN AS DIETARY SUPPLEMENT

Miroslav Šivel[1*], Stanislav Kráčmar[2], Bořivoj Klejdus[3]

[1 2] Department of Biochemistry and Food Analysis, Faculty of Technology, Tomas Bata University, Zlin, Czech Republic
[3] Department of Chemistry and Biochemistry, Mendel University of Agriculture and Forestry, Brno, Czech Republic
[*] Corresponding author–E-mail: m.sivel@act.de; Phone: +420 602 536 264

Lutein is a yellow pigment belonging to the chemical family of carotenoids. Lutein is present in green vegetables, fruits like mango and papaya, red paprika, alfalfa, algae, yellow corn, marigold flower, and egg yolks and also in the macular region of the eye retina. Humans do not synthesize lutein and depend entirely on natural sources such as vegetables and fruits or dietary supplements.

Lutein derives its medical importance due to its presence in the macula of the eye where it is strongly implicated in maintaining eye health. Lutein has been linked to promoting healthy eyes through reducing the risk of Age-related Macular Degeneration (AMD). While no recommended daily allowance currently exist for lutein as for other nutrients, positive effects have been seen at dietary intake levels of 6 to 10 mg per day.

Marigold flower petals (*Tagetes erecta*) offer as a rich source of lutein. The dried marigold flower contains approximately 0.1 to 0.16% carotenoids and the lutein esters account for 90% of the total carotenoids. The dried flowers are used for obtaining marigold oleoresin which is subjected to further purification for enrichment of lutein esters or subjected to a saponification process for obtaining lutein free of esters.

The HPLC method was applied to lutein in different forms (powder, oil and beadlets) of marigold flower extracts. There were tested several sources of marigold flower extracts from India and China which are commercially sold as raw materials for dietary supplements applications.

Keywords: lutein, carotenoids, marigold flower, AMD

N-22

EFFECTS OF THE ESSENTIAL OILS OF SOME HERBS ON GROWTH AND SURVIVAL OF LISTERIA MONOCYTOGENES IN CHEESE ALONE AND IN COMBINATION WITH MONOLAURIN

Hassan Hamedi[1*], S. Mehdi Razavi-Rohani[2]

[1] Department of Food Hygiene, University of Tehran, Tehran, Iran
[2] Department of Food Hygiene, University of Urmia, Urmia, Iran
[*] Corresponding author–E-mail: Hamedih@ut.ac.ir; Phone: 02161117048; Fax: 02161117023

Background and Aim: One alternative for chemical preservatives is plant-based materials. This study was undertaken to assess the inhibitory effect of essential oils of Tarragon (TE), Spearmint (SE) and Penny Royal (PE) alone and in combination with Monolaurin (ML) in Iranian white cheese.

Materials and Methods: To assess the effects of the essential oils, and starter culture on *L. monocytogenes* in cheese, the experiment was arranged in a factorial design. This design included three essential oils (TE, SE and PE), four concentration of each essential oil (EO: 0.1, 0.2, 0.4 and 0.6 *v/m*%), two levels of starter culture (ST: 0.0 and 5.0 µl ml^{-1}), four mentioned levels of essential oil plus two levels of ML (200 and 400 ppm), four control groups [C_1: two levels of ML (200 and 400 ppm), C_2: starter culture, C_3: ML and starter culture, and C_4: without any added material] in a food system (Fresh white cheese).

Results and Discussion: The essential oils reduced the number of bacteria in cheese and SE was more effective in comparison with others ($p < 0.05$), while difference between the TE and PE was not significant ($p > 0.05$). The effect of concentration of essential oils and time of incubation on the number of bacteria was significant ($p < 0.05$). The essential oils in combination with ML had a greater effect on *Listeria* and inhibitory effect of TE was superior to others. The inhibition was potentiated as concentration of essential oil increased. In the case of TE, the concentration of 0.6% essential oil with 400 ppm ML, completely inhibited *L. monocytogenes* and no growth was observed in the culture of cheese. Since the use of some herbs and their essential oils are restricted, and using high doses are also impossible, so combination of two or more substances could be more acceptable and effective.

Keywords: Essential oils, Monolaurin, Chesse

N-23

DEVELOPMENT OF A DNA-MODIFIED SENSOR TO EVALUATE THE TOTAL ANTIOXIDANT CAPACITY OF FLAVOURED WATERS

M. Fátima Barroso[1*], J. Paulo Noronha[2], M. Goreti F. Sales[3], Cristina Delerue-Matos[4], M. Beatriz P.P. Oliveira[5]

[1 5] Requimte/Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal
[2] Requimte/CQFB, Chemistry Dept, FCT/UNL, 2829-516 Caparica, Portugal
[3 4] Requimte/Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072, Porto, Portugal
[*] Corresponding author–E-mail: mfb@isep.ipp.pt; Phone: +351 228 340 500; Fax: +351 228 321 159

Most living organisms have developed complex antioxidant systems to counteract reactive oxygen species (ROS) and to prevent the deleterious effects of ROS. These antioxidant systems include enzymes; macromolecules and an array of small molecules.

Food constitutes are an excellent exogenous source of natural antioxidants. It is well known that vegetables, fruits, whole-grain and some beverages contain many antioxidant and bioactive compounds. Examples of antioxidants present in food are vitamins (particularly C and E), phenolic compounds and carotenoids including β-carotene. A healthy diet should provide an adequate and continuous supply of these antioxidants.

Recently, to answer to consumer's preferences and considering that water is the most consumed drink all over the world, flavoured waters were developed and commercialized. It consists in the addition of flavours, juices and sugar or sweeteners providing water singular tastes and smells appreciated by consumers. Flavours/aromas contain naturally occurring antioxidants that are extracted from vegetables or fruits, for which flavoured bottled waters carry these compounds. So, drinking these waters can increase the daily intake of antioxidants and maybe contribute for the exogenous protective system by reduction of oxidative DNA damage.

Several analytical methods have been developed in order to evaluate the Total Antioxidant Capacity (TAC) in food and beverage samples. The most commonly employed techniques are based in UV–Vis spectrometry, chemiluminescence, fluorimetry and chromatography.

In this work, an electrochemical voltammetric method based on a biosensing device was developed to assess the TAC in flavoured water samples [1]. This biosensor consisted in the electro-deposition of a purine base (adenine) on a glassy carbon electrode. In order to evaluate the oxidative lesions in the purine base, this biosensor was immersed in Fenton-type reaction. After addition of the iron and the hydrogen peroxide, they react together to generate some hydroxyl radicals according the following equation:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + {}^{-}OH$$

Then, the hydroxyl radicals react with adenine to oxidize it. Damage produced in the DNA layer was recorded by square wave voltammetry measuring the decrease in the oxidative peak current. The presence of ascorbic acid or gallic acid as antioxidants was evaluated and the protection effect was achieved. The developed method was applied to assess the antioxidant properties of flavoured waters.

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Keywords: Antioxidant; Radical; Biosensor; Flavours

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N-24
DETERMINATION OF WATERSOLUBLE B-VITAMINS WITH VITAFAST® TESTS IN FRUITS AND FRUIT PRODUCTS

Ronald Niemeijer[1], Sigrid Haas-Lauterbach[2], Sylvia Stengl[3], Wolfgang Weber[4*]

[1 2 3] R-Biopharm AG, Darmstadt, Germany
[4] Institut für Produktqualität, Berlin, Germany
[*] Corresponding author–E-mail: weber@roduktqualitaet.com; Phone: +49 30 76686012

Food products are often enriched with vitamins. Thus the industry expanded the variety of fruit products available on the market. The vitamin content is monitored by manufacturers and control authorities to check compliance with labelling regulation. R-Biopharm presents a rapid system for watersoluble B-vitamin determination which is based on AOAC, EN and DIN reference methods. With these microbiological VitaFast® test kits, which are in microtiter plate format, routine analysis can be carried out since the reagents contained in the kit are ready to use and therefore very user-friendly.

Among other food a range of fruit samples was successfully tested with VitaFast®. In cooperation with ifp (Institut für Produktqualität, Berlin) folic acid, vitamin B12, biotin, niacin, pantothenic acid, vitamin B1, B2 and pyridoxine were validated for these matrices mentioned above. The coefficient of variation is below 10% and the recovery rate after spiking is about 95–105%. In conclusion these microbiological tests can be used successfully to determine the total vitamin content, added and natural vitamins, after specific enzymatical treatment or only the added vitamins.

Currently AOAC RI approvals for VitaFast® folic acid, cyanocobalamin, biotin, pantothenic acid and riboflavin are under preparation.

Keywords: vitamins, fruit samples, VitaFast

N-25

SEPARATION AND PURIFICATION OF NATURAL ANTIOXIDANTS OBTAINED FROM A PVPP SLUDGE GENERATED DURING THE BREWING PROCESS

Letricia Barbosa-Pereira[1], José M. Cruz[2*], Inmaculada Angulo[3], Raquel Sendón[4], Perfecto Paseiro-Losada[5]

[1 4 5] Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy. University of Santiago de Compostela, Spain
[2] Department of Chemical Engineering, E.T.S.E.I. University of Vigo, Spain
[3] 3GAIKER Technological Centre. IK4 Research Alliance. Zamudio–Bilbao. Spain
[*] Corresponding author–E-mail: jmcruz@uvigo.es; Phone: +34981598450

Beer contains a great variety of phenolic compounds. Numerous studies have shown that polyphenols are extremely important in regard to the physical stability, fundamental quality parameter, of beer. During beer storage, colloidal haze development is predominantly a complex formation of proteins and polypeptides with polyphenols. In order to prevent haze formation and thus extend the shelf life of beer, polyphenols are partly removed from beer by treatment with polyvinylpolypyrrolidone (PVPP). However, non-haze active polyphenols that are also removed by this treatment are considered to be important antioxidants, with several applications for the food industry [1, 2, 3].

Natural extract of phenolic compounds with high antioxidant activity can be obtained from the residual stream generated after the cleaning of the PVPP in brewery industry. A suitable purification process leading to the removal of fractions with limited antioxidant activity allows using lower amount of natural extract to obtain the same effect as food antioxidant. Moreover, it is also important to get extracts with high purity to ensure the identity and safety of antioxidant compounds to be used as food additives.

The aim of this work is to evaluate the yield and the radical-scavenging activity of fractions of the crude extract obtained after the cleaning of the PVPP, as well as to identify and quantify the polyphenols responsible for the antioxidant activity.

The PVPP loaded with polyphenolic compounds was cleaned with a NaOH solution (2% *w/w*). This stream, generated after the PVPP cleaning process, was kindly supplied by Mahou-San Miguel, Spain. Aqueous phase was acidified to pH 1.5 with HCl (37%) and polyphenolic compounds were extracted with ethyl acetate by stirring during 45 minutes at room temperature. Organic phase was separated and evaporated to dryness. The residue obtained was lyophilized and the dry extract was used in fractionation experiments. This residue was dissolved in water and loaded in a Supelclean LC-18 20 ml tubes (Supelco) to be eluted with solutions with different methanol:water ratio. All fractions were collected, then evaporated to dryness and dissolved in methanol. Yield of each fraction was determined gravimetrically. The antioxidant activity of extracts was measured by the DPPH radical-scavenging test. Fractions were characterized using HPLC-DAD-UV.

In this purification process eleven fractions were obtained and the fractions with highest yield were the most active. About 45% (*w/w*) of the crude extract has very poor antioxidant activity and the fraction eluted with 30:70% (*v/v*) methanol:water showed the highest antioxidant activity similar to BHA. The main polyphenolic compounds found in the extracts were: ferulic acid, p-coumaric acid, caffeic acid, protocatechuic acid, gallic acid and gallocatechin.

These natural extracts obtained from this residual stream of the brewing process are a promising source of natural antioxidant to be used as food additives.

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Keywords: Beer, polyphenols, polyvinylpolypyrrolidone, antioxidant activity

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N-26
CHALLENGES IN DIETARY FIBRE ANALYSES

Kommer Brunt[1*]

[1] Department Carbohydrate Analyses, Eurofins Food B.V., PO BOX 766, 8440 AT Heerenveen, The Netherlands
[*] Corresponding author–E-mail: k.brunt@eurofins.nl; Phone: + 31 88 831 00 00

It is generally recognized that dietary fibre (DF) is an essential part of the human food. In our western world the daily intake of DF is considerably less than the recommended daily intake. The different DF categories have shown to benefit amongst others diabetes, blood cholesterol levels, body weight control (obesities) and reduction of risk of coronary hart disease.

Nowadays many new products are launched with statements regarding their DF content. Presently food products are fortified with different DF ingredients, ranging from classical natural high molar weight (HMW) DF (e.g. cereal based ß-glucans, hemicelluloses and pentosans, and fruit based pectins) to low molar weight (LMW) soluble DF/prebiotics (e.g. inulin/fructose-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and polydextrose). Also resistant starch (RS) and resistant maltodextrins are considered as DF and applied in food products.

Different analytical protocols are available for the different DF classes. Up to now, the golden standard is the AOAC 985.29 protocol for the determination of the total (soluble and insoluble) HMW DF. However resistant starch is just partly measured applying this method or the extended AOAC 991.43 protocol for distinguishing in soluble and insoluble HMW DF. The resistant starch categories RS1, RS2, and RS4 are not measured/determined by these two commonly applied methods. It is only the retrograded starch, the RS3 category resistant starch, which is also quantified with the AOAC 985.29 and 991.43 methods. And also the prebiotics are not quantified with the above mentioned classical methods. For the different LMW DF or prebiotica (inulin/FOS, GOS, polydextrose, resistant maltodextrins), different dedicated analytical tests are available.

For these reasons the authorities have withdrawn the legislation in which the AOAC 985.29 and/or 991.43 were assigned as the official methods.

It is evident that this diversity in tests for the determination of the (specific) fibre content in food products is difficult to understand. It will result in the use of inappropriate tests for the measurements of the real total DF content in food products especially in those which have been fortified with LMW DF constituents (prebiotics), mostly resulting in a (severe) underestimation of the reported fibre content on the label.

