

# Deliberate contamination of food

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## New chemical markers for the assessment of egg products freshness

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Egg products freshness is a crucial issue for the production of safe and high quality commodities. Up to now, European legislation declares that, from a chemical perspective, this parameter must be verified with the quantification of few compounds (1); however, the possibility to evaluate this topic with a group of molecules that should or should not be simultaneously detected could provide more robust results. In this study, new compounds responsible of freshness and of not freshness of egg products are identified with an UHPLC-HRMS metabolomic approach, using an Orbitrap Q-Exactive instrument (Thermo Scientific) and different data processing software.

Samples were collected directly from the production plant, extracted immediately after the receipt, left at room temperature and extracted again after 1 day and 2 days. A total amount of 79 samples was used for the model creation.

The same molecules were detected in a group of new egg products batches subjected to the same experimental design but not used for model creation; this certifies that these compounds can be considered reliable freshness markers, regardless of the chemometric model used to identify them.

In addition, appearance, disappearance or strong intensity variations of these markers was evaluated also in egg product batches stored at 2-8 °C and in eggs subjected to incubation processes, expanding the aim to the detection of other frauds related to the eggproducts chain

### References

1. D.L. no. 65, February 1993 Italian Legislation reception of European Union.

## “Non-targeted” analytical methods to detect food frauds: new markers for egg products freshness evaluation

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The topic of my research project is the development of innovative “non-targeted” analytical methods for the detection of food frauds. My PhD is strictly related to the “Food Integrity” European project, where I’m co-author of a Scientific Opinion related to the standardization of the validation workflows for “non-targeted” methods. My research is now focused on the egg products freshness, that is a crucial issue for the production of safe and high quality commodities. Up to now, European legislation declares that, from a chemical perspective, this parameter must be verified with the quantification of few compounds (1); however, the possibility to evaluate this topic with a larger group of molecules could help to provide more robust results.

In the study that we performed, new compounds responsible of freshness and of not freshness of egg products were identified with an UHPLC-HRMS metabolomic approach.

The same molecules were detected in a group of new egg products batches subjected to the same experimental design but not used for model creation; this certifies that these compounds can be considered reliable freshness markers, regardless of the chemometric model used to identify them.

In addition, appearance, disappearance or strong intensity variations of these markers was evaluated also in egg product batches stored at 2-8 °C and in eggs subjected to incubation processes, expanding the aim to the detection of other frauds related to the egg products chain.

### References

1. D.L. no. 65, February 1993 Italian Legislation reception of European Union.

## Industrial self-control for a safe and fair worldwide fruit juice market.

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Fruit and vegetable juices are healthy and nutritious food products but are considered within the 10 most susceptible food products to be adulterated in the European Union. The complicated supply chain also contributes to difficult the product traceability and the product integrity. In a global market, juices are produced in all continents and shipped all over the world.

SGF implements since more than 40 years a system based on risk analysis that reduces the occurrence of food fraud and safety issues in the global supply chain. The system is based in a combination of on-site inspections of the producers, product assessments through targeted and non-targeted analysis of randomly taken samples and the application of corrective actions when deviations on safety, quality or authenticity are found. With the periodical controls the number of typical frauds such as over dilutions, mixing with cheaper raw materials and adding no authorized substances, are not so frequently found in the markets. The SGF system controls on the supply chain include the use of pesticides and herbicides in the farm and the proper cleaning of the bulk transport vehicles. The supply chain actors that voluntarily take part in the system are granted with a certification after checking that all requisites are successfully fulfilled. The SGF model could be implemented in other food chains such as honey, confitures and olive oil.

## Detection of chemical and microbiology composition of white fish combined with the differentiation of pangasius

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The high quality of fish and fishery products necessitates the development simple handled and precise tools that can test in real time the quality and evaluated the fish species. There is a need for objective methods to identify the different types of fishes by species, determine the chemical composition (fat, protein and moisture) and microbiological content (freshness, spoilage, and nematodes). Near infrared (NIR) spectroscopy as a non-destructive and cost effective analytical method is widely used in food industry for rapid measurement of quality attributes, however, there is still a vast need for simple low cost NIR instruments usable by non-technical personnel in everyday situations.

The goal of the present study was to develop multivariate models for differentiation of different white fish species and prediction of chemical and microbiological composition based on the NIR spectra acquired with a handheld scanner and a user friendly mobile app.

## Rapid Detection of Herb and Spice Adulteration

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High end food commodities such as Herbs and Spices are susceptible to fraudulent activity as they command a premium price for retail. It is estimated that the global herb and spice market is worth in the region of US\$4 billion. Supply chains in this global marketplace are complex and vulnerable to fraud from criminals dealing in economically motivated adulteration. It is therefore in the best interests of stakeholders throughout the supply chain to detect and deter this kind of activity. Screening techniques to detect adulteration are being developed using the spectroscopic techniques, Fourier Transform Infra-Red (FTIR), and Near Infra-Red (NIR) combined with chemometric modelling. These techniques are gaining prominence due to their ease of use, rapidity and minimal sample preparation with potential to be used by stakeholders in the field. Typically, the raw spectral data of a range of samples along with possible known adulterants are collected, pre-processed and used with chemometric algorithms to convert into qualitative models which can be used to determine if a sample has been adulterated. Recent developments in the analysis of Herbs and Spices, regarded as high risk from fraud, will be presented.

## Development Of Detection Technologies For Foodborne Toxins

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Toxins are the some of the major pathogenicity factors for bacterial pathogens and plants. In an era of concern over foodborne contamination through bioterrorism, the sensitive and accurate detection of toxins is clearly essential for both food safety and biodefense. Our research unit designs and develops methods and reagents such as high-affinity monoclonal antibodies against high-consequence toxins including shiga toxins, botulinum neurotoxins, abrin and staphylococcus enterotoxins. We combine the use of antibody-based techniques with activity assays such as cell-based and mouse bioassays that can detect enzymatic activities and provide sensitive and rapid validation for toxin detection. These detection assays are further optimized for evaluation in food matrices and evaluated for possible field-deployable use. Developing new toxin detection technologies will facilitate advancements in preventing, diagnosing, and treating foodborne intoxications. New methodologies and their further development with commercial partners will provide food processors and regulatory agencies with tools for enhanced food safety and biosecurity.

## Fish species identification by PCR using parvalbumin gene as a platform

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Food fraud is a significant and growing problem. The increase in international trade, increasing global consumption of fish and different level of supplies and demands for some species, have led to many cases of economic frauds. In this case one type of fish product is illegally replaced by another. This is big economical problem, because mislabeling can result as fraudulent substitution of meat with high value with some less expensive fish. Moreover, proper labeling is also important in terms of the impact on health, certain people can consume only specific fishes, because of allergic sensitivity. The most common determination of fish species is based on morphological traits. This approach faces more and more complications as the level of processing fish flesh into products of food industry and/or complex dishes in gastronomy makes morphological markers less available.

Methods based on the polymerase chain reaction (PCR) are the most spread for control purposes, due to the high level of sensitivity and specificity. The presented analysis is performed as an amplification of nuclear gene encoding, important protein of fish muscles parvalbumin, also known as a major fish allergen. Conventional PCR method for the differentiation of the following fish species were developed: black seabream (*Sponyliosoma cantharus*), Atlantic mackerel (*Scomber scombrus*) and iridescent shark (*Pangasianodon hypophthalmus*). Specificity was verified on panel of 19 different fish species. This method can be employed as a routine tool for species origin determination irrespectively of morphological traits.

This research was supported by grants MZe (NAZV) QJ1530272 and MZe RO0318

## Use of DNA analysis for the study of meat food fraud in the Czech Republic

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Meat and meat products are one of the most expensive foods and therefore fall into the category of the adulterated commodities. The falsification of meat is a current and serious problem on a global scale. Molecular-biological methods based on the detection of DNA are techniques applied in the analysis of food ingredients due to their high sensitivity and specificity. DNA is a universal molecule present in most cells of the animal organism, analysis of samples of various animal species and tissues can be therefore carried out under the same conditions.

In this work, the polymerase chain reactions (PCR) were successfully used for an identification of cattle, pig, horse and poultry (chicken, duck and turkey) meat. The target molecules of amplification were mitochondrial cytochrome b gene for quadruplex PCR (mPCR), chromosomal DNA coding interleukin-2 for triplex PCR of poultry (mPCR) and single copy chromosomal genes as cattle cyclic-GMP-phosphodiesterase, pig beta-actin, interleukin-2 for chicken and myostatin from mammal and poultry for multiplex qPCR (mqPCR) analyses. The functionality of methods was proved by mixed DNA samples and by mixed meat with and without heat treatment. Both methods, mPCR and mqPCR, were verified on several commercially available samples typical for the Czech Republic including sausage, meatloaf and salami. The discrepancies between results of DNA analysis and content of the sample declared by producer were determined. This study was supported by a grant MZe (NAZV) QJ1530272.



## Verification of presence of inhibitory effect in qPCR in DNA analysis

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Nowadays many different methods which allow us to differentiate species in food product exist. These one can be used for food fraud disclose. One of the possibilities is to use PCR based on DNA analysis with extraction of high-quality DNA as one of the key step.

In this work, three DNA isolation methods were compared. First one was based on cetyltrimethyl ammonium bromide (CTAB), second on CTAB with phenol addition and the third one was commercial kit NucleoSpin Food Kit. The risk of inhibitors presence from the known chemicals needed for isolation was considered together with possibility of the inhibitors' presence originated food samples such as meat and oilseeds. In addition to the comparison of the inhibitory effect between DNA isolation methods, the effect of adding different concentrations of inhibitor, NaCl solution, to the reaction mixture was also tested. The presence of the inhibitory effect was evaluated by calculating the efficiency of the amplification reaction and/or changing the threshold cycle (Ct). Furthermore, a melting curve which may indicate a change in the PCR product (probably due to inhibitor binding) was analyzed.