In this presentation helpful schemes will be discussed which have been developed to facilitate the decision which (combination) of the dedicated specific DF tests have to be applied for a correct determination of the DF content in the DF enriched food products.

Keywords: dietary fibre analyses, high molar weight, low molar weight

N-27
FLOW INJECTION ANALYSIS DEVICES FOR TOTAL PHENOLIC COMPOUNDS MEASURE

Jorge Ferrer[1], Felix Amarita[2*]

[1 2] Azti Tecnalia
[*] Corresponding author–E-mail: famarita@azti.es; Phone: +34 94 657 40 00; Fax: +34 94 657 25 55

The basic design of the proposed biosensor was based on the measurement of the consumed oxygen in the reaction catalysed by the enzyme Benzenediol:oxygen oxidoreductase (polyphenol oxidase, laccase). In such reaction, two adjacent hydroxyl groups, placed on the aromatic ring of a phenolic compound, react with molecular oxygen releasing two water molecules and a semiquinone. The commercial laccase Suberose, kindly offered by Novozymes, was immobilised in Eupergit C by random covalent binding between epoxy groups from the matrix and hydroxyl, sulfhydryl and amine groups from the enzyme. Three hundred milligrams of Eupergit C and 1500 µl of Suberose were put in an eppendorf tube and maintained for 3 days at 4ºC. Once immobilisation process was finished the enzymatic matrix was transferred into a chromatographic minicartridge (mobicol from MoBiTec GmbH). Minicartridge was placed “on line” in a flow injection analysis system, comprising a peristaltic Masterflex C/L pump that flows a carrier buffer (continuously saturated with air by a pump) to an Bio-Rad injection valve, used to inject the samples into the system, and “push” through the enzymatic minicartridge where the immobilized enzyme oxidises the phenols (the consumption of oxygen is proportional of the quantity of phenols oxidised). The consumption of oxygen on the buffer carrier is registered hy using a oxygen meter probe. This probe is settled in a flow cell and conected to the correspondent oxigen controller (Strathkelvin Instruments) The system permits to work in two different modes. First option flow injection analyse method (FIA) is very quick as it allows to get the results in less than 2 minutes per sample. This method can be used for the analysis of total phenols in wines and fruit juices injecting 100 µl of a diluted sample. Second option, sequential flow injection analyse (SFIA) is also quik as it takes no longer than 7 minutes per sample and it is capable of detecting very small concentrations of polyphenols. In this case, once the sample is injected into the enzyme minicartridge, system stops, and the sample gets in contact with the enzyme during five minutes. After that time, system is re-started and the reaction can be eventually observed. The best flowing for the carrier buffer has been studied and set up for both analytical methods (SFIA & FIA), plus the effect of the ethanol and the enzymatic reaction interferences caused by other molecules which may appear in the wine. Also the effect of several phenols has been studied.The main ones present in wine show a very similar response, with no statistical differences among them.

Keywords: Phenolic compounds, Flow injection analysis, Laccase

N-28
PROFILE ANALYSIS OF GREEK HONEY EXTRACTS AND THEIR BIOACTIVITY ON CANCER CELLS

Mari Jaakkola[1], Tiina Tolonen[2], Anna V. Tsiapara[3], Ioanna Chinou[4], Konstadia Graikou[5], Paraskevi Moutsatsou[6], Vesa Virtanen[7*]

[1 2 7] Biotechnology Laboratory, Kajaani University Consortium, University of Oulu, Salmelantie 43, FI-88600 Sotkamo, Finland
[3 6] Department of Biological Chemistry, Medical School, University of Athens 75 MikrasAsias Str, Goudi 11527, Athens, Greece
[4 5] Laboratory of Pharmacognosy, Department of Pharmacy, University of Athens, Panepistimioupolis, GR 15771 Zografou, Athens, Greece
[*] Corresponding author–E-mail: vesa.virtanen@oulu.fi; Phone: +358 8 63321311; Fax: +358 8 63321313

Honey products demonstrate antioxidant, chemopreventive, antiatherogenic, immunoregulatory, antimicrobial and wound healing properties as well as metabolic effects in diabetes. Honeys from various floral sources have been shown to be rich in phenolic compounds, which are substances known to exert estrogenic effects by modulating the estrogen receptor activity, being estrogen agonists or antagonists in a cell and tissue type specific manner. In this study we investigated the chemical composition, total phenolics, phenolic acids, fatty acids, hydroxymethylfurfural, sugars and volatile compounds (GC-MSD) of three Greek honey (pine, thyme and fir) extracts (ethyl acetate). Furthermore, we investigated the potential of the extracts to influence the estrogenic activity and cell viability of breast (MCF-7), endometrial (Ishikawa) and prostate (PC-3) cancer cells. Sugars (sucrose, glucose and fructose) contributed over half of the dry weight of the honey extracts. Thyme and pine honey extracts had two times higher total phenolic content (ca. 1000 mg/100 g) than fir honey. Vanillic acid and p-hydroxybenzoic acid were found in all studied honey extracts, whereas protochateuichuic acid was found only in fir and pine extracts. Hydroxymethylfurfural was found in all honeys, but the content was significantly higher in thyme extract than in other honey extracts. The profiles of volatile compounds (GC-MSD) were similar in all studied honeys. Thyme, fir and pine honeys showed both antiestrogenic and weak estrogenic effect at low and high concentrations, respectively, in MCF cells. Furthermore, thyme honey reduced the viability of Ishikawa and PC-3 cells, whereas fir honey stimulated the viability of MCF-7 cells. In conclusion Greek honeys are rich in phenolic compounds and they modulate estrogenic activity. Thyme honey-enriched diet may prevent cancer related processes in breast, prostate and endometrial cancer cells.

Reference:
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Keywords: honey, chemical composition, cancer cells

N-29

RAPID FLOW CYTOMETRY ANALYSIS OF ANTIMICROBIAL PROPERTIES OF HYBRID ARCTIC BRAMBLE (RUBUS ARCTICUS NOTHOSSP. X STELLARCTICUS G. LARSSON) AND CLOUDBERRY (RUBUS CHAMAEMORUS L.)

Mari Jaakkola[1], Johanna Korhonen[2], Maarit Hattuniemi[3], Jarkko Räty[4], Kalle Hoppula[5], Vesa Virtanen[6*]

[1 2 3 4 6] Biotechnology Laboratory, Kajaani University Consortium, University of Oulu, Salmelantie 43, FI-88600 Sotkamo, Finland
[5] MTT Agrifood Reseacrh Finland, Sotkamo Research Station, Kipinäntie 16, FI-88600 Sotkamo, Finland
[*] Corresponding author–E-mail: vesa.virtanen@oulu.fi; Phone: +358 8 63321311; Fax: +358 8 63321313

Plants of the genus *Rubus* have been found effective to inhibit the growth of several bacteria strains (i.a. *Salmonella* and *Staphylococcus*). Hybrid arctic bramble (*Rubus arcticus* nothossp. *stellarcticus* G. Larsson*,* also known as all-fieldberry) is a less studied berry of genus *Rubus* and it is a hybrid between the arctic blackberry (Rubus arcticus *ssp*. stellatus *(Sm.) Boivin*) and the arctic bramble (*Rubus arcticus* ssp. *arcticus* L.). Cloudberry (*Rubus chamaemorus* L.) is one of the most valuable wild berries grown especially in Nordic countries and it is used in foodstuffs due to its nutritional properties and distinctive flavour. The aim of this study was to investigate the antimicrobial properties of arctic bramble and cloudberry against *E. coli* and, furthermore, develop a rapid and reliable method to enumerate the amount of total bacteria using flow cytometry (FCM). Antimicrobial activity of berry materials (lyophilized berry powder) against *E. coli* was measured in liquid cultures (LB-broth) by FCM (Beckman-coulter Cell-Lab Quanta SC). Samples were stained with two fluorescent colors, PI and SYTO9 (LIVE/DEAD Backlight kit, Invitrogen), before measurements. Reproducibility was assured by triplicate analysis of each sample. The inhibitory effects of berries on growth of *E. coli* were estimated by comparing the results of control sample (*E. coli)* with those obtained from *E. coli* samples with berry material. Hybrid arctic bramble and cloudberry inhibited significantly the growth of *E. coli*. Flow cytometry results were confirmed by reference analysis performed by accredited plate count method in commercial laboratory. Flow cytometry results of bacteria enumeration corresponded well with the results obtained by plate count method. Compared to the plate count method FCM provides significantly faster technique to enumerate bacteria and requires less working time. Furthermore, plate count techniques are sensitive to culture conditions like temperature, media and duration of incubation, which were avoided in FCM. FCM is an excellent tool to study the kinetics of the growth of bacterium, since subsamples can be taken from the same liquid medium during the growing period. Furthermore, FCM together with fluorescent dyes offers a rapid method to investigate viability of the bacterium.

Keywords: flow cytometry, antimicrobial, Rubus genius

N-30
ANTIOXIDANT CAPACITY OF PEPTIDE HYDROLYZATES OBTAINED BY POULTRY LEFTOVERS

Gianni Galaverna[1*], Francesca Lambertini[2], Stefano Sforza[3], Arnaldo Dossena[4], Rosangela Marchelli[5]

[1 2 3 4 5] Department of Organic and Industrial Chemistry, University of Parma, Italy
[*] Corresponding author–E-mail: gianni.galaverna@unipr.it; Phone: ++39-0521-906196; Fax: ++39-0521-905472

In the process of production of meat for human consumption approximately 50% of the animal is turned into animal by-products, leading every year to the loss of many tons of proteinaceous material having a potential high nutritional value. In the work of an european project (PROSPARE), a new technology of poultry processing product conversion into peptide hydrolyzates has been recently developed. In particular, unmarketable poultry secondary resources (carcasses, meat trimmings, bone residue) can be converted into added value peptide hydrolyzates through different types of enzymatic and chemical hydrolysis. Beside their nutritional value, it is known that protein hydrolyzates exhibit a variety of biological activities, therefore they could also be considered as a promising ingredient for functional products. In this work the antioxidant capacity of different peptide hydrolyzates, obtained from the fermentation of bone and meat trimmings by different type of enzymatic digestion, has been studied. 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical-scavenging assays were carried out to determine the antioxidant activity of 3 different matrices and the results were expressed in terms of Trolox equivalents. The results obtained have shown that the highest antioxidant capacity is directly correlated with the enzymes weight ratio used during the fermentation step. The hydrolyzates were also fractionated in 10 selected subfractions by semipreparative RP-HPLC and the subfractions have been assayed by DPPH method. The fraction with the highest antioxidant activity is the first one which account for more than 60% of the total weight of the sample. Its impact on the total antioxidant activity, from 25 to 40%, increase with the hydrolysis degree. An extensive study was carried out by HPLC-ESI/MS/MS in order to identify the principal components of the first four fractions and the sequences analysis shown that only free amino acids, small polar peptides and other low molecular weight molecules (MW<300 Da) are presents. This findings allowed to conclude that poultry left-over hydrolyzates have antioxidants properties: their possible use as additives in meat products is currently investigated.

Keywords: poultry leftovers, antioxidant activity, peptides

EU VII Framework Program: "Prospare" project (www.prospare.eu)

N-31

A RAPID ANALYTICAL METHOD FOR THE COMBINED DETERMINATION OF VITAMINS AND METALS IN DIETARY SUPPLEMENTS

Salvador Maestre[1*], Eduardo Paredes[2], Jose Luis Todolí[3], Soledad Prats[4]

[1 2 3 4] University of Alicante, Department of Analytical Chemistry, Nutrition and Food Science, Alicante, Spain
[*] Corresponding author–E-mail: salvador.maestre@ua.es; Phone: +34965903777; Fax: +34965903527

The use of an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) as a detection method for organic compounds after High Performance Liquid Chromatography (HPLC) separation has not been extensively studied. The detection of these compounds by ICP-AES has been usually performed by means of the measurement of the carbon emission intensity at 193.03 nm. The main drawback of this detection method is the relatively high limits of detection, LODs (i.e., typically between 1 and 10 mg L^{-1} of carbon), thus making impossible the detection of organic compounds at low concentrations. However, when organic compounds are present at concentrations high enough the ICP-AES can be advantageous for several applications provided that it allows the determination of both organic compounds and metals in a single analytical run. Vitamins are present in most foods at concentration lower than LODs achieved by ICP-AES. For this reason, these compounds have not been studied by HPLC-ICP-AES. However, this hyphenated method could be used to control the composition of multivitamin and mineral supplements since these samples contain high concentrations of vitamins.

The aim of the present work was thus to use the HPLC-ICP-AES coupling for the combined determination of water-soluble vitamins and minerals in a single chromatographic run. With the method developed it was possible to quantify 5 water-soluble vitamins (i.e., thiamine, riboflavin, pantothenic acid, nicotinamide and ascorbic acid) and 10 minerals (i.e., Cr, Mo, Se, Mn, Zn, Fe, Cu, Mg, Ca and K) in several dietary supplements. Results obtained for water-soluble vitamins were compared against those provided by a photodiode array detector. Finally, the concentrations determined were compared with the labelled values.