Comparison of the yield, purity, time demands and inhibition effect present upon DNA analysis by given methods show the best results by first CTAB method according to ČSN EN ISO 21571. PCR analysis of DNA isolated by the method using CTAB showed the optimal effectivity of PCR (90-109%). The least effective was the commercial kit (78-89%).

Study was supported by grants: MZe (NAZV) QJ1530272 and QK1720263.

## The Food Authenticity Research Network (FARNHub) for sharing and accessing information on food authenticity activities

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One of the objectives of the Authent-Net project is to establish a dynamic and sustainable European information platform, named the "Food Authenticity Research Network Hub (FARNHub). This platform is a web-based portal where users can get an overview of currently available resources related to food authenticity for each country or for each food sector. This includes papers and documents (scientific or other), ongoing projects, online databases, an overview of funding bodies with contact points, news stories and regulations on food authenticity. Analytical methods are addressed by the Food Integrity project (WP2) through the Food Integrity Knowledge Base

The FARNHub application is now available online on <http://farnhub.authent.cra.wallonie.be/> for search and view content. By giving open access to this web tool, all possible users who have an interest in food authenticity (funding bodies, industries, regulatory authorities, research organisations and other stakeholders) can benefit from the hub and its content.

A map available on [http://www.authent-net.eu/AN\\_FARNH\\_click\\_map.html](http://www.authent-net.eu/AN_FARNH_click_map.html) gives statistics on the number of publications, projects, news, ... and includes links to the 14 National status reports and 3 commodity status reports developed by groups of experts from the resources stored in the FARNHub tool.

Any update or adding can be suggested and provided to email [farnhub@cra.wallonie.be](mailto:farnhub@cra.wallonie.be). A network of national representatives involved in the Authent-Net project has been created to approve new entries and update the database.

### Acknowledgments

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## Fraud Vulnerability Assessment of the UK Seafood Supply Chain

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Food fraud is an intentional act for economic gain, which poses a risk to food integrity. It has gained increasing concern due to economic, public health and ethical risks. Seafood, is one commodity which has been reported to endure extensive fraudulent activity due to its increasing popularity, resource limitations, high value and complex supply chain. Vulnerability Analysis Critical Control Point (VACCP) has been identified as a fundamental procedure to reveal the fraudulent opportunities, assign countermeasures and make the food supply chain a hostile and difficult environment for the offender to operate in. In order to implement VACCP, this research systematically maps the seafood supply chain to identify all the nodes which can be exploited for economic gain, their likelihood of occurrence and the severity of potential consequences. The aquatic supply chain comprises of multiple stakeholders in numerous countries producing a diverse range of products distributed globally. At each of the supply chain points the opportunity for fraud in terms of; species substitution, fishery substitution, illegal, unreported and unregulated fishing, species adulteration, chain of custody abuse, catch method fraud, undeclared product extension, modern day slavery and animal welfare, have been identified and evaluated. These mappings and risk evaluations provide the foundations for a proactive mitigation plan to assign control measures and responsibility where vulnerability exists. Fraud is a dynamic and continuous process, thus intelligence gathering and timely reviews of the VACCP is essential to ensure consistent food integrity.

## Tackling shrimp fraud with Mass Spectrometry

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Unfortunately, fishery products occupy the second highest ranking position among commodities that are at most risk of food fraud. Among these, marine shrimps and prawns, especially the penaeid shrimps, accounts for more than 17% of the global seafood consumption. As per Article 35 of the EC regulation 1379/2013, the mandatory information to be provided to consumers for all categories of fishery and aquaculture products includes the commercial designation of the species, production method and geographical origin. DNA based assays are established methods for determination of fish species identity whereas stable isotope based assays have been the methods of choice for provenance determination. However, high resolution mass spectrometry based food metabolomics holds potential to test all these authentication issues in a single analytical platform.

Authentic samples were collected either directly from aquaculture farms or through local supermarket chains. Six species of shrimps namely Tiger Prawn (*Penaeus monodon*), King Prawn (*Litopenaeus vannamei*), Indian Prawn (*Fenneropenaeus indicus*), Pink Speckled Shrimp (*Metapenaeus monoceros*), Argentinian Red Shrimp (*Pleoticus muelleri*) and Red Prawn (*Solenocera crassicornis*) were included in the study. Among Tiger Prawns, wild caught prawns from India and Madagascar; and farmed prawns from Vietnam were sources as were farmed King Prawns were from India, Thailand, Vietnam and Honduras.

Different mass spectrometry methodologies to tackle shrimp fraud including holistic profiling with LC-HRMS and subsequent confirmatory LC-MS/MS as well as ambient MS with REIMS were developed and will be presented.

## Real time identification of the adulteration of processed meat using rapid evaporative ionisation mass spectrometry

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Processed meat adulteration with low cost materials is a global issue most recently highlighted by the 2013 European horsemeat scandal and the subsequent Elliott Review of food networks in the United Kingdom (UK). Deliberate, economically motivated product substitution can be in full or partially but must be conducted at levels of at least 10-20% for criminals to make a profit from such fraud. In developing methods for detecting food fraud, speed is as important as specificity and sensitivity. In this study, we present an effective, near real-time method to identify the adulteration of beef patties (20g) with goat, lamb, pork and various offal cuts using rapid evaporative ionisation mass spectrometry (REIMS). Detection of goat, lamb and pork at levels ranging from 2-20% adulteration was identified depending on patty preparation. The identification of multiple meat species within a sample was also correctly identified at levels ranging from 25-33% adulteration. Database search associated with MS/MS fragmentation did not result in the identification of any unique species-specific markers, however, significant ions were found to occur more prominently in certain species and therefore, assigned the following lipid classes; phosphatidic acid (PA); phosphatidylcholine (PC); phosphatidylethanolamine (PE); phosphatidylinositol (PI) and phosphatidylserine (PS). Preliminary results of offal (brain, heart, kidney and liver) detection in beef patties identified that approximately 10-20% adulteration is detectable, sufficient enough to screen for economically viable meat adulteration. Thus, we present a rapid screening method that could serve to detect meat adulteration in near real-time unlike that of PCR or protein marker techniques.

## Isothermal recombinase polymerase amplification for rapid identification of *Pangasius hypophthalmus*

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Fish mislabelling involving substitution of valuable species with cheaper ones has been described worldwide. Therefore, there is a need to apply fast, reliable, and cost-effective methods for species verification. PCR-based assays are the most widely used methods for this purpose, requiring highly-trained staff and complex instrumentation. To overcome this problem, Recombinase Polymerase Amplification (RPA) has been shown to be highly suitable for species identification in non-laboratory settings. This study investigates RPA technology to identify the widely marketed *Pangasius hypophthalmus* in a portable and rapid dipstick format.

### Material and Methods

Primers and a probe specific to the Cytochrome oxidase I (COI) gene of *P. hypophthalmus* were designed. The reaction was performed using the RPA-nfo kit (TwistDx) and the result was detected by lateral flow strip (LF-RPA) (Millennia). The result was obtained within 15' including DNA extraction and the specificity and repeatability were evaluated.

### Results

A Lateral Flow-RPA assay specific for *P. hypophthalmus* has been developed. A clear positive test line in the dipstick is obtained when DNA from *P. hypophthalmus* is applied. No cross-detection was observed when DNA from other white-fish species was applied. The sensitivity and specificity obtained were comparable to PCR-based methods.

### Conclusion

In conclusion, the non-destructive LF-RPA assay developed is suitable to identify *P. hypophthalmus* with high sensitivity and specificity. The reaction time and requirements are extremely low, holding the potential to become a point-of-care test.

### Acknowledgments

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## Strategy to Improve Food Safety in Bangladesh

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Food safety has been a burning issue in Bangladesh and the situation seems to be more severe as almost every day the leading newspapers are covering a number of news about the contamination of food. The government is ensuring food security but also giving priority to food safety with quality and nutritious value. Antibiotics are used for rapid growth of poultry and cattle farms in the country. Residue of the drugs above allowable limit reduces food safety & quality. To assess amount of residual antibiotics, chicken meat and beef samples were analyzed for the presence six sulpha drugs i.e, sulfadiazine, sulfadimethoxime, sulfamethazine, sulfamerazine, sulfamethiazole and sulfamethoxypradiazine by LC-MS/MS coupled with ESI and TQ mass analyzer. Identification of each of the drug was done by MRM method. Six point calibration curves were linear with correlation coefficients ( $r^2$ ) 0.9978, 0.9985, 0.9986, 0.9991 and 0.9993 for sulfadiazine, sulfadimethoxime, sulfamethazine, sulfamerazine, sulfamethiazole and sulfamethoxypradiazine, respectively. Method was validated by recovery experiments by spiking control sample (poultry meat and beef) at 2 different concentration levels (5 & 10 ng mL<sup>-1</sup>). Extraction was done by QuEChERS method (methanol-water;40:60 ratios), cleaned up with C-18 cartridge & PSA and analyzed LC-MS/MS. Intra- (n=6) and inter day (n=6; 2 days) recoveries were found to be in the range of 86-104%. Chicken meat and beef samples from ten different shops were analyzed under the same conditions and no residues of six drugs were found to be present in the real chicken meat and beef samples.