Keywords: ICP-AES, Carbon, Vitamins, Metals

N-32

EFFECT OF ACTIVE FILMS CONTAINING NATURAL ANTIOXIDANTS ON THE LIPID STABILITY OF BEEF MEAT

Letricia Barbosa-Pereira[1], José M Cruz[2*], Inmaculada Angulo[3], Raquel Sendón[4], Perfecto Paseiro-Losada[5]

[1 4 5] Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy. University of Santiago de Compostela, Spain
[2] Department of Chemical Engineering, E.T.S.E.I. University of Vigo, Spain
[3] GAIKER Technological Centre. IK4 Research Alliance. Zamudio–Bilbao. Spain
[*] Corresponding author–E-mail: jmcruz@uvigo.es; Phone: +34981598450

Lipid oxidation is the major cause of quality deterioration of meat during refrigerated storage, reducing shelf stability and acceptability. Lipid oxidation may produce changes in meat quality parameters such as organoleptic properties and loss of nutritional value. Therefore it is a major cause of quality deterioration in meat products, and generates and accumulates compounds that may pose continual risks to human health. [1] The shelf life of meat can be extended by using antioxidants and proper packaging materials. Active packaging is currently one of the most dynamic technologies that can be used to preserve the quality of food where the releasing of the active agents incorporated on the film can be controlled in an extend period of time. [2] Because of the growing consensus on the potential health hazard caused by synthetic antioxidants, there is a renewed interest in the use of naturally occurring antioxidants. Waste products from fruit and vegetables processing offers a practical and economic source of potent antioxidants that could replace synthetic preservatives. [3] The aim of this work is to evaluate the antioxidant effect of an active film containing an extract obtained from a residual stream from brewing industry that contain natural antioxidants and consequently the extension of the shelf life of beef meat packaged with this film during refrigerated storage. The residual stream generated after the PVPP cleaning process in brewing industry was kindly supplied by Mahou-San Miguel, Spain. Aqueous phase with polyphenolic compounds were extracted with ethyl acetate and organic phase was separated and evaporated to dryness. The residue obtained was lyophilized and incorporated on the film, by coating with a nitrocelulosic resin, in different concentrations. Beef steaks were wrapped with different preparations of films containing natural antioxidants and stored at 4ºC for 16 days. Lipid oxidation, were determined by measuring the TBARS-reacting substances, at selected times during storage. The thiobarbituric acid reactive substances (TBARS) value (mg malonaldehyde/kg) of beef meat was determinate by using the extraction method described by Witte *et. al*. (1970) with slight modifications. The use of an active film with natural antioxidant obtained from brewing industry resulted in enhanced oxidative stability of beef steaks compared with the film control. The results showed that higher concentrations of polyphenols result in higher antioxidative effects. In samples in which films with the highest concentration of extract were used is where the greatest differences were observed. The film containing the high concentration of extract delayed the onset of oxidation until the ninth day, after which slight oxidation is observed, but much less than the observed in the other samples under study. These active film containing natural extracts obtained from this residual stream of the brewing process are a promising active packaging to be used in food industry.

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Keywords: Lipid oxidation, beef, natural antioxidants.

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N-33
THE MICRO-EXTRACTION AND DETECTION OF PHENOLIC ANTI-OXIDANTS FROM CEREAL PRODUCTS USING MEPS-GCMS

Paul Wynne[1*], Naza Lahoutifard[2]

[1] SGE Analytical Science Ltd, Ringwood, Australia
[2] SGE Europe, Courtaboeuf, France
[*] Corresponding author–E-mail: pwynne@sge.com; Phone: +61 3 98374230

Synthetic phenolic antioxidants are increasingly rejected as acceptable food additives because of their demonstrable or suspected adverse effects on human health. Among the compounds of concern are the butylated hydroxyphenols such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) and bisphenol A. Simple methods for the removal of food matrices are necessary for the detection of these compounds in regulatory compliance programmes.

Micro-extraction Packed Sorbent (MEPS) is a solid-phase technique that allows rapid sample extraction by reducing the volume of sample processed. Because the sorbent device is incorporated directly into a liquid handling syringe, it may also used with robotic autosamplers for on-line chromatographic analysis.

In this example, rice crackers manufactured with canola oil that was stabilised with BHA were crushed and extracted with either water or methanol-water. The liquid recovered was extracted using a C_{18} MEPS cartridge that had been conditioned sequentially with methanol and water. The retained fraction was eluted with methanol, dichloromethane or mixtures of both and analysed directly by EI-GCMS in full scan mode on a BPX-5 column.

The method was found to be suitable for the rapid extraction and detection of BHA, which was readily detected in the extracts from 100 mg of rice cracker. Sample processing time, including sorbent conditioning and recycle time, was less than 5 minutes for samples of 1mL in volume. The technique also permitted the detection of phenolic and other compounds that were common to the plastic packaging in which the crackers were presented.

Keywords: BHA, MEPS, GCMS, phenolics

N-34
LC/MS/MS ANALYSIS OF WATER-SOLUBLE B VITAMINS IN FORTIFIED FOODS AND BEVERAGES

Jim Krol[1], Brent Lefebvre[2], Sneh Bhandari[3], Jim Carlson[4], Andre Schreiber[5*]

[1 3 4 5] Applied Biosystems
[2] MDS Analytical Technologies
[*] Corresponding author–E-mail: andre.schreiber@lifetech.com; Phone: +1-905-660-9006

Several publications describe the analysis of water-soluble B (WSB) vitamins, as stan-dards or in clean matrices such as beverages, analyzed in a single LC/MS/MS injection. There is much interest in quantifying the WSB vitamins in more complex matrices, like food products. However, a major difficulty with a single WSB vitamin assay is that sam-ple preparation depends both on the vitamin and the sample matrix. There is consider-able interest in a generalized sample prep method for all WSB vitamins, but each vitamin's chemistry differs and a single extraction may not appropriate. Another consid-eration is that the B Vitamins also have differing optimal electrospray ionization volt-ages. We present a method for identifying and quantifying the WSB vitamins in fortified foods and beverages. The method for all WSB vitamins and methods for individual vitamins utilize a C18 col-umn, water/methanol gradient and positive ion ESI with differences in spray voltage and gradient. The experiment for analysis of all WSB vitamins is under 10 minutes. Two MRM transitions per analyte were used for analyte quantification and confirmation. The MRM transitions can also trigger a full-scan analyte product ion spectrum for library matching validation. Samples of vitamin-enriched water, a fortified energy beverage, a low-fat white milk, chocolate diet "shake", and multigrain cereal flakes were extracted with 0.1 N HCl (pH 1), 0.1% formic acid (pH 3), 0.1% formic acid / 5 mM ammonium for-mate (pH 5), or 5 mM ammonium bicarbonate (pH 9) as appropriate. Comparison of MS/MS results with microbiological methods shows that each extraction solution has it own efficiency, and extraction solution recovery of a spiked sample matrix varies with each vitamin, matrix, and pH. Major interferences are carbohydrates, vari-ous polymeric additives, and mineral content. In some cases, the MS/MS results are significantly higher than the microbiological (im-munoassay?) result and in some cases lower. However, the confirmatory ion ratios suggest the MS/MS results are valid. Lower results from the mass spectrometric analy-sis may be due to ionization suppression or increased specificity for the analyte over the microbiological assay. The low cost, routine MS/MS system gives linear standard L injection.μcurves for the WSB vitamins with from 0.5 to 1000 ng/mL using a 10 LODs of less than 1 ng/mL were obtained for each analyte. The fortified liquid samples were diluted between 10- and 50-fold and the cereal extract was diluted 1,000-fold to bring the concentration range of the analytes within the range of the standard curve. Additional sensitivity might be gained by increasing the sample dilution factors as this will also dilute away the suppression effects of the matrix. Dilution should simplify the sample preparation and make the analysis more reproducible. The current method is designed for the analysis of fortified food products, but the potential sensitivity gain sug-gests that the analysis of WSB vitamins at natural levels may be possible. Faster scan-ning, more sensitive instruments may provide a means to do faster chromatography and therefore improve sample throughput. The potential of additional sensitivity suggests that analysis of WSB vitamins at natural levels may be possible.

Keywords: vitamins, LC/MS/MS, quantitation, identification

N-35

CHARACTERIZATION OF POLYPHENOLICS COMPOUNDS IN ARGENTINEAN RED WINE USING HPLC-MS/MS WITH RELATION TO ANTIOXIDANT ACTIVITY

Daniel Wunderlin[1*], Romina Di Paola Naranjo[2], María Verónica Baroni[3], Carolina García Ferreyra[4], Santiago Otaiza Gonzalez[5], Micaela Ruiz Smania[6]

[1 2 3 4 5 6] Universidad Nacional de Córdoba, Argentina
[*] Corresponding author–E-mail: dwunder@fcq.unc.edu.ar; Phone: 0054 351 4333193 ext 141; Fax: 0054 351 4333194

Polyphenolic compounds are ubiquitous in foods of plant origin, constituting an integral part of the human diet. Recently, the interest in polyphenols has greatly increased, since these phytochemicals have been implicated in suppressed rates of degenerative processes, such as cardiovascular disorders and cancer. Wine is an excellent source of various polyphenols, including phenolic acids, flavonols, anthocyanins and flavanols. Big effort has been expended on the analysis of red wine polyphenols, looking the relationship between polyphenol content and their antioxidant capacity. Furthermore, the evaluation of phenolic profile could help to evaluate which compounds are mainly involved as ROS scavengers, acting as chemoprotectors that could be emphasized for dietary purposes.

Thus, we analyzed twenty nine Argentinean wine samples, produced from five different varietals (*Cabernet Sauvignon, Syrah, Merlot, Malbec, Malbec, Tempranillo*). Total phenolics contents were measured by the oxidation-reduction reaction, using Folin-Ciocalteu reagent and gallic acid as the standard. Besides, HPLC-MS/MS (triple quadrupole) was used for the identification and quantification of individual compounds in wine samples. We examined several sub-classes of polyphenolic compounds: anthocyans, flavonols, flavan-3-ols and phenolic acids. Furthermore, we also evaluated the antiradical activity (TEAC) and the reducing power (FRAP) of studied wines.

The reducing power of samples (FRAP) was highly correlated with total phenols (r2: 0.86 $p<0,01$). By contrast, antiradical activity did not show correlation. This could be explained since TEAC depends on polyphenolics profile and not on its total content.

Thus, we evaluated the polyphenolic profile as well as the content of each compound by HPLC-MS/MS, looking to evaluate their contribution to the total antioxidant capacity. Results show that different subclasses of polyphenols correlated quite well with the antioxidant activity of wine samples raising questions on the efficiency of individual compounds to scavenge ROS, synergism, antagonism, etc.

Keywords: wine, polyphenols, antioxidant, ROS scavenger

CONICET & TRACE consortia EU-FP6

N-36

DETERMINATION OF 12 PHENOLIC ACIDS BY A VALIDATED HPLC METHOD: ITS APPLICATION TO SOME PLANTS FROM IDA MOUNTAIN (KAZ MOUNTAIN/ KAZDAĞI)

Necati Barış Tuncel[1*], Neşe Yilmaz[2]

[1 2] Onsekiz Mart University, Çanakkale, Turkey
[*] Corresponding author–E-mail: baristuncel@comu.edu.tr; Phone: +90 286 2180018 (2262); Fax: +90 286 2180541

Phenolic acids are a group of natural products that have been found to be strong antioxidants against free radicals and other reactive oxygen species, the major cause of many chronic human diseases such as cancer and cardiovascular diseases. Phenolic compounds synthesized in the plant by secondary metabolism and interest in antioxidant and bioactive properties has increased due to their health benefits. Kazdağı, a mountain located in the North-Western Turkey, is ecologically diverse, containing a number of plant species endemic to Turkey.

Gallic acid (GA), protocathechuic acid (protoCA), p-hydroxy benzoic acid (p-hydBA), vanillic acid (VA), caffeic acid (CA), chlorogenic acid (ChA), syringic acid (SA), p-coumaric acid (p-COU), ferulic acid (FA), rosemarinic acid (RA), o-coumaric acid (o-COU) and trans-cinnamic acid (tr-CIN) were determined. The analysis was performed using a gradient program with a two solvent system A: methanol:water:formic acid (10:88:2 *v/v*); B: methanol: water: formic acid (90:8:2 *v/v*). The signals were detected at 280 nm. The internal standard (IS) technique was applied to the analysis to increase the repeatibility; propylparaben was the suitable IS.

Herbal parts of Sideritis trojana, Salvia tomentosa, Origanum vulgare subsp. hirtum, Lavandula stoechas subsp. stoechas, Sideritis athoa, Mentha pulegium, Abies nordmannia subsp. equi-trojani (cone), Hypericum perforatum, Achillea nobilis subsp. sipylea and Mentha spicata which were collected from Ida Mountain were investigated in terms of their phenolic acids content.

Keywords: Phenolic acids, HPLC, Ida Mountain

N-37

SCREENING OF ANTIMICROBIAL ACTIVITY OF MARINE SPONGE EXTRACTS AGAINST POTENTIAL FOOD-BORNE PATHOGEN BACTERIA: PRELIMINARY RESULTS

Martiña Ferreira[1], Ana G. Cabado[2], Paula Fajardo[3], María-José Chapela[4], Juan Manuel Vieites[5], Lucia Blanco[6], Jorge Lago[7*]

[1 2 3 4 5 6 7] ANFACO-CECOPESCA, Vigo, Spain
* Corresponding author–E-mail: jlago@anfaco.es; Phone: 00 34 986 469 301

The increasing spread of antibiotic-resistant bacterial strains brings about the need for alternative antibacterial compounds. Natural sources may provide a broad range of molecules with antimicrobial, cytotoxic, anti-inflammatory or immunoregulator properties that constitute an interesting start point for pharmacological research, though very few of such attempts result on the development of new drugs. In particular, marine sponges synthesize a large number of bioactive compounds. We have recently started a project aimed to searching for bioactive substances from sponges of Galicia (NW Spain) coast. In this work, we present some of the results of the screening for antibacterial activity of sponge extracts against potential food-borne pathogens. Several species of marine sponges (Ophlitaspongia seriata, Grantia compressa, Adreus fascicularis, Hymeniacidon sanguinea, Halichondria panicea, Tethya aurantium, Haliclona oculata, Phorbas plumosum plus three unidentified species) were collected by hand in the intertidal zone of Baiona or by SCUBA diving at 10–12 m depth in Ria de Ferrol (both sites located in Galicia, NW Spain). Individuals were homogenized in different organic solvents and extracts were concentrated and subsequently used in microtiter plates assays against Escherichia coli, Vibrio parahaemolyticus, V. cholerae, Listeria monocytogenes and Salmonella enterica. Bacterial growth was assessed by absorbance measuring at 600 at 5 min intervals for 8 h. Some of the chloroform and acetone extracts showed inhibitory activity against the bacterial species tested at concentrations ranging from 0.25% to 1%. Tethya aurantium acetone extract totally inhibited the growth of V. cholerae at a concentration of 0.25%, whereas a 1.5% concentration of the chloroform extract of Ophlitaspongia seriata reduced the growth of E. coli and L. monocytogenes to 30% of control cultures. All ethanol and methanol extracts tested were ineffective. Results suggest that screened sponge species may contain substances with potential antibacterial applications. Further research would aim to the identification and isolation of the compounds responsible for this activity, as well as the optimization of extraction procedures.