## Isotope Ratio Measurements for Food Authentication and Provenancing

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The stable isotopes of carbon, nitrogen, hydrogen, oxygen and sulphur are used in food authenticity and provenancing studies to determine geographical origin, compliance with stated growth and manufacturing processes and to detect post-growth adulteration. Both bulk and compound specific isotopic measurements are of interest, and instrumentation which is highly sensitive, precise and stable enable food suppliers, consumers and researchers to have confidence in food forensic data. Here we present the developments which have recently been made to our instrumentation which is relevant to the food industry, and data from a range of food and drink samples to demonstrate how stable isotopes are used in food authentication studies



## Multiplex analysis of mycotoxins using Mass Sensitive Micro-Array Technology

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Naturally occurring food and feed contaminants are inevitably unavoidable making them a major issue in global food security, particularly those which pose health concerns to humans and animals. Mycotoxins are one such example, mainly entering the food chain as a result of fungal colonization of pre-harvest susceptible crops, during the time between harvesting and drying or during storage. Whilst visual and reader based ELISA and lateral flow assays are the main mycotoxin detection methods used for direct on-site analysis by importers, traders, and food and feed manufacturers, these methods require expensive labelling of reagents. This project aims to develop a novel rapid, handheld, multi-mycotoxin detection device based on Mass Sensitive Micro-Array (MSMA) technology, where detection of analytes is by mass making it both a label free method and cost efficient. The sensing platform consists of an array of microscopic weighing scale pixels that can be used as a functionalised surface for the immobilisation of reagents such as antibodies or hapten conjugates. The technology will be evaluated and assessed using key antibody reagents for important mycotoxins: deoxynivalenol, HT/T2, zearalenone and fumonisins that have been developed at Queen's University, to show in principle the MSMA technology performance in buffer reagents. A novel sample preparation method will be developed for the simultaneous extraction of these toxins. The technology will be evaluated for feed and food matrix interference effects as well as limit of detection and results will be compared to other biosensor platforms or commercial lateral flow devices for its suitability for use.

## Common and emerging fraud in the olive oil sector: advancements from the OLEUM project

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Olive oil adulteration has become one of the biggest sources of food fraud at a global scale. This is mainly due to the higher price, related with the mechanical extraction, the high quality of the raw material, the peculiar sensory, nutritional and healthy properties of this product, especially when is extra virgin. The H2020 EU funded OLEUM project ([www.oleumproject.eu](http://www.oleumproject.eu)) seeks to better guarantee olive oil quality and authenticity by improving the detection and fostering the prevention of olive oil fraud. OLEUM started on 1st September 2016 and will run for four years, with twenty project partners committed to developing advanced solutions and networking actions to assure the authenticity and quality of olive oil. In order to understand the growing threat to the integrity of the olive oil scenario from fraud, one of the specific objective of the OLEUM project is to collaborate with the competent authorities and control bodies for acquiring a consolidated and updated report on the occurrence of common and emerging frauds in this sector. The information herein reported will be transferred to the relevant stakeholders (OLEUM Network), uploaded in the OLEUM Databank and disseminated to the consumers for providing a reliable source of information, thus improving their trust that has been mined by the frequent olive oil scandals during the last years.

## The UK Food Authenticity Programme - Combatting food fraud through scientific innovation

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The prevention of food fraud features highly on the UK Government's policy agenda. Protecting consumers, maintaining the resilience of the food chain and preventing fraudulent practices are significant challenges facing policy makers, regulators, enforcers and the food industry. Maintaining our strong reputation in delivering high standards of traceability and high quality British food and drink is vital to ensuring consumer confidence and supporting a productive food and drink sector.

Defra's Food Authenticity Programme develops fit for purpose analytical methods for use by official control laboratories and other competent laboratories engaged in food authenticity testing. The programme has been instrumental in investing in the development of novel scientific methods using cutting-edge analytical technologies and in the validation, technology and knowledge transfer of newly developed methods to make them widely available for detecting food misdescription, including misleading claims about food quality, composition, geographic origin and method of production; this requires a 'tool-box' of different methods to address the wide variety of fraud issues and presents a plethora of technical challenges in terms of analytical method development.

There is a need to make use of the latest innovative technology which builds on the existing science base to develop flexible and effective tools that will enable us to respond to future food fraud incidents. A recent focus has therefore been on developing non-targeted methods for higher-throughput, more efficient screening of food products to identify a range of potential adulterants and to overcome some of the limitations associated with current targeted testing approaches.

## Almond or Mahaleb? Orthogonal allergen analysis during live incident by ELISA, PCR and protein MS

**Dr. Michael Walker<sup>1</sup>**, Dr Malcolm Burns, Dr Milena Quaglia, Ms Kirstin Gray

<sup>1</sup>*Laboratory Of The Government Chemist, Newtownabbey, United Kingdom*

It is now well known that an incident investigated in the UK in 2015 of cumin alleged to be contaminated with almond, a risk for people with almond allergy, was caused by the Prunus species, P. mahaleb. In the UK the Government Chemist offers a route of technical appeal from official findings in the food control system. Findings of almond in two official samples, cumin and paprika, which had prompted action to exclude the consignments from the food chain, were so referred. We describe the approaches deployed to resolve the analytical issues during live investigation of the incidents. ELISA (enzyme-linked immunosorbent assay) is useful for screening for Prunus species, but owing to cross reactivity there is a need for orthogonal confirmation. Two novel PCR (polymerase chain reaction) assays have been developed, one specific for P. mahaleb and the other a melt curve method capable of identifying common Prunus DNA. Peptides unique to almond and mahaleb have been identified permitting LC-MS/MS with developed for peptide identification to forensic standards. This work enables a staged approach to be taken to any future incident thought to involve Prunus species and provides a template for the investigation of similar incidents.

## Food Fraud Prevention: Harmonized Terminology and Public-Policy Development Research

**Dr. John Spink<sup>1</sup>**, TBD- Chris Elliott was a co-author on 1 article TBD Christopher Elliott, TBD- MARS & Danone are MSU Food Fraud Think Tank members Frederic Rene

<sup>1</sup>*Michigan State University Food Fraud Initiative, Okemos, United States*

Conference: ASSETT2018 (note: I am also submitting a proposal for the Global Food Fraud Prevention conference)

Food Fraud prevention has continued to evolve and formalize in the terminology, focus on prevention, and public policy development. This presentation will review current research projects on terminology, the implementation of prevention strategies, and public policy development needs. First the MSU/GMA food fraud terminology survey research examined common definitions and provided insight on unmet needs. This was conducted in parallel with an INFOSAN (WHO/FAO)/MSU survey and in support of the CODEX electronic working group on food fraud. The terminology builds a foundation that can support policy-making. The second part of the presentation covers public policy development theory and application to Food Fraud. Dye's public policy development model identifies specific milestones and need from for the stages from "definitions and scope" through to "implementation." This is a follow-up from the MSU works for ASSETT2015 (presentation on "A Global Perspective on Food Fraud Prevention" and Food Chemistry special edition article "Introducing Food Fraud including Translation and Interpretation to Russian, Korean, and Chinese Languages").

### References:

- SpinkJ, HegartyVP, FortinND, ElliottCT &MoyerDC, (Under Review). The Application of Public Policy Theory to the Emerging Food Fraud Risk

### References from ASSETT2014

- Spink, J. (2014). Keynote Speaker, A Global Perspective on Food Fraud Prevention, Centre for Assured, Safe and Traceable food (ASSET)
- SpinkJ, ETAL. (2015), Introducing Food Fraud including Translation and Interpretation to Russian, Korean, and Chinese, Food Chemistry

## Food Fraud Prevention Strategy Based Decision-making (for Global Food Fraud Conference)

**Dr. John Spink<sup>1</sup>**, TBD- Co-author Guangtao Zhang, Mars TBD-Guantao Zhang, TBD-Co-author Doug Moyer, MSU Douglas C Moyer

<sup>1</sup>*Michigan State University Food Fraud Initiative, Okemos, United States*

Conference: Global Food Fraud Prevention Conference (submitted in the ASSETT2018 system for coordination of content)

Summary: Food Fraud is "bad" and "more" should be done. There is tremendous innovation and investment in related countermeasures and controls systems but “what should be implemented?” and “how much is enough?” The most basic question is, if there was only \$1 million for your entire company to spend on any project, could you define or defend that the best enterprise-wide expenditure is on Food Fraud Prevention? This session will present requirements that the “C-Suite” had instructed the presenters to address. This seemingly simple and non-scientific question is the keystone for investment in development or implementation. This presentation is based on previous and current research into the “resource allocation” decision-making process that applies to both industry and governments. The basic starting point for an organization is: (1) do I need to act, (2) how to start, (3) what to do, (4) how much is enough and (5) how to define – and defend – success.

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- Spink, John, Zhang, Guangtao, PENDING1, & PENDING2 (Under Review). Introducing the Food Fraud Prevention Cycle (FFPC)
- Spink, John, Moyer, Douglas C (MSU Public Health), & Speier-Pero, Cheri (MSU Business), (2016). Introducing the Food Fraud Initial Screening Model (FFIS), Food Control, Volume 69, November 2016, Pages 306-314.

## Can metabolomics enable wine authentication according to the grape varieties used for production?

**Mr. Leos Uttl<sup>1</sup>**, Ms. Kamila Hurkova<sup>1</sup>, Mrs. Irena Kolouchova<sup>1</sup>, Mrs. Jana Hajslova<sup>1</sup>

<sup>1</sup>*UCT Prague, Prague, Czech Republic*

In 2008, the European Commission highlighted the risk of wine mislabelling regarding geographical origin and varietal identification. While analytical methods exist for the identification of wine by geographical origin, a reliable strategy for authentication of wine variety is still missing. Here, we investigate the suitability of the metabolomic fingerprinting of ethyl acetate wine extracts using U-HPLC-HRMS/MS. Red and white wines (three varieties of each, 85 samples in total) were analysed within our study. In addition to variety authentication, the possibility to recognize the ratio in varietal admixtures, was tested, too. The generated data, after automated data mining and alignment, were processed by principal component analysis (PCA) and then by partial least squares discriminant analysis (PLS-DA). The resulting statistical models were validated and assessed according to their recognition and prediction abilities. The most promising model was based on a positive ionization mode (ESI+), providing 100% recognition ability and 96.7% prediction ability for Riesling and Pinot gris wine varieties. Moreover, using constructed models, prepared wine mixtures were successfully distinguished from single varietal samples. The characteristic markers of the examined varieties were identified, and found to belong mainly to groups of cinnamic acid derivatives, stilbene derivatives and flavonoids. Our results indicate that a HRMS based metabolomic fingerprinting of wine is a promising strategy for wine variety authentication, of course, additional data are needed to verify the models.

## Authentication of CBD oil by using convergence chromatography coupled with high resolution mass spectrometry (SFC-HRMS)

**MSc Frantisek Benes<sup>1</sup>**, MSc Jiri Hricko<sup>1</sup>, PhD Vojtech Hrbek<sup>1</sup>, Prof. Jana Hajslova<sup>1</sup>

<sup>1</sup>*University of Chemistry and Technology, Prague, Prague, Czech Republic*

In the recent decade, the interest in hemp plants (*Cannabis sativa*) and products thereof has been rapidly growing. One of the popular groups of the hemp-based products are various food supplements, especially the CBD (cannabidiol) oils, which are available mainly through internet shops. Although the producers have proposed to classify CBD as a 'novel food', the process of approving has not been completed yet. Worth to notice that the quality / safety of these oils is currently not under official control, therefore, the health risk for consumers due to fraudulent practices cannot be excluded. In our study, we used a convergence chromatography coupled with high resolution mass spectrometry (SFC-HRMS) for cannabinoids analysis in CBD-oils purchased at the European markets in years 2016 (29 samples) and 2017 (25 samples). We determined not only CBD content and compared it with label declaration, but we also monitored major psychotropic cannabinoids and their precursors (such as  $\Delta^9$ -THC,  $\Delta^8$ -THC and  $\Delta^9$ -THCA-A). The results showed in many cases lower than declared CBD content, moreover, some products contained relatively high levels of the mentioned psychotropic cannabinoids. Under these conditions, more attention should be paid to these products, regulatory action is obviously needed.



## UHPLC-HRMS/MS metabolomics approach to differentiate *Cannabis sativa* varieties

**Mr. Jiri Hricko<sup>1</sup>**, Ms Alena Novakova<sup>1</sup>, Ms. Milena Stranska-Zachariasova<sup>1</sup>, Mr. Zbynek Dzuman<sup>1</sup>, Prof. Jana Hajslova<sup>1</sup>

<sup>1</sup>*UCT Prague, Karlovy Vary, Czech Republic*

*Cannabis sativa* plants are known to produce a broad spectrum of biologically-active secondary metabolites, many of which are specific to the plant and known as phytocannabinoids. Based on the hypothesis that the representation of such metabolites is characteristic for particular *C. sativa* varieties, we developed a fingerprinting method to differentiate between them. This study optimizes non-target screening using separation and detection by ultra-performance liquid chromatography coupled with high resolution tandem mass spectrometry (UHPLC–HRMS). UHPLC conditions were optimized for effective metabolite separation. The resulting method was then applied to the analysis of 128 dry *C. sativa* samples comprising five varieties; the samples differed in cultivation location and harvest season. The acquired data were processed by multivariate analysis methods and the resulting models validated. The best classification was obtained for the following *C. sativa* variety groups: (i) Santhica, (ii) Finola + Bialobrzeskie, and (iii) Carmagnola + Uniko B, for all of which excellent recognition (R<sup>2</sup>) and prediction (Q<sup>2</sup>) ability was achieved. Then we validated two models differentiating between Finola, Bialobrzeskie and between Carmagnola, Uniko B also with good recognition and prediction ability. In all cases, the markers primarily responsible for species classification belonged to the phytocannabinoid group. Thus, our method reliably differentiated between the five tested varieties, thereby confirming the hypothesis that individual cultivars are specific in terms of compound representation.

## A novel approach to assess quality and authenticity of Czech wine based on SPME-GC-HRMS

**Mr. Michal Stupak<sup>1</sup>**, Prof. Jana Hajslova

<sup>1</sup>*UCT Prague, Prague 6, Czech Republic*

Wine authenticity has become a subject of great concern over the last few years. Therefore, the reliable identification of the final product composition is a key point to combat fraudulent practices and to assure commercial fairness. To document wine quality parameters, a number of laboratory tests employing various techniques including chromatography and spectroscopy have been developed. In most cases, the analytical methods are based on targeted screening strategies based on 'classic' markers. In our study, we focused on volatile metabolome components of wine isolated from the wine headspace by solid phase microextraction (SPME). For non-target fingerprinting, gas chromatography coupled to tandem mass spectrometry (Q-TOF mass analyzer) was employed. The data obtained by analysis on a set of 48 white wine samples (Pinot gris, Traminer Musque and Riesling) and 50 red wine samples (Blauer Portugieser, Pinot noir and St. Laurent) provided directly from manufacturers were assessed by advanced chemometric methods. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were applied for classification model construction between different red and white wine varieties separately and, subsequently, characteristic markers for different groups were tentatively identified. Moreover, the oxidation stability of selected wine was also tested. The best separation according to the variety within the white wines sample set was achieved for Pinot gris and Riesling (prediction and recognition ability were 100% and 97%, respectively). In case of red wine samples, Blauer Portugieser and St. Laurent have been very well separated (prediction and recognition ability – 100%).

## Development of Immunosensor Technology for Nut Allergen Detection as a Food Surveillance Tool

**Mr. Matthew Mcgoldrick**<sup>1</sup>, Dr Philippe Delahaut<sup>2</sup>, Dr Micheal Lochhead<sup>3</sup>, Prof Christopher Elliott<sup>4</sup>, Dr Katrina Campbell<sup>5</sup>

<sup>1</sup>*Institute for Global Food Security, Queen's University Belfast, Belfast, United Kingdom*, <sup>2</sup>*CER Groupe, Marloie, Belgium*,

<sup>3</sup>*MBio Diagnostics Inc, Boulder,, USA*, <sup>4</sup>*Institute for Global Food Security, Queen's University Belfast, , United Kingdom*,

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Substitution of ingredients with those of lower value or quality can be a very attractive economic motivator for food adulteration. Recent insecurities in the food supply chain and new labelling legislation has drawn attention to allergens as an adulterant of concern to food safety. Food allergens and particularly as adulterants need to be effectively controlled through rapid detection systems to prevent contaminated food from reaching the market and affecting highly susceptible individuals.

The aim of this study was to develop an immunoassay for the detection of nut allergens simultaneously but where individual nut types can be identified.

Incorporating advanced nano-spotting technologies, in-house produced nut standards were printed on a nano-array for development of a competitive assay format. Rapid extraction techniques were constructed into the assay design enabling the detection of nuts from sample to result in under 15 minutes, without the use of laboratory equipment suitable for field development. Individual assays were developed showing sensitivities (IC50) of; 16.1ppm, 0.9ppm, 1.6ppm, 4.6ppm and 16.7ppm for peanut, almond, pine nut, Cashew and Macadamia nut respectively. Multiplex analysis combining all nut assays on the platform shows similar sensitivity.

The immunosensor system is capable of detecting less than 0.1% nut allergen adulteration without the requirement of laboratory equipment. This is a powerful tool which can be applied to HACCP management systems along the industrial food chain to help prevent product cross-contamination or adulteration with high risk allergen food groups. Future work will examine the feasibility for personalised food safety testing with tailored multiplexed assays.

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## Biosensors into the limelight: Towards a user-friendly interface with The Bio End user Sensor Tree

**Mr. Joost Nelis<sup>1</sup>**, Mr. Aris Tsagkaris<sup>2</sup>, Mr Yungfeng Zhao<sup>1</sup>, Mr. Javier Lou-Franco<sup>1</sup>, Ms Philene Nolan<sup>1</sup>, Prof. Christopher Elliott<sup>1</sup>, Dr. Cuong Cao<sup>1</sup>, Dr. Karen Rafferty<sup>1</sup>, Dr. Huiyou Zhou<sup>3</sup>, Prof. Jana Hajslova<sup>3</sup>, Dr. Katrina Campbell<sup>1</sup>

<sup>1</sup>Queens university; Institute of global food security, Belfast, United Kingdom, <sup>2</sup>University of Chemistry and Technology, Prague, Czech Republic, <sup>3</sup>University of Leicester, Leicester, UK

Point of site (POS) devices can be seen as the beau ideal for contaminant screening in foodstuffs since the devices bring the power to the consumer and potentially open the door to bottom up big data accusation regarding food safety and integrity. However, the currently used complex classification system combined with purely scientific dissemination of information regarding novel sensors can conceal POS cognisance from the larger public which occludes integrated decision making. As a counter measure we propose a novel end-user orientated system for biosensor classification better equipped to deliver useful information to all stakeholders in the biosensor production chain. The system applied is the “Bio End-user Sensor Tree” or BEST decision tree. BEST is an online decision tree and website that uses 4 criteria in the following order 1) expert training needed 2) sensor portability 3) quantification ability 4) single or multiplex screening ability. These criteria form the building blocks for the questions in the decision tree that directs one to a page featuring summaries of current scientific literature and consumer reviews regarding sensors tailored to the user’s needs. BEST is currently being designed for sensors targeting marine biotoxins, mycotoxins, pathogens and pesticides. However, other branches can be created since the system is kept open source and participation is invited from other enthusiasts. Finally, it is hoped that this approach will promulgate cognisance regarding the DIY possibilities for companies and consumers for food testing and help set the bearings for a future with globally ensured food integrity.