Keywords: marine sponges, antimicrobial compounds, pathogens

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N-38

ANALYSIS OF β-CASOMORPHINS IN CHEESE AND MILK BY NANOELECTROSPRAY WITH ION-TRAP MASS SPECTROMETRY

Cristina Juan García[1*], Guillermina Font Pérez[2], Ana Juan García[3], Jordi Mañes Vinuesa[4]

[1 2 3 4] Laboratori de Bromatologia i Toxicologia, Facultat de Farmàcia, Universitat de València
[*] Corresponding author–E-mail: cristina.juan@uv.es; Phone: 0034 654742761

β-casomorphins (β-CMs) are peptides yielded by the digestion of β-casein that present opioid agonist effects similar to morphine and are generated in natural way like in fermentation process of milk products. [1]. These bioactive peptides have high content on proline residues which it is related with effects as opioid (casomorphins), antithrombotic, antihyperthensive, inmunomodulators or mineral carriers [2,3]. Under the hypothesized damage that β-CMs cause, it is necessary to develop sensitive techniques that unequivocal confirm of the presence of them.

In this study, a selection of analytical methods were developed for the four most commons β-CMs: β-casomorphin (1–5) bovine, β-casomorphin (1–7) bovine, [d-Ala2, D-Pro4,Tyr5]-β-casomorphin (1–5) amide and β-casomorphin (1–5) amide[D-Ala2,Hyp4,Tyr5] using multiple tandem MS with electrospray ionisation (ESI) and nano ESI sources with quadrupole ion-trap mass spectrometry (QIT-MS) and applied for cheese and milk samples.

Multiple tandem mass spectrometer was examined for the determination of β-CMs in both matrices; a quadrupole ion-trap (QIT) MS permitted structural studies on casomorphins since mass fragmentation pathways can be studied using MS^n. The major product ions obtained in QIT-MS were used to construct fragmentation pathways for the above β-CMs mentioned and different collision energies using automated nanoESI ion source NanoMate and conventional LC in QIT-MS were studied.

Calibration data for β-CMs, using spiked milk or cheese samples (10 g or 10 mL), were: NanoMate/MS (25–1000 µg/L), r^2= 0.998; NanoMate/MS^2 (5–1000 µg/L), r^2=0.9992; NanoMate/MS^3 (2.5–1000 µg/L), r^2= 0.9998. Reproducibility data (% RSD, N=5) for NanoMate/MS^n mode ranged between 2.0 at 500 µg/L and 7.0 at 10 µg/L.

Our results showed that QIT-ESI-MS permits to elucidate the main fragmentation pathways for β-CMs and structures for the major fragment ions that were observed in the mass spectra and an improvement detection sensitivity using LC and automated nanoESI (NanoMate), linked to the QIT-MS, to determine β-CMs in cheese and milk samples.

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Keywords: beta-casomorphins, milk, cheese, nanoelectrospray

N-39

AGROBACTERIUM RHIZOGENESE,PLANT TRANSFORMATION, HAIRY ROOTS INDUCTION OF THEOBROMA CACAO

Sumaryati Syukur[1*], Zozy Aneloi N.[2]

[1] Laboratory of Bioteknologi, Department of Chemistry, Fac.of Natural Sciences, Univ.of Andalas, Padang,Indonesia
[2] Laboratory of Plant physiology, Department of Biology.Fac.of Natural Sciences, Univ.of Andalas, Padang, Indonesia
[*] Corresponding author–E-mail: sumaryatisyukur@yahoo.com; Phone: 0812661 5601; Fax: 0751 71691

The used of transformed root cultures as a genetically and biochemical stable system for the study and production of secondary metabolites have been achieved. *Agrobacterium rhizogenes* is known to induce the proliferation of rapid growing, highly branching roots (hairy roots) in most dicotyledonous plants. Transformation of Ri T-DNA Plasmid *Agrobacterium rhizogenese* strain LBA 9457 to 3 different varieties of *Theobroma cacao* (Criollo, Forestero, and Trinitario), growing in west Sumatra Indonesia has been successfully observed. Pure cacao seeds known as good supplement of antioxidant bioflavonoid polyfenol such as catechin, olygomer procianidin (OPC) and other minerals, but contain more than 50% saturated fatty acid. So far no report concerning hairy root induction and antioxidant polyfenol can be obtained from cacao hairy roots.

The aim of this paper is to study the early respond of three diffrent varieties of *T.cacao* embryos, namely varietis of (Criollo, Forestero or Trinitario), and also have better and quick respond of hairy root formation. Secondly, to determine the antioxidant content of polyfenol catechin in hairy roots. The conformation of Ri plasmid T-DNA in some transform roots also will reported.

The results shows that all varieties of *T.cacao* (Criollo, Trinitario and Farestero) can induce hairy root formation after 6 to 7 days after infected. But the *T.cacao* Trinitario was the earliest hairy root formation. All *T.cacao* varieties can produce hairy root, as percentages of 100%, with 9 treatments and 3 repeated experiments. The antioxidant polyfenol catechin content in hairy roots transformant give significant increase (10 times) in transform roots of *T.cacao*, compared to non transform.

The conformation of plasmid Ri T-DNA of hairy roots some transformants analyses by PCR methods. The primers rol B1 (5^2ATGGATCCCAAATTGCTTCCCCCACGA3^2) and rol B^2 (5^3TTAGG CTTTCATTCGGGTTTACTGCAGC 3^3) was used. For TR-DNA the primes used is TRI (5^3GGAAATTGTGGCGTTGTTGTGGAC3') and TR2 (5' AATCGTTCAGAGAGCGTCCGA AGTT 3'). DNA electrophoresis shows the band of TL region at 780 bp and TR at 1600 bp using DNA Ladder as standard.

Keywords: Agrobacterium rhyzogenese, Hairy Root, T.cacao

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APPENDIX

LAST MINUTE POSTERS

L-36
METHODS TO DETECT DNA IN RELATION TO FOOD SAFETY AND AUTHENTICITY

Rosangela Marchelli[1*]

[1] Dipartimento di Chimica Organica e Industriale dell'Università di Parma
V.le G.P.Usberti 17/A, 43100 Parma, Italy
Corresponding author - E-mail: rosangela.marchelli@unipr.it

With the development of genomics and trascriptomics in molecular biology and biomedicine, methods developed for these disciplines became available and very useful also for food analysis, in connection with the evaluation of safety and authenticity. In this context, a number of new methods have been developed aiming at improving the sensitivity and/or rapid methods of detection in order to comply both with the regulations and with the screening purposes of industries.

In this presentation I will describe several methods, developed in our laboratories, based on PNA (peptide nucleic acids), new probes with a high affinity for DNA and RNA and a particularly high ability to discriminate SNPs (single nucleotyde polymorphisms).

In particular, PNAs were used as probes to build microarrays for the detection of GMOs (1), allergens (2) and for the recognition of olive oil authenticity and tomato varieties. More recently the method has been applied to to the detection of a food-born virus, the Norovirus, not easy to detect since it cannot be cultivated.

With the purpose of detecting Norovirus RNA in solution, Light-up and FIT-probes based on PNAs, modified with fluorophores such as Tiazole Orange or Pyrene have also been developed. The recognition is based on the fluorescence switch-on occurring upon formation of the duplex PNA-RNA:

PNA probes have been used also in connection with HPLC (3), with CD (circular dicroism) (4) and with Surface Plasmon Resonance (SPR) (5). In collaboration with Spoto (University of Catania) a microfluidic device has been developed which allows to detect DNA without previous amplification (PCR free) down to zepto-Molar concentrations.

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B-11

ABSENCE OF DETECTABLE GENETICALLY MODIFIED (GM) MAIZE SEED SAMPLES IN NEPAL

Hari Kumar Shrestha[1], Men-Chi Chang[2], Kae-Kang Hwu[3*]

[1 2 3] Department of Agronomy, National Taiwan University, Taiwan, R.O.C
* Corresponding author - E-mail: khwu@ntu.edu.tw; Phone: +886-2-33664761 (309); Fax: +886-2-2362-0879

Maize is the second major cereal in Nepal and its biosafety as food resource and ecologically conservation is an important concern in Nepal. To address this issue and establish the trade and biosafety monitoring system, GM maize detection is necessary. Currently, in Nepal none of any publicly available GM maize detection platform was build up, neither comparable legal regulations nor labeling directives and information. Therefore, we aimed to provide an updated and complete assessment of GM maize in Nepal. This work would be helpful in setting up regulation policy of GM maize in Nepal and renewing the latest information of GMO database for cereal grains. To address the uncertainty of transgene in Nepal maize seed samples, first, forty six maize seed samples were collected from sixteen different localities of Nepal in August/September 2008. Among them twenty-five, twelve and nine samples were from seed markets; the National Maize Research Programme and farmers saved seeds respectively. Then, a highly specific and sensitive multiplex polymerase chain reaction (mPCR) method was used to monitor the presence of event-specific GM maize lines, including Event176, Bt11, TC1507, NK603, MON863, MON810; T25 and GA21. Our screening result showed that after testing bulk samples composed of 2000 seeds each from standard sampling procedure, none of the GM maize sample had detectable GM content. We concluded that transgenic maize seeds were either absent or extremely rare in Nepal. This was the first report of GM maize risk assessment in Nepal and would provide a preliminary baseline for understanding, formulating and implementing maize seed quality control and food safety regulation in Nepal.

Keywords: GMOs, risk analysis, maize, mPCR

We thank Nabin Chand Tara Devi Shrestha and staff at National Plant Quarantine Programme, Nepal for providing phytosanitory certificate of samples. We thank to Hsin-Yi Chang for handling importation paper work in Taiwan. We acknowledge Laxmi M. Shrestha, Padam K. Shrestha, Som N. Shrestha, Jeevan K. Shrestha and Kamala D. Shrestha for helping to collect some maize samples, and delivering samples from Nepal to Taiwan.

LM-1

MIGRATION OF CAPROLACTAM FROM NYLON INTO MIGLYOL CONTAINING EMULSIFIERS

William Limm[1*], Timothy Begley[2], Anting Hsiung[3], Gregory Noonan[4]

[1 2 4] US FDA, College Park, MD
[3] Univ of Maryland, College Park, MD
* Corresponding author - E-mail: william.limm@fda.hhs.gov; Phone: (301) 436-1678; Fax: (301) 436-2634

Migration of caprolactam from nylon films at 40ºC to four food simulants is reported. The food simulants are: isooctane, Miglyol and two Miglyol solutions containing an emulsifier, either 2.3% lecithin or 2.3% polysorbate 60 (or, Tween 60). Multiple LC-MSD analyses of caprolactam in the food simulants indicate measurable differences between caprolactam concentration in Miglyol and those in two Miglyol solutions containing either of the two emulsifiers. A greater reduction in caprolactam concentrations in emulsifier solutions in comparison to that in Miglyol is observed at 22ºC and 5ºC than at 40ºC. At lower temperatures, migration of caprolactam to Miglyol is expected to be reduced due to its lower solubility. However, for Miglyol solutions containing emulsifiers, caprolactam concentration in Miglyol phase seems to be further reduced by increased partitioning of caprolactam into emulsions.

LM-2

ESTIMATION OF FE AND ZN CONCENTRATION FROM 3 NOURISHING SUPPLEMENTS TO ORGANIZING A NUTRITION EXPERIMENT ON WEANED PIGLETS

Arabela Untea[1], Rodica Diana Criste[2*], Margareta Olteanu[3], Raluca Olaru[4], Lenuta Enache[5]

[1 2 3] National Research and Development Institute for Biology and Animal Nutrition, Balotesti, Roumania
[4] Institute of Hygiene and Veterinary Public Health, Bucharest, Roumania
[5] National Institute for Nonferrous and Rare Metals, Bucharest, Roumania
[*] Corresponding author - E-mail: arabelapatrichi2002@yahoo.com; Phone: 0040726279268

Three nourishing supplements from wild environment (Origanum vulgare; Vaccinium myrtillus L.; Tribulus terestris) were used in a nutrition experiment with the main purpose the enhancement of mineral status of weaned piglets. The calculating approach of the experimental diets and the inclusion rate of the supplements in diet are the stages of experimental protocol based on chemical composition of the plants. For an accurate determination of the mineral composition of plants, we made an interlaboratory study, with 7 participants – laboratories in the field "feed / food". A homogeneity test was applied to the samples and they were distributed to each participant on sealed plastic bags. All laboratories accomplished ten measurements for each sample for iron and also for zinc. The determination method was established by protocol, flame atomic absorption spectrometry. The reference value was established by consensus (value established directly from the reported results – we calculate the central trend of results and then the standard deviation is used for uncertainty determination). The appearance frequency of the results was determined by Kernel distribution plot and the remove of the doubtful results was done by the t test. The participants performance was evaluated by Z score (21 values of Z = 7 laboratories x 3 plants samples - in all cases $z < 3$, for one value $2 < z < 3$ for iron and for another value $2 < z < 3$ for zinc) and Zeta score (for 2 values zeta < 2, for 2 values 2 < zeta < 3 si for 3 values 3 <zeta). The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories offer as possible explanation of unsatisfactory zeta score an underestimation of the uncertainty. This hypothesis is confirmed in case of laboratories with z score satisfactory and a zeta score unsatisfactory. From the results obtained, we concluded that one participant provide questionable results for iron for Origanum Vulgare and another participant for zinc for Tribulus terestris.