## The application of ambient mass spectrometry to rapidly determine production systems used in poultry production

**Mr. Nicholas Birse**<sup>1</sup>, Dr. Olivier Chevallier<sup>1</sup>, Dr. Steven Pringle<sup>2</sup>, Dr. Sara Stead<sup>2</sup>, Prof. Christopher Elliott<sup>1</sup>  
<sup>1</sup>*Institute For Global Food Security, Queen's University Belfast, Belfast, United Kingdom,* <sup>2</sup>*Waters Corporation, Wilmslow, United Kingdom*

Poultry production in the UK and Europe suffered from a series of negative events in 2017, damaging consumer confidence in the poultry and wider food industries. Events included the contamination of eggs with Fipronil, poor quality turkey products entering the food chain, and the alleged incorrect handling and labeling of chicken products at processing plants, which may have resulted in production method labelling on packaging being incorrect.

There is currently no analytical platform which can rapidly determine the main production systems (conventional, free-range, organic or other) used in poultry production to confirm labeling accuracy. Differences in fatty acid profiles (particularly the omega-6:omega-3 ratio) of meat samples from the main production systems have been previously determined by use of a number of analytical techniques, including GC-FID and HPLC-DAD. These techniques require significant sample preparation time and have low-throughput capability. This project aims to use ambient mass spectrometry and a chemometric modelling to develop a high-throughput, direct sampling platform capable of rapid confirmation of labelling accuracy. Rapid evaporation ionization mass spectrometry (REIMS) with an iKnife sampling and the LiveID chemometrics package will be used to build multi-variate models sampling meat from known production systems, against which meat from unknown systems can be compared. The commercial applications of such a system will be assessed in collaboration with a major poultry producer, with a view to the application of this technology as part of a high-throughput quality control and authenticity verification system.

## Leaving the laboratory behind: Rapid in-field food authenticity screening using handheld spectroscopy

**Dr. Terry F. McGrath<sup>1</sup>**, Dr Simon A. Haughey<sup>1</sup>, Prof Chris T. Elliott<sup>1</sup>

<sup>1</sup>IGFS, Queens University Belfast, Belfast, United Kingdom

Food fraud is estimated to cost the world economy \$US49b annually. The British Retail Consortium suggest 1 in 10 consignments of imported basmati rice has been adulterated, whilst our work on oregano showed 25% of retail product, on UK shelves, was adulterated and this pattern is similar globally.

Economic gain is often the goal. Authentic products are substituted with inferior products, eg the 2013 European horsemeat scandal. However, there are also health implications, eg the 2008 adulteration of Chinese infant formula with melamine.

Conventional methods used to determine food authenticity are laboratory based, require skilled operators, are expensive or time consuming and can take days or even weeks to complete. Meanwhile products pass along the supply chain and in many cases reach the consumers tables before results are reported. New and better ways of checking the authenticity of foods and their ingredients are required. Methods capable of rapid detection anywhere, anytime, in the food supply chain are what industry are demanding. This can be achieved through the use of handheld spectroscopic analysis in conjunction with chemometric modelling.

We present our latest research in developing spectral databases and chemometric models, on handheld portable spectroscopic instruments, to enable stakeholders to determine food authenticity in high risk commodities.

This research will cause a paradigm shift in food fraud detection by taking authenticity testing out of the laboratory and putting it in the hands of end users who can test on-site, anytime, in the supply chain and get immediate results right at their fingertips.

## The Food Authenticity Network; the one-stop-shop that can help protect the integrity of your food

**Mrs. Selvarani Elahi**<sup>1</sup>, Dr Stephen Ellison<sup>1</sup>, Dr Mark Woolfe<sup>1</sup>

<sup>1</sup>LGC Limited, Teddington, United Kingdom

The Food Authenticity Network (<http://www.foodauthenticity.uk/>) is a free toolkit for the detection of food fraud that can help to fight food fraud and build a more resilient food supply chain.

The Food Authenticity Network is a UK government funded initiative that was born out of the 2013 horsemeat issue and brings together all those with an interest in food authenticity testing. The network aims to raise awareness of the tools available to check for mislabelling and food fraud, and to ensure that stakeholders have access to a resilient network of laboratories providing fit for purpose testing to check for food authenticity so consumers can have confidence in the food they buy.

Membership is free and it's very quick to join so if you're not a member then please visit [www.foodauthenticity.uk](http://www.foodauthenticity.uk) and sign-up today for the latest information on food authenticity.

The Network is now 2 and half years old and has over 750 members from 39 countries, and its Twitter account (<https://twitter.com/fauthenticity>) has over 970 followers.

This talk will highlight plans to transform the Network from a UK government funded initiative into an industry led global Network aimed at fighting food fraud.



## Authentication of edible oils using non-targeted spectroscopic fingerprinting techniques – Comparison of measuring instruments

**Dr. Susanne Esslinger<sup>1</sup>**, Mr Sebastian Drescher<sup>2</sup>, Dr. Carsten Fauhl-Hassek<sup>1</sup>, Ms Carolin Lörchner<sup>1</sup>  
<sup>1</sup>Federal Institute For Risk Assessment BfR, Berlin, Germany, <sup>2</sup>Technical University of Berlin, Berlin, Germany

Non-targeted analytical methods aim to capture as many features as technically possible within one measurement to deliver a comprehensive insight in the composition of food samples enabling their authentication, e. g. in view of the declared geographical/botanical origin. Although these approaches are upcoming and seem to have a high potential in authentication processes, the routine use is currently restricted to certain products, often in conjunction with commercial solutions. A number of requirements regarding the application in routine analysis have to be addressed, e. g. (i) strategies for method validation and quality assurance measures, (ii) reliable databases of representative samples, and (iii) uniform data exchange formats for jointly usable databases.

In order to fulfil these prerequisites, the comparability of non-targeted measurements as well as the outcome of respective chemometric data evaluation was investigated within the national funded project FoodAuthent. For this, the same set of edible seed oil samples was analysed by two identical FTIR-spectrometers. The acquired fingerprints were used to define data spaces of authentic samples with the aim to distinguish oils of different botanical species. It was also aimed to combine the data obtained in one database and to develop strategies to jointly use data from the two instruments. The respective results will be presented and discussed.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

## Beyond the state-of-the-art in multivariate classification analysis: building instrument-agnostic methods

**Dr. Tassos Koidis<sup>1</sup>**, Mrs Konstantia Georgouli<sup>1</sup>, Dr Jesus Martinez-delRincon<sup>1</sup>

<sup>1</sup>*Queen's University Belfast, Belfast, United Kingdom*

The great majority of the studies specially in the area of food authenticity that involve vibrational spectroscopy, are training the chemometric models on the spectral data acquired from one instrument, e.g. the in-house FTIR or NIR. If the same method/model is used to predict 'food properties' from spectral data acquired from a different instrument the results will hardly be the same even if using the same acquisition parameters. This often leads to model overtraining and prevents their applicability in real-world scenarios such as in a food testing laboratory or in the QA/QC of a food producing factory. In this paper we are introducing the results of an inter-laboratory trial that was conducted on a FTIR method for the identification of botanical origin of vegetable oil blends, the lessons learned and the painstaking approach to build an instrument-agnostic method by introducing a totally new concept: augmentation with synthetic samples. The novel spectral data augmentation framework that is proposed here in fact was able to increase the performance of the typical classification model described previously by generating realistic data augmented samples. More specifically, the feasibility of the proposed data augmentation framework has been evaluated on three different core experiments. Results demonstrate a significant ~40% improvement in classification when testing in more than 10 different spectroscopic instruments. Although not new information is added after augmentation, careful application of the framework can increase the variability in the data, balance the group membership when applicable and build more tolerant and definitely more instrument-agnostic models.

## Isotope Fingerprints: Authentication of Honey by LC Coupled with Isotope Ratio MS

**Dr. Mario Tuthorn<sup>1</sup>**, Dr. Dieter Juchelka<sup>1</sup>, Dr. Jens Griep-Raming<sup>1</sup>, Dr. Andreas Hilker<sup>1</sup>

<sup>1</sup>*Thermo Fisher, Bremen, Germany*

Honey is considered as a value-added food of natural origin, yet it is simply structured regarding its composition, which makes it prone to economically motivated adulteration by addition of sugars of other sources.

The introduction of bulk <sup>13</sup>C/<sup>12</sup>C isotope analysis by White and Doner in 1978 was a major step towards establishing better detectability of adulteration. But carefully selected mixtures of sugars can mimic both, the bulk <sup>13</sup>C composition and the sugar profile of the natural product.

Compound specific isotope analysis can refine authenticity fingerprints of honey. The methodology based on the chromatographic separation of the carbohydrates and carbohydrate fractions and the subsequent <sup>13</sup>C/<sup>12</sup>C isotopic analysis by coupling LC with isotope ratio mass spectrometry (IRMS).

The <sup>13</sup>C/<sup>12</sup>C isotope ratio of bulk honey is determined by analyzing pure honey samples by an elemental analyzer coupled with IRMS (Thermo Fisher Scientific, Bremen). Samples are prepared by encapsulation in tin foil and introduced into the elemental analyzer without additional treatment. Additionally, the protein fraction is prepared by Na<sub>2</sub>WO<sub>4</sub> precipitation from aqueous sample solution, dried and analyzed using the methodology described above.

<sup>13</sup>C/<sup>12</sup>C isotope ratios of the individual carbohydrates, bulk honey and protein fraction are compared for authenticity evaluation. A large difference in the values (on the order of 1‰ or greater) might indicate adulteration and requires further investigation.

This work describes a multi-parametric methodology, looking at both, bulk and compound specific <sup>13</sup>C/<sup>12</sup>C, deducing isotope fingerprints and identifying adulteration.

## Isotope Fingerprints: Origin of Tequila with GC Coupled with Isotope Ratio MS

**Dr. Mario Tuthorn**<sup>1</sup>, Dr. Dieter Juchelka<sup>1</sup>, Dr. Christopher Brodie<sup>1</sup>, Dr. Andreas Hilker<sup>1</sup>

<sup>1</sup>*Thermo Fisher Scientific, Bremen, Germany*

The blue agave (*Agave tequilana* Weber var. *Azul*) is a native plant of the Jalisco region in Mexico and is an important economic product that, by law, is the only one allowed to be used in the production of tequila. Globally, tequila is a popular alcoholic beverage with a subsequent increase in export value to the Mexican economy. This provides an opportunity of economically motivated fraud either by adulteration and mislabeling of original tequila or production of fake tequila.

Gas chromatography/ isotope ratio mass spectrometry provides a powerful tool for determining carbon, oxygen and hydrogen isotope fingerprints in beverages and food. Thermo Scientific™ TRACE™ 1310 GC coupled with Thermo Scientific™ GC IsoLink II™, Thermo Scientific™ ConFlo IV™ Universal Interface and a Thermo Scientific™ DELTA V™ isotope ratio mass spectrometer offers a solution for identifying the purity and adulteration of products.

Oxygen isotope fingerprint of the *A. tequilana* plant, and local sugars used in mixed tequilas, is primarily given by the rainfall water in those regions and can provide a geographical tool for origin. Here we report carbon and oxygen isotope fingerprints from commercial tequila, sugar cane and the *A. tequilana* plant. Coupled  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of ethanol allow differing the original branded mixed tequila from pure tequila, which derives 100% from *A. tequilana*. In addition, it also shows the difference between *A. tequilana*, original mixed tequila and sugar sources, meaning that adulterated and mislabeled tequila can be differentiated from original tequila and original source ingredients.

## Fraud vulnerability assessment in the Dutch milk supply chain

**Ms. YUZHENG YANG<sup>1,2</sup>**, Prof. Saskia van Ruth<sup>1,2</sup>, Prof. Wim Huisman<sup>3</sup>

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Food fraud is not new for us. It brought not only large economic losses to companies, but also had an influential impact on human health and public confidence. The raised awareness has strengthened the need to evaluate the food fraud vulnerability. Facing the urgent demand, SSAFE has developed a Food Fraud Vulnerability Assessment (FFVA) tool in 2015 to help all actors across the supply chain to conduct fraud vulnerability assessments.

Milk is in the top of the most adulterated food products all around the world. The current study aims at understanding the fraud vulnerabilities of the Dutch milk supply chain, and profiling the critical risk factors in the chain, with the application of SSAFE FFVA tool. The main tier groups in the Dutch milk chain were investigated and the risk factors for each tier group were identified.

The FFVA tool provides a practical way to elucidate the vulnerability of food industry or certain actors in the food supply chain. The outcome of the current study helps to understand the potential fraud threat in the Dutch dairy chain and to develop the corresponding fraud mitigation schemes. Furthermore, it provides feasible way to ensure the integrity of local and global food system.

## A direct link between organic milk and feed - from grass to glass

**Mr Ningjing Liu<sup>1</sup>**, Dr. Annemieke Puschens<sup>2</sup>, Prof. Saskia van Ruth<sup>1</sup>

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Organic milk is drawing more and more attention in recent years because of its ecology, sustainability and safety. Due to its higher retail price and the strict requirements for organic products compared with conventional milk, organic milk is susceptible to fraud. To guarantee the authenticity of organic milk and preserve consumers' trust, several studies have been conducted to portray the differences between organic milk and other milks. Nevertheless, there is still a lack of information regarding the cause of these differences. Therefore, the main interest of this research was to find the factors leading to the unique composition of organic milk. The present work analysed milks and related feeds from 41 dairy farms (including 17 organic farms, 12 pasture farms and 12 conventional farms ) in The Netherlands. The fatty acid compositions and volatile organic compound profiles of different milks and feeds were obtained and the correlation between milk, feed and farm management was investigated. Overall, this work identified the relevance of factors affecting the compositional characteristics of organic milk.

## Registration-based analysis to detect discrepancies in the food supply chain

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Seafood is one of the most valuable and highly traded commodities worldwide, and thus highly susceptible to fraud. The Norwegian fisheries sector is subject to a wealth of regulatory requirements ensuring an environmentally and economically sound fishery. Nonetheless, studies have shown that there exists a, at times significant, discrepancy between imports/landings and exports/domestic consumption. Parts of this gap between input and output can be explained by intentional acts of fraud, such as landings of illegal, unreported and unregulated (IUU) fish. However, as both the production process and the supply chain is highly complex, portions of the gap might also be attributed to non-fraud related issues, such as gaps in the regulatory framework, inaccurate reporting, production process errors, human error, or other unintentional factors.

By combining a documentary study of the regulatory framework with a material flow analysis and an in-depth case study, we map and analyse the Norwegian cod fishery supply chain to identify possible sources of discrepancies and inconsistencies. We estimate the scope of the discrepancy within the Norwegian cod fisheries, highlight weak points within the supply chain, and identify weaknesses within the regulatory framework regarding product registrations. The study exposes a highly complex framework with varying demands for data submission, a lack of registrations in certain crucial stages of the supply chain, overlapping registrations, and no central system set up for coordination and data sharing between authorities aimed at combatting food fraud.

## Food fraud early warning systems and bigdata trend analysis: from design to validation

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Economically motivated adulteration of food is, by definition, motivated by profit. Therefore, there is a strong expectation that occurrence of such incidents be sensitive to prevailing macroeconomic conditions, and that the disruption of trade relationships (e.g. due natural disaster or crop failure) may present novel opportunities for fraudsters. As part of Work Package 8 of the Food Integrity project, we developed an Early Warning System (EWS), which monitors global signals of over 5000 foodstuff commodities utilising network analysis and unsupervised machine learning to identify ongoing disruptions, and provides intelligence allowing directed response by affected stakeholders. But how reliable is this system? How can we validate the system's prediction given that fraud is in nature a concealed activity? This presentation attempts to provide an overview of the system, followed by discussing the results of validation process against the occurrence of reported instances of food fraud within FERA's incident monitoring system HorizonScan. Considering only events with the strongest evidence for trade disruption, we have estimated the accuracy of the system based on matches (i.e. same nations and commodities) between reported fraud incidents and EWS warnings up to three and six months before the event. The improved availability of "big-data" in relation to food trade and sophistication of learning algorithms opens untapped opportunities to improve our understanding of conditions leading to economically motivated adulteration in foodstuffs. As supply chains become increasingly global such automated tools have the potential to become a key component in strategic response to fraud risk within the industry.



## Mystery foods on the menu: can the restaurant industry limit risks?

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Not only food industry and retail have been hit by food fraud, in the restaurant/catering sector many cases about swaps of expensive fish species, frozen foods being passed as fresh, substitution of olive oil on the table, artisanal soup straight from a can, etc. have been reported. Consumers dining in good faith are deceived, but the restaurants themselves are targets too, especially for criminals earlier in the chain. Large food service businesses, airline catering companies, wholesalers for the restaurant industry, and high end restaurants are vulnerable but also local fish and chip outlets. Their reputations are at stake. The large number of ingredients, poor visibility in the chain beyond the first supplier, lack of testing and limited awareness make their situation unique. As a preventive measure, a simple fraud vulnerability checklist was developed in collaboration with the catering industry. It is divided in three categories of questions to avoid unnecessary repetition: those dedicated to the own company, the product, and the product suppliers. The checklist was validated in practice for olive oil, meat, black pepper and a complex food with various industry actors. This approach, focusing on prevention rather than detection, helps to raise awareness in the restaurant industry and eventually limit the risk of food fraud.

## Species identification of fish fillets using portable NIR: a pilot study

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Selling a fish species different from that declared on the label is the most frequent fraud in seafood. This fraud can have strong economical, health, and ecological implications. Currently the two most used countermeasures are visual inspection by experts and DNA analysis. The former is hard to apply to fish fillets, the seafood category at highest risk of substitution, while the latter is expensive and not applicable on site. Within the FoodIntegrity project, we thus carried out a pilot study to explore the possibility of applying Near-infrared spectroscopy (NIR) in order to identify the species in fish fillets. To measure the NIR spectrum and to perform a classification model of the fish fillets we adopted the SCiO molecular sensor, developed and distributed by Consumer Physics<sup>3</sup>. In connection with the instrument the SCiO Developer Toolkit enable to customize and collect the spectra of the desired materials via mobile application. We selected 10 fish fillets for each considered species: *Solea solea*, *Pleuronectes platessa* and *Pangasianodon hypophthalmus*. Every species was sampled 60 times, with 5 different scans for each sample. The validation set was carried out over 3 fish fillets per species, comprising 30 samples per species with 3 different scans per sample. The acquired scans were realized on different points of the fillet in order to evaluate possible variation with respect to the portion of the fillet side. The obtained global accuracy was 98.3%, suggesting the SCiO as a promising tool to use in the context of on-site controls.

## Food authentication by DNA based methods: a document classifier of the literature database

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Food safety and food quality have increasingly come to the forefront of consumer concerns, industry strategies, and government policy initiatives. In this context DNA technologies represent a useful tool in food inspection and regulation. In fact, molecular markers are of extreme importance to traceability since they allow the outcome of a given item to be monitored at each stage in the food chain.