Keywords: iron, zinc, nourishing, supplements, piglets

LM-3
PRODUCTION OF POLYCLONAL ANTIBODY AGAINST AFLATOXIN M1 IN THAILAND

Amara Chinaphuti[1*], Suppara Aukkasarakul[2]

[1 2] Postharvest and Processing Research and Development Office, Bangkok, Thailand
[*] Corresponding author - E-mail: amarachina@yahoo.com; Phone: 662 579 6009

Aflatoxin M1 (AFM1) is a major metabolite of aflatoxin B1 in mammalian system. Contamination was found in milk and their products which had been classified as carcinogen by IARC. AFM1 is considered to be the pose certain hygienic risk for human health. Therefore, the rapid, sensitive and accuracy methods for routine assay AFM1 in milk and milk products are necessary. The recent study was developed the rapid technique Enzymed-Linked immunosorbent Assay (ELISA) in order to detect AFM1 in milk. Polyclonal antiserum specific for AFM1 was produced against AFM1–BSA in eight white New Zealand rabbits of 2 months old. Intradermal injection was done for the initial injection at the back of rabbits followed by 4 times booster injections (muscular injection) with 1 month interval. Bleeding from the ear of rabbits was first done one month after initial injection and weekly bled for 21 weeks. The amount of 720 mililitre of antiserum was produced. The highest concentration of collected serum when tested by indirect ELISA was 1:250,000. Percentage cross reaction against AFB1 AFB2, AFG1 and AFG2 were 67.34, 4.85, 23.57 and 2.20 respectively. AFM1-HRP conjugate was prepared by water soluble carbodiimide method and yielded 7.2 milliliters with the concentration 1:640.The produced antiserum and the prepared AFM1-HRP conjugate were used to analyze AFM1 milk sample by direct competitive ELISA with the proper concentration of 1:2,000 for antiserum and 1:20 for enzyme conjugate . Moreover, the indirect competitive ELISA was also tested for their efficacy in comparison to direct competitive ELISA for AFM1 detection. The better result was obtained when indirect competitive ELISA was used. The recovery test for the efficacy of the technique to detect added AFM1 in the sample were 0, 74.6, 76.8, 69.4 and 81.2% for 0.1,0.2, 0.5,1 and 2 ng/ml of added AFM1. ELISA technique for AFM1 detection in milk had been developed in this study could be successfully done within 2 hours.

Keywords: Polyclonal Antibody, ELISA, aflatoxin, milk

LM-4

IDENTIFICATION OF APPROPRIATE WILD YEASTS FOR SPONTANEOUS WINE FERMENTATIONS AND DETERMINATION OF WINE ORIGIN MARKERS

Ana María Molina[1*], Amadée Mathier[2], Fadri Kuonen[3], Gisela Boehle[4], Rainer Peters[5], Elizabeth Hardebusch[6], Björn Seidel[7]

[1] Universidad San Sebastián, Santiago, Chile
[2 3] Albert Mathier et fils, Salgesch, Switzerland
[4 5 6 7] Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany
[*] Corresponding author - E-mail: ana.maria.molina@ime.fraunhofer.de; Phone: +49/2972/302-441; Fax: +49/2972/302-319

The winemaking process requires the presence of wine yeasts to ferment the grape must. In the ancient winemaking tradition, grape juice was left to ferment spontaneously by the endogenous grape flora in a natural and uncontrolled manner. In the last decades, industrialization of the winemaking process and renovation of the food and brewage market have encouraged the practice of inoculating wine yeasts into the must in order to standardize the fermentation process. This practice allows a better control of the fermentation starting time, prevention of stuck and sluggish fermentation and prevention of spoilage of undesired microorganisms. However, inoculated fermentations produce wines with more reproducible flavor and homogeneous aroma than no-inoculated wines, and thus, with less unique and distinctive characters.

Nowadays, an increasing consumer proportion is preferring exclusive and gourmet foodstuffs. Limited edition and Premium wines are high-priced high quality products, characterized by small scale production, high-cost treatments, special terroirs, or exclusive designation of origin. Some wineries willing to produce elite wines favor the uniqueness of their wines and exceptional flavor combinations by performing spontaneous non-inoculated fermentation. However, with this method longer fermentation times occur, as well as higher risk of stuck or sluggish fermentation and of off-flavor formation, and thus, the risk of economical losses is high. Therefore, traditional wineries are interested on decreasing the risk of unsuccessful fermentation.

Spontaneous fermentations can originate defective wines when the grape wild flora is unable to complete the fermentation, or when microorganisms producing negative flavor compounds are predominant. Thus, efficient monitoring and identification of yeasts in the grape flora and in the fermenting must could facilitate the detection and prediction of problematic fermentations, and the opportune incorporation of adequate solutions. In this context, we try to develop a cost effective detection kit for on-site monitoring of wild yeasts in spontaneous fermentations. In addition, suitable chemical markers for objective identification of the wine origin will be determined.

Keywords: spontaneous wine fermentation, wild yeast

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LM-5

TACKLING THE POSSIBLE ABUSE OF ILLEGAL DYES IN AQUACULTURE: 2 NEW ANALYTICAL METHODS FOR THE DETERMINATION OF CHRYSOIDINE AND 17 TRIARYLMETHANE AND PHENOTHIAZINE DYES IN FISH AND FISHERY PRODUCTS

Tim Reyns[1], Stéphanie Fraselle[2], Désiré Laza[3], Joris Van Loco[4*]

[1 2 3 4] Scientific Institute of Public Health, Brussels, Belgium
[*] Corresponding author - E-mail: joris.vanloco@iph.fgov.be; Phone: +32(0)26425353; Fax: +32(0)26425691

Due to the growing concern dealing with the usage of dyes as biocide in aquaculture a project has been launched to investigate the possible abuse of illegal dyes in aquaculture. Although most attention is paid to malachite green, other members of this class of dyes have also concern to possible toxicological, teratological and carginogenetic properties. Moreover, they could also be used in aquaculture industry for the treatment of bacterial, fungal and parasite infections. A remarkable fact is that several of the dyes are commercially available in local pet shops for the usage of pet and ornamental fish. Possible abuse in aquaculture may be expected. The alerts for Malachite Green (MG) and Leuco Malachite Green (LMG) in the Rapid Alert System for Food and Feed (RASFF) has reached a maximum in 2005 with 57 alerts and has been decreased significantly to 2 alerts in 2008. 10 announcements in the RASFF for Cristal violet, one of the possible alternatives for MG, have been announced up to now. The use of brilliant green is suspected but not yet reported within the RASFF. An UPLC-MS/MS method for the determination of 15 triarylmethane, xanthene, phenoxazine and phenothiazine dyes was developed with emphasis on low detection limits. Components can be quantified at 0.25 ppb (LOQ). The LOD is estimated to be 0.05 ppb or lower.

Chrysoidine, a dye used as colouring agent for wool and silk paper, may be illegally used to colour fish skin to make lower quality aquaculture fish resemble natural fish. A strict monitoring program has been installed by the Chinese government for this compound. In Europe no rapid alerts have been announced until now. An UPLC-MS/MS method has been developed and was validated in accordance with the decision 2002/657/EC.

In a first attempt to investigate the possible abuse of illegal dyes in fish 50 samples have been collected on the Belgian market and will be analysed with these 2 analytical methods.

Keywords: dyes chrysoidine residue fish

LM-6

ABSENCE OF DETECTABLE GENETICALLY MODIFIED (GM) MAIZE SEED SAMPLES IN NEPAL

Hari Kumar Shrestha[1], Men-Chi Chang[2], Kae-Kang Hwu[3*]

[1 2 3] Department of Agronomy, National Taiwan University, Taiwan, R.O.C
[*] Corresponding author - E-mail: khwu@ntu.edu.tw; Phone: +886-2-33664761 (309); Fax: +886-2-2362-0879

Maize is the second major cereal in Nepal and its biosafety as food resource and ecologically conservation is an important concern in Nepal. To address this issue and establish the trade and biosafety monitoring system, GM maize detection is necessary. Currently, in Nepal none of any publicly available GM maize detection platform was build up, neither comparable legal regulations nor labeling directives and information. Therefore, we aimed to provide an updated and complete assessment of GM maize in Nepal. This work would be helpful in setting up regulation policy of GM maize in Nepal and renewing the latest information of GMO database for cereal grains. To address the uncertainty of transgene in Nepal maize seed samples, first, forty six maize seed samples were collected from sixteen different localities of Nepal in August/September 2008. Among them twenty-five, twelve and nine samples were from seed markets; the National Maize Research Programme and farmers saved seeds respectively. Then, a highly specific and sensitive multiplex polymerase chain reaction (mPCR) method was used to monitor the presence of event-specific GM maize lines, including Event176, Bt11, TC1507, NK603, MON863, MON810; T25 and GA21. Our screening result showed that after testing bulk samples composed of 2000 seeds each from standard sampling procedure, none of the GM maize sample had detectable GM content. We concluded that transgenic maize seeds were either absent or extremely rare in Nepal. This was the first report of GM maize risk assessment in Nepal and would provide a preliminary baseline for understanding, formulating and implementing maize seed quality control and food safety regulation in Nepal.

Keywords: GMOs, risk analysis, maize, mPCR

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LM-7

DETERMINATION OF GLYPHOSATE AND AMPA BY LIQUID CHROMATOGRAPHY COUPLED TO ELECTROSPRAY TANDEM MASS SPECTROMETRY: DERIVATIZATION, PRECONCENTRATION AND APPLICATION IN NATURAL WATER

Samuel Cantarero Malagón[1*], Teresa Blazquez Málaga[2], Antonio Sanchez Sanchez[3], Pedro Aranzadi Cortina[4]

[1 2 4] Laboratorios medioambientales Denga, S.A., Madrid, Spain
[3] Infraestructura y Ecología, S.L., Sevilla, Spain
[*] Corresponding author - E-mail: scantarero@denga.net; Phone: 0034913839536

Glyphosate [N-(phosphonomethyl)glycine] is broad spectrum, nonselective, post-emergence herbicides extensively used in various applications for weed control in aquatic systems and vegetation control in non-crop areas. Aminomethylphosphonic acid (AMPA) is the major degradation product of glyphosate found in plants, water and soil [1]. Although glyphosate and AMPA are thus not expected to be exported to groundwater and surface water, both were detected at levels of up to several µg·L-1 in surface water [2]

Due to the extensive worldwide use of these compounds and the restrictive regulations for water in the European Union [3], very sensitive methods for the determination of glyphosate and AMPA are required. However, the determination of these two herbicides at the sub µg·L-1 level is difficult due to their ionic character, low volatility, low mass and lack of chemical groups that could facilitate their detection.

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is currently the method of choice for polar analytes due to its high selectivity and sensitivity. However, when using LC-MS/MS, derivatization of glyphosate and AMPA is required to enable analysis and enrichment by reversed phase (RP) sorbent phases [4].

The present research work is based on the development of a novel methodology to determine both analytes in natural water. The proposed method comprises sereval steps: i) Derivatization of Glyphosate and AMPA with FMOC-Cl and acetonitrile as a reagents in order to generate suitable response, ii) Preconcentration step so as to obtain low limit of quantification (0.01 µg·L1), iii) Separation and detection by Liquid Chromatography coupled to electrospray tandem mass spectrometry.

Finally, the methodology was successfully applied in a monitoring study carried out in Andalucia (Spain), analyzing more than forty samples.

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LM-8

ASSESSMENT AND COMPARISON OF TWO COMPLEMENTARY METABOLOMIC STRATEGIES BASED ON LC-HRMS FINGERPRINTS AS A TOOL TO SCREEN FOR ANABOLIC TREATMENT IN CALVES

Gaud Pinel[1*], Stefan Weigel[2], Arjen Lommen[3], Lauriane Rambaud[4], Martien Essers[5], Linda Stolker[6], Frédérique Courant[7], Jean-Philippe Antignac[8], Michel Nielen[9], Bruno Le Bizec[10]

[1 4 7 8 10] LABERCA, ENVN, France
[2 3 5 6 9] 2 RIKILT – Institute of Food Safety, Wageningen, The Netherlands
[*] Corresponding author - E-mail: pinel@vet-nantes.fr; Phone: 33 2 40 68 78 80; Fax: 33 2 40 68 78 78

Anabolic steroids have been widely used over the last past 50 years in cattle breeding practices with beneficial effects such as animal growth promotion and feed efficiency. They are banned in food producing livestock in Europe. Efficient methods based on mass spectrometry detection have been developed to ensure the control of such veterinary drug residues. Nevertheless, the use of "cocktails" composed of mixtures of low amounts of several substances as well as the synthesis of new compounds of unknown structure prevent efficient prevention. New analytical tools able to detect such abuse are today mandatory. In this context, metabolomics may represent new emerging strategies for investigating the global physiological effects associated to a family of substances and therefore, to suspect the administration of steroids. The purpose of the present study was to set up, assess and compare two complementary mass spectrometry-based metabolomic strategies as new tools to screen for steroid abuse in cattle and demonstrate the feasibility of such approaches. The protocols were developed in two European laboratories in charge of residues analysis in the field of food safety. Apart from sample preparation, the global process was different in both laboratories from LC-HRMS fingerprinting to multivariate data analysis through data processing. The reproducibility of both sample preparation and MS measurements were assessed in order to guarantee that any differences in the acquired fingerprints were not caused by analytical variability but reflect metabolome modifications upon steroids administration. The protocols were then applied to urine samples collected on a large group of animals consisting in 12 control calves and 12 calves administrated with a mixture of 17β-estradiol 3-benzoate and 17β-nandrolone laureate esters according to a protocol reflecting likely illegal practices. The modifications in urine profiles as indicators of steroid administration have been evaluated in this context.