To assess critically the scientific literature reporting application of analytical DNA methods to authenticity testing of complex food products, a bibliographic research was performed using PubMed and EBSCO libraries by keywords. The articles were analyzed with "Lucene 4.0.0" and carried out on "lukeall-4.0.0-ALPHA" to have a user-friendly interface. This library allowed us to search within index by using a simple keyword query or a complex query with Boolean operator and multiple keywords. We have analyzed 117 articles, published in the last 20 years.

This database was used to understand the current state-of-the-art of know-how and methodologies.

Detection of fraud, especially in meat products, is important not only for economic problem, but also for health, religious and ethical reasons. In the last decades, the main DNA methods are based on the detection of species-specific DNA sequences. Most of these methods rely on the polymerase chain reaction (PCR) technique for its specificity, sensitivity, simplicity, and rapidity, allowing the identification of species of origin even in complex and processed foods.

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## Innovative multiplex organic photonic sensor for plasmonic-based detection of contaminants in milk: the MOLOKO project

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The increase of contaminant levels in food has led to negative human health effects from exposure to toxic substances. The interactions between environment and the food supply chain that mainly occur at primary production level can cause serious short- and long-term detrimental effects on human health. In particular milk and dairy products may contain contaminants or adulterants from different sources and testing for these is compulsory, based on risk assessments. In this scenario, the EU H2020-ICT granted MOLOKO project aims at manufacturing and implementation of a miniaturized organic photonic sensor for low-cost standardized and validated screening of analytes for sustainable food safety. In particular, the multiplexed and (semi)quantitative detection is expected of up to 10 analytes including food safety parameters such as antibiotics (i.e. penicillin, cephalonium), toxins (i.e. mycotoxins) and food quality parameters (i.e. lactoferrin and caseins). The rapid, compact and reusable final prototype sensor is based on an opto-microfluidic detection scheme and designed for milk production and distribution end-users. These challenging objectives are achieved by the unprecedented integration within the same device platform of multiple key-enabling technologies such as organic photonics (as light-emitting transistors), nanoplasmonics, immunoassay diagnostics and microfluidics.

MOLOKO lab-on-a-chip has the potential to allow low-cost early detection capabilities of affected food supplies setting a higher quality standard along the whole food supply-chain from producers to retailers. This work has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 780839 (MOLOKO Project).

## Hapten synthesis and production of a monoclonal antibody for ribavirin

**Professor Zhanhui Wang**<sup>1</sup>, Dr. Xiya Zhang<sup>1</sup>, Prof. Suxia Zhang<sup>1</sup>, Dr. Kai Wen<sup>1</sup>, Prof. Jianzhong Shen<sup>1</sup>, Dr. Jianyu Zhu<sup>1</sup>

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In this study, two novel ribavirin haptens with different spacer arms were synthesized and identified. Those haptens were conjugated to KLH and BSA by active ester method for preparing immunogens and coating antigens. An obtained monoclonal antibody against ribavirin named 4C3 was produced and was of the immunoglobulin G1 (IgG1) isotype possessing a kappa light chain. A rapid and sensitive indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) was developed based on the antibody, aiming to detect ribavirin residue in chicken muscles. Under optimized assay conditions, the standard curve of icELISA was in the range from 1.53 to 38.5 ng mL<sup>-1</sup> with an IC<sub>50</sub> value of 7.07 ng mL<sup>-1</sup>. The LOD of the assay was calculated to be 0.35 ng mL<sup>-1</sup>. The recovery rate of the developed icELISA for ribavirin in real samples ranged from 79.2% to 107.3% with the coefficient of variations less than 15.6%.

## Cues of food authenticity; Supporting consumer food choice judgements in the absence of structural trust.

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The Chinese food system has been beset with cases of intentional deception and fraud that have threatened the integrity of its food system. In an environment where trust in the domestic food supply chain is low, this research explores how consumers make judgments about a products authenticity. Three levels of product cues 'indexical', 'iconic' and 'integrity' (The 3 'I's') are used by consumers to assess the authenticity of foods. 'Indexical' cues (i.e. certifications and county of origin claims) can be objectively verified through analytical methods and provide the legislative framework for supporting product authenticity. However, they require a trusted regulatory framework and whilst they provide the greatest assurances of authenticity, they are not easily substantiated by consumers. 'Iconic' cues, convey authenticity through product packaging and imagery (i.e. labeling). Consumers recognise that they are easy to falsify, although, they are widely understood and trusted. 'Integrity' cues are designed to specifically reassure consumers of a products authenticity. They signal that a product has not been tampered with (i.e. tamper-proof seals), can be traced through the production process (i.e. QR codes) and provide reassurances if a product does not meet expectations (i.e. customer care contacts). 'Integrity' cues require greater levels of consumer involvement, but provide the only tangible mechanism for assessing a products credibility. 'Integrity' cues therefore, act as a key deterrence against fraud, and food manufactures operating in China are encouraged to invest in the development of sophisticated but easy to interpret 'integrity' cues and ensure effective communication of these to consumers.

## On-Site Species Authenticity: Integrating DNA Testing into the Food Supply Chain

**Doctor Amanda Naaum**<sup>1</sup>, Dr. Robert Hanner<sup>2</sup>

<sup>1</sup>*Qub, Belfast, United Kingdom*, <sup>2</sup>*University of Guelph, Guelph, Canada*, <sup>3</sup>*TRU-ID Inc., Guelph, Canada*

Food fraud, including the inaccurate labeling of species ingredients, continues to present a threat to the food industry. Incidences of species mislabeling can reduce consumer confidence, mask sale of illegal or endangered species, cause health problems, and ultimately cost consumers and industry billions of dollars. Recent prosecutions have made it clear that willful ignorance of potential issues in food fraud still constitute criminal negligence. New regulations in multiple countries and across multiple industry sectors mandate attention to managing food fraud. However, specific details around how industry can best do this are limited. DNA-based testing for species authenticity is one way to help ensure products contain the listed ingredients. However, it can be difficult for industry to access and understand the options and results from this type of testing so that they can be successfully integrated into existing manufacturing and supply chains. We have developed assays for on-site DNA authenticity for species verification in product ingredients using simplified, lab-free protocols and hand-held instrumentation. This presentation will review case studies for key species, and review how the testing might be implemented in an industrial setting with minimal cost and disruption. These novel options allow industry to access DNA testing and easily integrate it as a tool in risk management related to food fraud.

I have been working in species differentiation for detecting food fraud for nine years, with a recent focus in development of simplified methods for non-expert users. I have also recently published and edited volume on seafood fraud.

## The Use of Blockchain Technology and DNA to Assure Origin in the UK Pig Sector

**Dr. Stephanie Brooks<sup>1</sup>**, Mr Kieran Kelly<sup>1</sup>, Ms Jackie Carter<sup>2</sup>, Mr Andrew Caines<sup>2</sup>

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Food fraud is believed to cost the UK economy billions of pounds per year, with reported losses of up to £12 billion attributable to fraud in the food and drink sector (Crowe Clark Whitehall, 2017). These losses have a significant reputational and financial impacts on legitimate actors in the food and drink industry. In addition, fraud scandals create mistrust among consumers, and in some instances, can result in serious food safety and ethical concerns.

As part of the EU China Safe programme, this project focuses on innovations in traceability to detect and prevent food fraud. arc-net are working with Cranswick Country Foods to establish the use of a blockchain platform, coupled with DNA, to track, trace and verify origins of British pigs. Blockchain technology is a distributed and immutable digital ledger that can be used to present a single view of the traceability path, compared to the fragmented means of traceability that typically exist in the food supply chain presently.

Currently, pigs are tracked in groups between farms and within processing facilities rather than individually. By tagging individual pigs with RFID ear tags, this project aims to track pigs on an individual level between farms and into the batching system at processing facilities. By testing and capturing the DNA of the sow and the movements of progeny on the blockchain, coupled with sampling and random testing of progeny DNA, there is an ability to make a parental link between progeny and sow, thereby proving authenticity, origin and traceability.



## Food Fraud Prevention: The Effectiveness of Combining Certification Data, Production and Trade Volumes

**Mr. Gerald Herrmann<sup>1</sup>**

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Based on 4 case studies and interviews with food supply chain actors and food industry experts, we can describe the many different techniques used to commit food fraud as resulting in the sale of a less valuable product disguised as a more valuable product. That means that supply chain actors increase the volume of the more valuable product by using fraudulent practices. Instead of following the assumption that food fraud is taking place and detecting fraudulent practices as they manifest in the product, food fraud defense programs should use this knowledge to prevent food fraud by comparing produced volumes to traded volumes. When combined with audit/ certification data, this mass balance approach detects when traded volumes are not plausible and therefore prevent food fraud. System-wide solutions based on registration or certification of all system participants have been implemented in organic certification systems and can be envisioned in many different settings. Based on our feasibility study, we identified three prerequisites for Check X system applications:

1. All supply chain actors within the system must participate,
2. All production and trade must be recorded, and
3. All relevant product qualities must be recorded.

With these prerequisites, the mass balance approach requires much less data than for example a (batch-)traceability approach, is less cumbersome and prone to gaps than other approaches such as internal audits and/ or the building of close business relationships and can prevent fraud from happening rather than detect it once it has happened.