Keywords: Metabolomics, Untargeted profiling, steroids, calves

BioCop FP6 Project

LM-9

INCREASED PESTICIDE RECOVERY IN FRUIT AND VEGETABLE PRODUCTS USING THE GENO/GRINDER WITH THE QUECHERS METHOD

Lea Anderson-Smith[1*], Patricia Atkins[2], Grahame Mowatt[3]

[1] SPEX SamplePrep, Metuchen, USA
[2] SPEX CertiPrep, Metuchen, USA
[3] SPEX CertiPrep, London, England
[*] Corresponding author - E-mail: gmowatt@spexcsp.com; Phone: +44 208 204 6656

Samples prepared for pesticide analysis according to the QuEChERS method are typically combined with solvent and buffering salts, then mixed by shaking for 1 min. by hand. In this study, GC-MS results of samples prepared using the standard, manual QuEChERS methods were compared with results for samples mechanically mixed using the Geno/Grinder.

Keywords: Pesticide, quechers, GC-MS, food

LM-10
FOOD SAFETY ISSUES AND HACCP FOR MILK PRODUCTS

Kakhaber Nadiradze[1*]

[1] Tbilisi, Georgia
[*] Corresponding author - E-mail: knadirad@geo.net.ge; Phone: +99532654878; Fax: +99532650871

Hazards can occur in milk due to unsanitary food contact surfaces that can contaminate milk with pathogens or with residual allergens from product processed on the equipment in prior runs that can cause allergic reactions in sensitive individuals. Hazards that arise from unsanitary food contact surfaces have the potential to affect the safety of a milk product because they arise from points within the process and not from general conditions within the facility. Control of these hazards may be accomplished by the use of Prerequisite Programs. For example, an appropriate PP could be to establish a procedure for cleaning equipment with a cleaning solution, e.g. a pre-rinse followed by a caustic wash followed by a rinse. The procedure could include maintaining a log of which foods, e.g. juice, eggnog, soy drinks, were processed on the equipment, the sequence in which the foods were processed, and how/when the equipment was cleaned. The operator could check that log prior to starting any production run for milk. The procedure could provide that the equipment would not be used for milk until the prescribed cleaning procedure was carried out, recorded in the log, and the equipment was visually checked for cleanliness.

Keywords: Hazards, Milk, Safety, Food, Controle

LM-11

ELEMENTAL CONTENT IN WHEAT PRODUCTS OF ASIR REGION, SAUDI ARABIA

Omar A.F. Al-Dayel [1*], Saad A. Al-Kahtani [2]

[1 2] King Abdulaziz City for Science and Technology (kacst), P. O. Box 6086, Riyadh 11442, Saudi Arabia
[*] Corresponding author - E-mail: oaldayel@kacst.edu.sa; Phone: +966-1-4813624; Fax: +966-1-4814750

Wheat is one of the most grown crops in Saudi Arabia. It is grown in various regions of the country. Accurate knowledge of the various elemental concentrations in wheat and its products (bran and flower) is of great importance from nutrition point of view.

Wheat samples were obtained from Riyadh region in Saudi Arabia and analyzed. Up to 50 elements (Al, Sb, As, Ba, Br, Cd, Ca, Cs, Cl, Cr, Co, Cu, Ga, Au, Hf, In, I, Ir, Fe, Mg, Mn, Hg, Mo, Ni, K, Rb, Sc, Se, Ag, Na, Sr, Ta, Te, Th, Sn, Ti, W, U, V, Zn, Zr, Ce, Dy, Eu, La, Lu, Nd, Sm, Tb and Yb) in wheat products were determined. It was observed that the mineral content of bran was much higher than white flour.

Keywords: Wheat, Elemental Analysis

LM-12

COLLOIDAL GOLD BASED FLOW-THROUGH RAPID TESTS FOR DEOXYNIVALENOL AND ZEARALENONE

Piet van Wichen[1*], Esther Grutters[2], Lucia Streppel[3], Ron Verheijen[4]

[1 2 3 4] EuroProxima BV, Arnhem, The Netherlands
[*] Corresponding author - E-mail: Piet.vanWichen@europroxima.com; Phone: +31(0)26 363 0 364; Fax: +31(0)26 364 5 111

Two easy to perform flow-through assays have been developed for the screening of cereals for the presence of the *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (Zea). The tests are competitive immunoassays based on monoclonal antibodies directed against DON (DON-FT test) and Zea (Zea-FT test).

Both the DON- and Zea-FT test are colloidal gold based flow-through immunoassays. DON(Zea), coupled to BSA, is immobilised as a distinct line on a flow-through membrane. The conjugate, consisting of mobile red colloidal gold particles labelled with a monoclonal antibody to DON(Zea), is deposited in dry form onto a glass fiber conjugate pad in a plastic vial. Prior to use, the conjugate is brought into solution by addition of reconstitution buffer. A sample, extracted from cereals that may contain DON(Zea), is then added to the conjugate solution. After a 10 minute incubation, the DON(Zea)-conjugate complex is transferred onto the flow-through membrane. If the sample contains no or a very low amount of DON(Zea), the anti-DON(Zea) antibodies in the conjugate can bind to the immobilized DON(Zea)-BSA and a red line will appear in the test zone. If the sample contains ≥ 500 ppb of DON (or ≥ 100 ppb of Zea), all antibodies will be captured by the conjugate and no line will appear. With any sample a control line should appear in the control zone. The control ensures that the conjugate is active. The sensitivities of the DON-FT test and the Zea-FT test have been set at a cut-off limit of 500 ppb and 100 ppb, respectively.

The main advantage of both the DON-FT test and Zea-FT test is that the results are obtained very quickly. The tests are easy to perform. Moreover, the sample preparation is fast and simple.The tests can be used to screen cereals for the presence of DON and zearalenone.

Keywords: mycotoxin, immunoassay, flow-through

LM-13

STUDY OF THE ANTIMICROBIAL ACTIVITY OF ACTIVE PP FILMS ADDITIVATED WITH CARVACROL AND THYMOL

Marina Ramos[1], Mercedes Peltzer[2*], Ma. del Carmen Garrigós[3]

[1 2 3] University of Alicante, Alicante, Spain
[*] Corresponding author - E-mail: mercedes.peltzer@ua.es; Phone: +34965903400

Active packaging systems interact with foodstuff by positively modifying the sensorial, nutritional and microbiological properties of food with the result of an increase in shelf-life with no damage to food quality (1). These systems have become a popular alternative to control undesirable molds in foods (2). Essential oils extracted from plants or spices are rich sources of biological active compounds such as terpenoids and phenolic acids. It has been long recognized that some of the essential oils show antimicrobial properties (3). Oregano essential oil is one of the most widely used and shows high antimicrobial activity (4), being carvacrol and thymol its major components (5). The polymer used in this study was polypropylene ECOLEN HZ10K (Hellenic Petroleum, provided by Ashland Chemical Hispania). Carvacrol 98% and Thymol 99.5% were used as active additives (Sigma-Aldrich). The following formulations were prepared: PP containing 8wt% of carvacrol (PPC8), 8wt% of thymol (PPT8), 8wt% of carvacrol and thymol (50%-50%) (PPCT8) and PP without any active compound as control (PP0). The different mixtures were obtained by blending the additives with the polymer in a Haake mixer at 190 ºC for 6 minutes and a rotation speed of 50 rpm. 200 µm (average thickness) films were obtained by compression-molding at 190 ºC. The antimicrobial activity of the active films was tested on three different foodstuffs: bread, cheese and strawberries. Foodstuffs were placed in Petri dishes with the active film in contact with them. The storage temperature was 25 ºC and foodstuff surfaces were observed for the presence of molds every three days. In addition, the effectiveness of the active films in bacteria: E. coli and S. aureus was evaluated by the disk diffusion method observing if there was any bacterial growing inhibition. The inhibition process of the different PP active films additivated with carvacrol and thymol was studied during 15 days, showing differences between samples depending on the antimicrobial agent used.

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Keywords: carvacrol, thymol, active packaging, antimicrobial

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LM-14

CONTRIBUTION OF INDOOR AIR, HOUSE DUST AND FOOD TO THE TOTAL PHTHALATE EXPOSURE OF ADULTS IN GERMANY

Hermann Fromme[1*], Ludwig Gruber[2], Wolfgang Körner[3], Sigrun Boehmer[4], Dieter Heitmann[5], Martin Schlummer[6], Gabriele Bolte[7]

[1 4 7] Department of Environmental Health, Bavarian Health and Food Safety Authority, D-85764 Oberschleissheim, Germany
[2 6] Department of Product Safety and Analysis, Fraunhofer-Institute for Process Engineering and Packaging, D-85354 Freising, Germany
[3 5] Bavarian Environment Agency, Unit Organic Analysis, D-86179 Augsburg, Germany
[*] Corresponding author - E-mail: hermann.fromme@lgl.bayern.de; Phone: ++49 89 31560231

Due to their widespread use as plasticizers, today phthalates are ubiquitous environmental chemicals with potential health effects on different endpoints, especially on reproduction. INES created a work for an integrated exposure assessment approach including data of different organic pollutants in various environmental media, in food, and the body burden of humans. The aim of this study was to quantify the exposure to phthalates using results from food duplicates, indoor air and house dust. Overall, 27 female and 23 male healthy subjects aged 14-60 years were included. Daily duplicate food portions (overall n = 350) prepared as for consumption were collected over 7 consecutive days. Phthalates were detected using GC/MS in SIM-mode. In the 34 residences in which these 50 individuals lived, air was sampled for 24 hours in the living room using a sampler with a flow rate of 50 mL/min. One vacuum cleaner bag was collected from each household on the day of indoor air sampling. The samples were sieved

Keywords: Phthalates, DEHP, food, air, dust

LM-15

PBDE EXPOSURE USING DATA FROM DUPLICATES, INDOOR AIR AND DUST IN GERMANY. RESULTS FROM THE INTEGRATED EXPOSURE ASSESSMENT SURVEY (INES)

Hermann Fromme[1*], Wolfgang Körner[2], Nabil Shahin[3], Michael Albrecht[4], Antonia Wanner[5], Gabriele Bolte[6]

[1 4 6] Department of Environmental Health, Bavarian Health and Food Safety Authority, D-85764 Oberschleissheim, Germany
[2 5] Bavarian Environment Agency, Unit Organic Analysis, D-86179 Augsburg, Germany
[3] Technical University of Munich, Department of Chemical-Technical Analysis and Chemical Food Technology, D-85354 Freising-Weihenstephan, Germany
[*] Corresponding author - E-mail: hermann.fromme@lgl.bayern.de; Phone: ++49 89 31560231

Polybrominated diphenyl ethers (PBDE) are substances extensively used as flame retardants in a wide variety of products. INES was carried out to create a work for an integrated exposure assessment approach including data of different organic pollutants in various environmental media, in food, and the body burden of humans. The study aimed to characterize the PBDE exposure of an adult German population using duplicate diet samples, collected daily over 7 consecutive days, indoor air and house dust measurements. The study population consisted of 27 female and 23 male healthy subjects, aged between 14-60 years, living in Germany. In 34 residences air was sampled on glass fibre filters and polyurethane foams and dust was derived from vacuum cleaner bags regular in use. The highest intake rates were observed for BDE 99, BDE 183 and BDE 47. The median (95th percentile) daily dietary intake of the sum of six Tetra- to HeptaBDE congeners was 1.2 ng/kg b.w. (3.4 ng/kg b.w.) or 69.8 ng/day (215 ng/day). BDE 47, BDE 209 and BDE 28 had the highest contribution to total air concentrations and BDE 209 was the predominant congener (90%) in dust. Concentrations in indoor air and dust (sum of 16 Tri- to DecaBDE congeners) ranged from 8.2 to 477 pg/m³ (median: 37.8 pg/m³) and 36.6 to 1580 ng/g (median: 386 ng/g), respectively. For some congeners a significant correlation between air and dust levels was observed. Based on these measurements and exposure assumptions we estimated for the sum of tetra- to hexabrominated congeners a mean (high) comprehensive total daily intake of 1.2 ng/kg b.w. (2.6 ng/kg b.w.), respectively. Overall, our results suggest that dietary exposure is the dominant intake pathway at least in our study population, responsible for 97% (mean intake) and 95% (high intake) of the total intake of an adult population. Our results were supported by findings from the U.K. where the median dietary exposure contributes 93% to the overall daily exposure. Our estimate of the total daily intake via all exposure pathways for adults in Germany was somewhat lower than reported before for the U.K. (1.6 ng/kg b.w.) and considerably lower as in the U.S. (7.7 ng/kg b.w.).

Keywords: PBDE, food, air, dust

LM-16

DETERMINATION OF PESTICIDES AND THEIR METABOLITES IN NATURAL WATER BY LIQUID CHROMATOGRAPHY COUPLED TO ELECTROSPRAY TANDEM MASS SPECTROMETRY

Samuel Cantarero Malagón[1*], Teresa Blazquez Málaga[2], Antonio Sanchez Sanchez[3], Pedro Aranzadi Cortina[4]

[1 2 4] Laboratorios medioambientales Denga, S.A., Madrid, Spain
[3] Infraestructura y Ecología, S.L., Sevilla, Spain
[*] Corresponding author - E-mail: scantarero@denga.net; Phone: 0034913839536

Pesticides are defined by FIFRA (Federal Insecticide, Fungicide and Rodenticide Act) as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any insects, or any other form of life declared to be pests, and any substance or mixture of substances intended for use as a plant regulator, or desiccant", with an annual worth market of 200 million in Spain [1, 2].

Despite their economical importance, the consumption of pesticides are decreasing in the last years [1, 2], due to their harmful effects on Human Health [3]. Taking into consideration these evidences, pesticides concentration in food and natural matrixes have been regulated by EU [4]. In spite of their regulation, some prohibited pesticides have been detected in natural water [5], causing the failure of the objetives that makes the EU to achieve good chemical status of natural water [6].

In order to control the concentration of pesticides in natural water, which can be used in agriculture, several analytical methodologies have recently been published [7].

The present research work is focused on the development and the validation of a new analytical methodology to analyze ultra trace levels of pesticides and their metabolites in natural water using Liquid Chromatography coupled to electrospray tandem mass spectrometry.

In comparison with others methodologies, the proposed method is able to obtain low quantification limit (0.01 $\mu g \cdot L^{-1}$), ten times lower than the permitted limit (0.1 $\mu g \cdot L^{-1}$) [4], without any preconcentration step, such as solid phase extraction (SPE).

Finally, the methodology was successfully applied in a monitoring study carried out in Andalucia (Spain), analyzing more than forty samples.

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Keywords: pesticides, natural water, mass spektrometry

LM-17

AF4-MALS-ICP-MS AND ELECTRON MICROSCOPY FOR THE CHARACTERIZATION OF NANOPARTICLES IN BIOLOGICAL STUDIES

Katrin Löschner[1*], Bjørn Schmidt[2], Keld A. Jensen[3], Xueyun Gao[4], Jens J. Sloth[5], Erik H. Larsen[6]

[1 2 5 6] National Food Institute, DTU, Søborg, Denmark
[3] National Research Centre for the Working Environment, Copenhagen, Denmark
[4] Chinese Academy of Science, Beijing, China
[*] Corresponding author - E-mail: kals@food.dtu.dk; Phone: +4535887029

A well defined characterization of engineered nanoparticles (ENPs) is essential for studying the influence of nanoparticles on biological systems in vitro and in vivo. For the determination of all relevant parameters, e.g. aggregation state, elemental composition, shape, size and size distribution, solubility, surface charge, a combination of different detection methods is required.

One important precondition for long-term nanotoxicity experiments is the stability of the stock solutions of ENPs. Both, agglomeration and dissolution of the ENPs can produce misleading results. We studied the stability of silver (uncoated as well as dextran-coated) and selenium nanoparticles in stock solutions of different concentrations over a time period of several weeks. The size distribution, the state of dispersion, and the zeta potential, were determined by asymmetric flow field flow fractionation (AF4) with multi angle light scattering (MALS) coupled to inductively coupled plasma mass spectrometry (ICP-MS), dynamic light scattering (DLS) and with transmission electron microscopy (TEM). Zeta potential measurements were carried out using laser Doppler electrophoresis (LDE). Because ENPs have a tendency to adsorb to surfaces the recovery of the ENPs following AF4 separation was evaluated.

The particle characterization by AF4-MALS-ICP-MS and TEM combined the advantages of both methods and their drawbacks canceled out each other. TEM is a single particle imaging method that provides information on the particle's primary structure in high resolution, but the sample preparation (drying) might lead to artifacts concerning the agglomeration state. In contrast, AF4 is a particle population method where the disturbance of the sample is kept to a minimum by directly analyzing the particle suspension.

Keywords: nanoparticles, nanotoxicity, ICPMS, electron microscopy

LM-18
CAN "DECONVOLUTION" IMPROVE GC/MS SENSITIVITY?

Chin-Kai Meng[1]*, Chris Sandy[2]

[1] Agilent Technologies, Wilmington, USA
* Corresponding author - E-mail: chin_meng@agilent.com; Phone: 302-633-8388

Deconvolution is a process of extracting compound spectra from a complex total ion chromatogram (TIC). Spectral Deconvolution of the MS data allows easy identification of analytes in the presence of overlapped matrix peaks. This is the goal of AMDIS (Automatic Mass spectral Deconvolution and Identification System) originally developed by NIST (National Institute of Standards and Technology) for trace chemical weapons detection in complex matrices. AMDIS de-skews the quadrupole TIC first and compares all ions' apexes and the rising and falling patterns throughout the TIC to associate all related ions into a deconvoluted spectrum (called a component). The matrix background or interference ions are therefore dropped out of the deconvoluted spectrum (component). Each deconvoluted (cleaned) spectrum is then searched against a custom library for hits. This allows analysts to find contaminants in the library but not on the method target compound list. The power of deconvolution will be explained and illustrated using results from sample extracts. Deconvolution increases accuracy and confidence of results, decreases processing time, minimizes user skill requirements, and improves productivity.

The ChemStation target compound hit is based on retention time, 4 ions, and ion ratios. Each AMDIS hit is based on full spectral library match, therefore, provides higher confidence results. In this study, the deconvolution was applied to spinach samples spiked with over 150 pesticides at 50, 250, and 500 ppb. The AMDIS quantitation results were compared to the ChemStation results for sensitivity comparison. Various AMDIS deconvolution settings (resolution, sensitivity and shape requirements) were compared for optimal results.

Keywords: AMDIS, deconvolution, pesticides, unknowns, non-target

The author wishes to thank Dr. Jon Wong of the US Food and Drug Administration (College Park, Maryland, USA) for providing numerous standards and food extracts for the study.

LM-19

COMPARING GC/QQQ TO GC/Q METHODS FOR THE ANALYSIS OF PESTICIDE RESIDUES IN FRUITS AND VEGETABLES

Chin-Kai Meng[1*], Philip Wylie[2], Chris Sandy[3]

[1 2] Agilent Technologies, Wilmington, USA
[*] Corresponding author - E-mail: chin_meng@agilent.com; Phone: 302-633-8388

This paper compares GC/single quadrupole-MS (GC/Q) and GC/triple quadrupole-MS (GC/QQQ) techniques for the analysis of pesticide residues in food extracts. The goal is to compare the sensitivity and selectivity of these two instruments. Various food extracts of increasing complexity were spiked with pesticide residues in decreasing concentrations to determine the limitations of each instrument for this type of analysis.

Two commonly-used GC/Q techniques were performed for this comparison. The first approach uses a GC/Q in the selected ion monitoring mode to analyze for a list of target compounds. The selectivity and sensitivity of this method are dependent upon sample cleanliness. When analytes co-elute with interferences that produce the same target or qualifier ions, identification and quantification are compromised.

The second approach employs a GC/Q in the scan mode with Deconvolution and a mass spectral library. This system is used to screen for over 900 pesticides, metabolites, and endocrine disruptors in a single injection. Deconvolution helps to extract clean, library-searchable spectra from background and interferences. Each deconvoluted spectrum is compared to the target library for identification.

For comparison, a GC/QQQ was used in the MRM mode to analyze the same samples. The instrument maintains the sensitivity of a SIM method while achieving the selectivity inherent in a triple quadrupole MS. Helium doping in the collision cell greatly reduces neutral noise and improves the instrument's sensitivity.

Several different crop extracts spiked with p,p'-DDE at 10 µg/Kg, were analyzed by GC/Q SIM and GC/QQQ MRM. The GC SIM method was able to identify DDE in apple, cabbage, and ginseng extracts, but matrix interference prevented its identification in orange and spinach extracts. The GC/QQQ had no problem with interferences in any of these samples. With an average peak-to-peak S/N of 370:1, it should be possible to identify DDE in the sub-µg/Kg range.

The high selectivity and sensitivity of this GC/QQQ makes it possible to confirm pesticide residues at the low ppb level even in the most complex extracts. Direct comparisons between these two GC/MS systems using the same samples illustrates the significant benefits afforded by the GC/QQQ.

Keywords: MRM, GC/QQQ, deconvolution, SIM

The authors wish to thank Dr. Jon Wong of the US Food and Drug Administration (College Park, MD, USA) and Dr. Steven Lehotay of the U.S. Department of Agriculture (ARS, ERRC, Wyndmoor, PA, USA) for providing numerous standards and food extracts for the study.

LM-20

HIGH SENSITIVITY MULTI-RESIDUE PESTICIDE ANALYSES IN FRUIT PRESERVE USING GC-MS/MS

Michael T. Hetmanski[1*], Richard Fussell[2], Michal Godula[3]

[1 2] Food and Environmental Research Agency, Sand Hutton, York, YO41 1LZ, UK
[3]Thermo Fisher Scientific, Slunečná 27, 100 00 Praha 10, Czech Republic
*E-mail: mike.hetmanski@fera.gsi.gov.uk

Fruit preserve (jam) is a complex, high sugar matrix. In this study, a simple, rapid method based on QuEChERS extraction and clean-up[1] and GC-tandem quadrupole mass spectrometry (GC-MS/MS) was evaluated for the multi-residue analysis of 96 pesticides in jam. QuEChERS extraction has been widely used for multi-residue LC-MS analysis, but many laboratories have reported difficulties when applied to GC-MS analysis. Solvent exchange or large volume injection using PTV have normally been required. However solvent exchange is time consuming and can result in losses of pesticides, and repeated injection of large volumes of extracts can cause degradation of the chromatographic system. The high sensitivity of the TSQ GC-MS/MS system enabled direct splitless analysis using low volume (1 µl) aliquots of acetonitrile extracts.

Recovery and CV data were within EU DG SANCO criteria[2] for almost all pesticides. The MRM chromatograms demonstrated excellent selectivity (no interferences observed) and good signal/noise ratio (>5:1), for all analytes at the lowest calibrated level (0.005 mg kg^{-1}). Thus the GC-MS/MS multi-residue method is suitable for monitoring the GC amenable pesticides in fruit preserves at high sensitivity.

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[2] 2. European Commission, Doc No. SANCO/10232/2006

LM-21

ION MOBILITY SPECTROMETRY FOR FOOD QUALITY CONTROL: SAUSAGES AT DIFFERENT STORAGE CONDITIONS

Stefanie Sielemann[1*]

[1] G.A.S. mbH, Dortmund, Germany
[*] Corresponding author - E-mail: sielemann@gas-dortmund.de; Phone: ++4923197426550;
Fax: ++4923197426555

The monitoring of real-time processes, as well as, the control of the quality of raw materials and products play a more and more important role in the food industry, especially with respect to food safety.

With analysis times of a few seconds and the ability to measure volatile organic substances (VOC) selective down to the low ppb_v-range ion mobility spectrometry (IMS) is considered as a powerful and low cost method to characterise food products without any sample preparation.

The combination of an IMS to a chromatographic column for selectivity enhancement was used to measure the headspace of slices of sausages (type “Lyoner”) at different storage conditions daily from day 0 to day 7. Whereas the one sample class was stored at room temperature, the other samples were kept in the fridge at 2.5 °C. To make the operation of sampling processing simple and transparent the system is set up with a sample injector unit.

As result of the measurements IMS-chromatograms with several peaks at different drift and retention times are achieved.

These IMS-chromatograms are the same for the sausage stored in the fridge from day 0 to day 7. Only for the peak with a drift time of 6.2 ms, which corresponds to the ion $NO^+(H_2O)_j$ (with j = 0,1,2), a slide increase of the peak height is achieved.

For the sausage stored at room temperature already at the second day dramatic changes are observed. A huge additional peak appears at day 2 at a drift time of 9.3 ms and increases within day 3. At day 4 an additional peak appears at 8.2 ms, while the peak height of the one at 9.3 ms decreases. Both peaks decrease with an increase of the peak at 6.0 ms, representing the ion of ammonia, $NH4^+(H_2O)_n$ (with n = 0, 1, 2). In sum: depending on the age of the sausage kept at room temperature the pattern of the headspace is changing and can be used for a classification using automatic chemometric tools.

The results of the measurements can be used to optimise storage conditions with respect to energy consumption or determination of the freshness of a product.

Next to this application, the system can be used to measure different kind of food materials as well as beverages or e.g. the emission of VOCs in packaging materials.

The theory of IMS, the experimental set-up and the results for the measurements will be presented.

Keywords: ion mobility, IMS, GC, VOC

LM-22

DEPLETION STUDY OF PCDD/FS AND DIOXIN LIKE PCBS CONCENTRATIONS IN CONTAMINATED HOME-PRODUCED EGGS: PRELIMINARY STUDY

Simonetta Menotta[1*], Margherita D'Antonio[2], Giuseppe Diegoli[3], Luigi Montella[4], Stefano Raccanelli[5], Giorgio Fedrizzi[6]

[1 2 6] Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna Reparto Chimico degli Alimenti- Bologna Italy
[3] Regione Emilia Romagna, Servizio Veterinario e Igiene degli Alimenti, Bologna Italy
[4] Azienda Sanitaria Locale, Servizio Veterinario, Bologna Italy
[5] Consorzio Interuniversitario La Chimica per l'Ambiente INCA, Venezia Italy
[*] Corresponding author - E-mail: simonetta.menotta@izsler.it; Phone: 0039 051 4200050; Fax: 0039 051 4200055

Dioxins (PCDD/Fs) and polychlorobyphenyls (PCB) are ubiquitous environmental contaminants. They are very persistent to chemical degradation, and accumulate in the food chain. The contamination of food products with dioxins and PCBs is a well studied issue, because food is generally considered as the major source of dioxin intake for humans. Eggs, in particular, contain an high fat percentage, and may accumulate persistent organic pollutants such as dioxin and PCBs. In Italy, the Regional Monitoring Plan used in the field for 2009, has also included the control of environmental pollutants in small egg producers (so called home-produced eggs). Following an irregular result, a private owner was recruited on a voluntary basis, and his birds, that were contaminated on a first sample (12 hens), were transferred from their free-range farming system into a lab controlled environment. Every day (from day 0 to 60) total eggs were collected: most of them were analysed for the evaluation of dioxins, dioxin like PCBs (DL PCBs), and non dioxin like PCBs (NDL PCBs six congeners) levels. The number of lay-eggs varied from four to 12 in a single day. All the eggs from each week were homogenised and lyophilised. The fat fraction was extracted by accelerated solvent extraction (ASE). The dioxins and PCBs contents were determined according to EPA 1613/94 rev B method by gas chromatographic determination, coupled with high resolution mass spectrometry. The content of PCDD/F, DL PCB and NDL PCB was evaluated by mean from week to week. The concentration of dioxins was lower than DL PCBs (2,5 pgTEQ/g of fat against 4,5 pgTEQ/g), but we observed the same depletion trend for both. After a steady level for the first two weeks, there was a small depletion until the sixth week, were PCDD/F and DL PCB showed similar concentration. Then, while a continuous depletion was seen for PCDD/F concentration, DL PCB levels decreased very slowly, and reached about 2 pgTEQ/g of fat. On the opposite, NDL PCBs had a different course: there was an increase between week six and seven, but the mean levels remained very low (about 20 ng/g of fat). The dioxins, and sum of dioxin and DL PCBs concentration were below the fixed European limits (i.e. 3 pgTEQ/g of fat for dioxins and 6 pgTEQ/g of fat for sum of dioxins and DL PCB), beginning from the third week of trial, because of their removal from the contaminated environment.

Keywords: Dioxins eggs depletions

LM-23

A NEW APPROACH FOR GENERAL SCREENING OF COMPLEX MIXTURES ON ION TRAP MSMS LIBRARY SEARCH

Roman Mylonas[1], Yann Mauron[2], Alexandre Masselot[3], Olivier Philippe[4], Pierre-Alain Binz[5], Veronique Viette[6], Marc Fathi[7], Denis F Hochstrasser[8], Frederique Lisacek[9], Carsten Baessmann [10*]

[1 2 5 9] Swiss Institute of Bioinformatics, Geneva, Switzerland
[3 4] Geneva Bioinformatics (GeneBio), Geneva, Switzerland
[6 7 8] Geneva University Hospital, Geneva, Switzerland
[10] Bruker Daltonik GmbH, Bremen, Germany
[*] Corresponding author - E-mail: cba@bdal.de; Phone: +49 421 2205 100; Fax: +49 421 2205 101

Novel Aspect: Robust integration of hardware and software for MS spectral library based toxicological screening applications

Introduction: Liquid Chromatography Tandem Mass Spectrometry (LC-MSMS) combined with library search appears in clinics, forensic, and food testing applications with the aim at replacing a LC-UV-DAD library search system. The approach does not require any chemical derivatization steps during the sample preparation. Compared to GC-MS, LC-MSMS covers a complementary in some respect, but clearly broader range of analytes. In routine laboratories, there is a need for an easy to use hardware and software solution coupled with simple SOPs, robust statistics and secured data storage and reporting methods. The system should also easily enable the creation, annotation and enhancement of spectral libraries.

Methods: Here we present an integrated hardware and software approach for acute clinical toxicology screening. The solution includes an ion trap MS instrument, a spectral library, a specially adapted search algorithm (SmileMS by Genebio) and predefined SOPs. Various SPE fractions of patient samples suspected for different drug intoxications as well as purified compounds were analyzed with an ion trap instrument coupled online with a modern ultra performance LC system. Both sample submission and evaluation of results is performed via a dedicated easy to use open access type software. The system was used to enrich a spectral library of dedicated analytes as well.

Preliminary Results: The implemented workflow allows a user to log in to open access software environment, register one or more samples and choose a processing method (including MS acquisition settings, data processing options and library search parameters). A technician launches the acquisition locally on the MS system. The recorded spectra are automatically sent to data processing. The user accesses the results in a summarized form via a web based browser and can visualize, validate and report the outcome of the analysis via hyperlinks. Finally, commented reports can be sent to the analysis requester via email. The summary results from the library search are made available in a simple table, while the details can be evaluated via hyperlinks. Due to its client-server architecture data can be accessed from any PC connected to the intranet. A library containing 300 common drugs and toxins is being created in collaboration with a clinical laboratory. Additionally an SOP was created that allows sample preparation, analysis and automated result evaluation in less than 30 min. In this work a selected set of clinical blood and urine samples has been spiked with known drug molecules. The identification quality was evaluated at different concentration levels.

Keywords: Ion trap, toxicological screening, mixtures

LM-24

APPLICATION OF AN ATMOSPHERIC PRESSURE CHEMICAL IONIZATION INTERFACE FOR GAS CHROMATOGRAPHY WITH TIME-OF-FLIGHT MASS SPECTROMETRY

Watson Rod[1], Thomas Arthen-Engeland[2], Carsten Baessmann[3*], Armin Holle[4]

[1] Bruker Daltonics, Coventry, United Kingdom
[2 3 4] Bruker Daltonik GmbH, Bremen, Germany
[*] Corresponding author - E-mail: cba@bdal.de; Phone: +49 421 2205 100; Fax: +49 421 2205 101

Novel Aspect: GC-APCI-TOF/MS; accurate mass analysis of the pseudomolecular ions of GC-separated compounds.

Introduction: The interfacing of GC with mass spectrometry is commonly performed in vacuo using electron or chemical ionization. This offers the benefits of library-searchable spectra and ionization e. g. of volatile and non-polar components. However, larger molecules often display no molecular ion and the spectra of unknowns can require a high level of skill to interpret. In particular, this is becoming a topic of concern in the pharmaceutical industry, where regulatory bodies require the certain identification of volatiles and other impurities throughout the production process. By applying an atmospheric pressure chemical ionization interface for time-of-flight mass spectrometry, molecular formula information is readily available for components separated by GC. Further MS/MS information can be gathered for quadrupole-TOF instruments.

Methods: A capillary GC column has been introduced to a modified Bruker API interface on micrOTOF and micrOTOF-Q mass spectrometers via a rigid heated transfer tube. A sheath nitrogen gas flow is used to ensure a focussed gas jet from the capillary exit and to make up the gas flow rate for introduction to the mass spectrometer. GC components can be mass-measured on the TOF detector. The SmartFormulaTM method is then applied, using accurate mass measurement and True Isotope Pattern (TIP) matching to give unprecedented certainty of molecular formula identity for GC-separated compounds

Preliminary results: Results from the analysis of typical GC volatile compounds will be presented. The range of compounds that can be ionised and determined by this technique will be demonstrated.

Keywords: GC-APCI-TOF/MS, microTOF, identification

LM-25

DETERMINATION OF BENZENE IN DIFFERENT FOOD MATRIXES BY DISTILLATION AND ISOTOPE DILUTION HS-GC/MS

Raquel Medeiros Vinci[1], Michael Canfyn[2], Ilse Van Overmeire[3], Bruno De Meulenaer[4], Joris Van Loco[5*]

[1 4] Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, B-9000 Ghent, Belgium
[2 3 5] Operational Direction Food, Medicines and Consumer Safety, Scientific Institute of Public Health, 1050 Brussels, Belgium
[*] Corresponding author - E-mail: Joris.VanLoco@iph.fgov.be; Phone: +32 2 642 53 53

Benzene is classified by the IARC as carcinogenic to humans. Several sources may contribute for the occurrence of benzene in foods, such as, environmental contamination and the reaction of benzoate salts and ascorbic acid (naturally present or added as food additives).

Matrix effect on benzene recovery (e. g. in fatty foods) and artefactual benzene formation during analysis are some of the challenges presented when determining benzene in a wide range of foodstuffs. Design of experiment (DOE) was used to determine the most important variables in benzene recovery. Based on the results of the DOE, a versatile method for the extraction of benzene from all kind of food commodities was developed. The method which consisted of distillation and isotope dilution HS-GC/MS was validated in accordance with the decision (2002/657/EC). Artefactual benzene was prevented by addition of a buffer solution (pH 11) under distillation conditions.

The method presented in this study allows the use of a matrix-independent calibration with detection limits bellow the legal limit established by the European Council for benzene in drinking water (1 µg L^{-1}).

Keywords: Benzene, food, headspace, GC/MS

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LM-26
IDENTIFICATION OF ROMANIAN WINE ADULTERATION

Gabriela Rapeanu[1*], Nicoleta Stanciuc (Sava)[2], Constanta Vicol[3]

[1 2] Faculty of Food Science and Engineering, Dunarea de Jos University, Galaţi, Romania
[3] Regional laboratory of wine control, Odobesti, Romania
[*] Corresponding author - E-mail: Gabriela.Rapeanu@ugal.ro; Phone: +40742038288

Wine is a widely consumed beverage in the world with thousands of years of tradition. Determination of its authenticity is one of the most important aspects in food quality and safety. Many successful studies have shown that it is possible to distinguish grape variety, vintage years or geographical zones on the basis of chemical parameters. Several papers have been published about classification of wines and recently, pattern recognition has become a useful and often applied method in food analysis.

The adulteration of wine is usually accomplished by addition of alcohol, water, dyes and aromas to wine of minor commercial value. As these wines are usually produced with inadequate conditions of hygiene, they become of high risk for the human health. Another risk for the consumer of adulterated wines is the ingestion of products not elaborated with raw materials controlled by control organs.

The authenticity of wine is guaranteed by strict guidelines laid down by the responsible national authorities who include official sensory evaluation, chemical analyses and examination of the register kept by the wine producer.

The purpose of this study was to identify the adulterated wines obtained in Cotesti vineyard, Vrancea County by the private and local producers during the period 2006-2008.

From the 341 wine samples analysed was noticed that the cheap wines are frequently adulterated. Some false labelled wines by integrating them in superior quality class of wines were observed. Moreover label mentions of the wine bottle are not in concordance with the wine content. The grape variety mentioned on the label has to be minimum 85% and a minimum alcohol concentration of 11% *v/v*. Even the wine contains more than 88-90% of a single grape variety but the synthetic colorants and flavors are detected, the wine can't be named controlled denomination of origin (CDO) wine. In this case, the wine is considered false labelled.

The dynamics of wine adulterations from Cotesti vineyard had a decreasing trend from 2006 (71.3%) to 2008 (29.7%). The reduction of the adulterated wines from 2006 to 2008 is probably due to the application of legislative regulations on wine quality.

Keywords: adulteration, quality, false labelled wines

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LM-27

DETECTION OF ROMANIAN SHEEP AND GOAT CHEESES ADULTERATION BY IMMUNOCROMATOGRAPHY

Gabriela Rapeanu[1*], Nicoleta Stanciuc (Sava)[2]

[1 2] Faculty of Food Science and Engineering, Dunarea de Jos University, Galaţi, Romania
[*] Corresponding author - E-mail: Gabriela.Rapeanu@ugal.ro; Phone: +40742038288

Authenticity testing of food products, such as meat, milk or fish, is important for labeling and assessment of value and is therefore necessary to avoid unfair competition and assure consumers protection against fraudulent practices commonly observed in the food industry.

Adulteration of milk and dairy products with different types of milk, other than declared, presents a big problem for food monitoring. The evidence of cheese adulteration is a difficult task considering similar compositions of various types of milk.

One of the main problems in the cheeses making is the adulteration of sheep and goat milk with cow's milk, because sheep and goat milk is, more expensive.

The aim of this study was to detect the presence of cow's milk in sheep's and goat's cheeses which are sold in retail markets of Galati, Romania.

For this purpose, a total of 64 sheep's cheese samples and 31 goat's cheese samples were purchased randomly from different local markets.

The presence of cow's milk in sheep's and goat's cheeses was studied by immunochromatographic test kit. The basis of the assay is the antigen - antibody reaction. The presence of cow's milk in a sample is determined by the immunological detection of bovine immunoglobulin G (Ig G bovine - an antibody class), which is a natural constituent of cow's milk.

The presence of cow's milk was detected in 53% of cheese samples, while no adulteration was found in 47% of cheese samples.

These obtained results were considered to be unacceptable. Therefore it was concluded that, routine controls on sheep's and goat's cheese adulterations have to be performed in Romania.

Keywords: adulteration, cheese, immunochromatography

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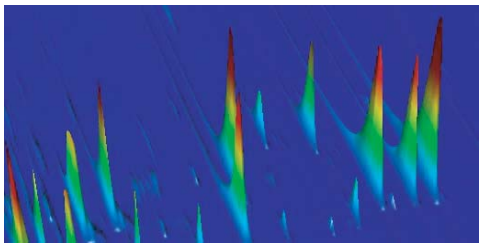
AN INTERLABORATORY STUDY FOR MEASUREMENT OF CU FROM 3 NOURISHING SUPPLEMENTS

Rodica Diana Criste[1], Arabela Untea[2*], Margareta Olteanu[3], Liliana Balind[4], Mariana Ionescu[5]

[1 2 3] National Research and Development Institute for Biology and Animal Nutrition, Balotesti, Roumania
[4] Institute of Food Chemistry, Bucharest, Roumania
[5] Institute of Food Bioresources, Bucharest, Roumania
[*] Corresponding author - E-mail: cristerodica@yahoo.com; Phone: 0040723585842

An interlaboratory study was made with 7 participants – laboratories in the field "feed / food". The main objective of the study was an accurate determination of the mineral composition of 3 plants from the wild environment (Origanum vulgare; Vaccinium myrtillus L.; Tribulus terestris). The three plants chosen as samples for the interlaboratory study are nourishing supplements used for the enhancement of mineral status in nutrition experiments on weaned piglets. The calculating approach of the experimental diets and the inclusion rate of the supplements in diet are the stages of experimental protocol based on chemical composition of the plants. A homogeneity test was applied to the samples and they were distributed to each participant on sealed plastic bags. All laboratories accomplished ten measurements for each sample. The determination method was established by protocol, flame atomic absorption spectrometry. The reference value was established by consensus (value established directly from the reported results – we calculate the central trend of results and then the standard deviation is used for uncertainty determination). The appearance frequency of the results was determined by Kernel distribution plot and the remove of the doubtful results was done by the t test. The participants performance was evaluated by Z score and Zeta score. From the 21st values of Z (7 laboratories x 3 plants samples) in 2 cases 3 < Z, both of them for Vaccinium myrtillus L. Zeta score confirm the unsatisfactory results of Z score just for one case and for another zeta value 3 < Zeta no other test confirm the hypothesis of unsatisfactory results. The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories offer as possible explanation of unsatisfactory zeta score an underestimation of the uncertainty. This hypothesis is confirmed in case of laboratories with z score satisfactory and a zeta score unsatisfactory. From the results obtained, we concluded that two participant provide questionable results for copper for Vaccinium myrtillus L.

Keywords: copper, interlaboratory, study, nourishing, supplements

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