## A metabolomics approach for assessment of fruit juices authenticity

**Dr. Jiukai Zhang<sup>1</sup>**, Dr. Ying Chen<sup>1</sup>

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Berry fruit juice, which is represented by blueberry and cranberry juice, has continued to rise and become more and more popular due to their reported nutritional and health benefits. However, adulteration of berry fruit juice with cheaper substitutes is frequently found in markets. In the present study, a metabolomics approach for assessment of fruit juices authenticity by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) was established. Metabolite fingerprinting was obtained and subjected to multivariate statistical analysis. Discrimination of blueberry juice, cranberry juice, and its adulterant apple juice, grape juice was carried out by principal component analysis-discriminant analysis (PCA-DA). Furthermore, 19 characteristic markers efficiently distinguishing berry fruit juice and its adulterants were selected by creation of profile plots displaying the abundances of markers. Determination of molecular formulae and tentative identification of marker compounds were conducted using elemental formula calculation and online database searches based on accurate MS mass and MS/MS fragmentation information. Moreover, targeted metabolomics analysis of juice bioactive compounds, such as flavonoid, anthocyanins, etc, exhibited good separation of berry fruit juices from adulterant juices. These results suggested that the combination of untargeted and targeted metabolomics approach has great potential for the rapid detection of berry fruit juice adulteration.

## Food Fraud: Risk-Ranking of Raw Materials using Data Mining

**Mr. John Points**<sup>1</sup>, Jordina Farrús Gubern<sup>2</sup>

<sup>1</sup>*Independent Consultant, Newbury, United Kingdom*, <sup>2</sup>*Fera Science Ltd, York, United Kingdom*

It is inherently difficult for food manufacturers and their suppliers to find metrics on food fraud incident rates, in order to help inform their risk-assessment and supply chain risk management policies. Problems include under-detection, under-reporting, and difficulty in interpreting whether reported incidents are innocent mistakes or deliberate fraud.

We present an analysis of the last 3 years publically-reported global food and feed incidents, as collated on Fera's Horizonscan database. We plot trends in regulatory non-compliance incidents using both a "charitable" interpretation (assuming innocent mistakes) and a "cynical" interpretation (assuming deliberate fraud) to give lower- and upper- bound estimates of food fraud. We normalise all data against the total number of food safety incidents in each territory, to mitigate national variations in testing frequencies.

This analysis shows that the most common offences are probably simple paperwork frauds; for example, extended expiry dates, or forged Health Certificates. Those ingredients with the highest risk of fraud prevalence are not necessarily the high-profile and high-value materials that gain the headlines, such as olive oil or meat species. They are those with a lower profit margin for the criminals but much lower profile and less emotive; materials such as ingredient-potatoes, or components of animal feed.

Certain types of food fraud are more prevalent in specific countries. We highlight some examples.

Despite the increased attention on food fraud in recent years, overall incidence has remained relatively static over the past three years.

## The Epidemiology of a Food Scare: Lessons from Fipronil

**Mr. John Points**<sup>1</sup>, Jordina Farrús Gubern<sup>2</sup>

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The supply chains of many food ingredients are international and complex. Food safety incidents can have a surprisingly wide impact. The 2017 contamination of eggs with fipronil illustrates how incidents that were initially believed to be isolated can expand in effect. There were interesting differences in the speed, proportionality and effectiveness of response in different countries, and some lessons that can be learned both for food manufacturers and for regulators.

We use the timeline of reported incidents and recalls in different countries to compare best practice, draw conclusions and make recommendations. Key aspects that had a clear effect on timelines, and hence public confidence in the industry, were the use of traceability data vs testing results as a trigger for withdrawal decisions, the speed and scale of follow-up testing, and an initial lack of appreciation of the complexity of the powdered egg supply chain.

## Tackling fraud in fish global supply chains using rapid evaporative ionisation mass spectrometry (REIMS)

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The increasing number of reports regarding global food fraud scandals has brought food authenticity and safety to the attention of regulators, industry and consumers worldwide. Fish fraud detection is mainly carried out using a genomic profiling approach requiring long and complex sample preparations and assay running times. Rapid evaporative ionisation mass spectrometry (REIMS), a new ambient ionisation technique, can circumvent these issues without sacrificing a loss in the quality of results. Our study investigated how the REIMS technology could aid the detection of fish fraud and whether quick and accurate results were obtainable. 478 samples of five different white fish species (cod, coley, haddock, pollock and whiting) were subjected to REIMS analysis using an electrosurgical knife. Each sample was cut 8-12 times with each one lasting 3-5 seconds. Chemometric models were generated based on the mass range  $m/z$  600-950 of each sample and a bin size of 0.5 Dalton was applied. The identification of 99 validation samples provided a 98.99% correct classification in which species identification was obtained near-instantaneously ( $\approx 2s$ ) unlike any other form of food fraud analysis. Significant time comparisons between REIMS and polymerase chain reaction (PCR) were observed when analysing 6 mislabelled samples demonstrating how REIMS can be used as a complimentary technique to detect fish fraud. Overall REIMS has been proven to be an innovative technique to aid the detection of fish fraud and has the potential to be utilised by fisheries to conduct their own quality control (QC) checks for fast accurate results.

## PCR based analyses for safety and quality assessment in the production processes of complex foods

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Nowadays many different analytical methodologies are available to identify plant and animal species in food and foodstuff. Among them, DNA-based analysis plays a central role due to its sensitivity, accuracy and reproducibility. Although more sophisticated molecular techniques (e.g, NGS, digital PCR) have been developed, these are still too expensive and/or difficult to be routinely implemented in an industrial process line. Thus, in the present study we focused on traditional and real time PCR methods to provide the industry with a reliable and feasible set of methods able to verify the compliance of tested food with the animal, plant and spice species listed in the labels. The 6 tested methods are: 1/2 endpoint singleplex and multiplex PCR for animal species; 3/4 quantitative PCR (SybrGREEN/TaqMan probes) for bovine and porcine species; 5/6 endpoint singleplex/multiplex PCR for plants species and spices.

Methods 1, 2, 3 and 4 were assessed on a panel of 13 animal species: cattle, pig, horse, chicken, turkey, goat, sheep, buffalo, rabbit, donkey, duck, deer, hare, whereas methods 5 and 6 on a panel of 8 plant species (tomato, carrot, celery, onion, sunflower, bay leaf, sage and black pepper).

Furthermore, Production Flow Diagrams of the two processed foods under study (Bolognese sauce and crude ham Tortellini) were analysed to identify the critical control point where these tests should be applied to detect potential frauds or adulterations of specific ingredients.

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## Rapid screening techniques for extra virgin olive oil authentication

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Extra virgin olive oil (EVOO) is a high value food commodity frequently found on the market in adulterated form. The most common adulterants include lower cost edible oils, such as rapeseed, sunflower, corn, soybean, hazelnut, and olive pomace oil. Various tests can be used to determine olive oil authenticity and the identity of the adulterant. Methods employing chromatography/mass spectrometry are often applied, but can be expensive, time consuming, and unknown adulterants may be missed.

Samples of authentic EVOOs and potential adulterants were scanned using a handheld near infrared spectroscopic device and FTIR-ATR. The performance of these instruments was evaluated for rapid screening for EVOO authenticity.

Admixtures of olive/rapeseed oils and olive/sunflower oils were prepared, ranging from 0 to 100%.

Estimation and classification models were generated using the data from the handheld spectrometer. Small spectral differences enabled detection of adulteration of EVOO with rapeseed/sunflower oil down to 5%.

FTIR was also applied for detection of EVOO adulteration. The FTIR test used an on-board algorithm called 'adulterant screen' which does not require further statistical analysis. Single spectra of pure adulterants (sunflower and rapeseed oil) were measured and stored under an adulterant library. Using adulterant screen, it was possible to detect adulteration of EVOO down to 2%.

Both methods are simple to use, require only the collection of the spectra (authentic samples and known adulterants) and require minimal additional statistical analysis, and could, therefore, be suitable as routine rapid screening techniques for adulteration of olive oil.

## Using the FoodIntegrity Knowledge base: case studies for food operators

### **Mr. Jean-François Morin<sup>1</sup>**

<sup>1</sup>*Eurofins Analytics France, Nantes, France*

Jean-François Morin is head of Innovation and Collaborative Research at Eurofins, one of the world's leading providers in the bioanalysis market. He is more specifically involved in food fraud prevention research. He is participating in a number of EU research projects, notably FoodIntegrity as Work Package leader, in the field of analytical sciences applied to food authenticity.

The latest GFSI Guidance Document includes recommendations on how industry should counter food fraud. Main food safety management schemes such as BRC and IFS have now included specific food fraud requirements in their certification schemes.

The FoodIntegrity Knowledge base, a Web-based tool available through the FoodIntegrity website ([www.foodintegrity.eu](http://www.foodintegrity.eu)), helps food operators take up both challenges of tackling food fraud in their own facilities and complying with these recommendations and schemes. It provides information on various food fraud practices together with recommended analytical strategies.

Several examples on different food matrices illustrate the Knowledge base added-value and how it will be used by stakeholders. Initiated either by an external alert or by a doubt on a raw material, a search in the Knowledge base helps stakeholders to identify, easily and rapidly, existing solutions for a given food product or ingredient and put testing actions into effect for ensuring authenticity and safety.

As part of a vulnerability assessment or during a mitigation plan set up, the FoodIntegrity Knowledge base will help put in place a documented procedure indicating when, where and how the integrity of food products entering or leaving the factory is verified.



## NMR and MIR-ATR approaches to assess the integrity of saffron: a FI WP18's case study

**Prof. Marco Arlorio**<sup>1</sup>, Dr. PhD Cristiano Garino<sup>1</sup>, Dr. PhD Monica Locatelli<sup>1</sup>, Prof. Luigi Portinale<sup>1</sup>, Dr. PhD Giorgio Leonardi<sup>1</sup>, Prof. Vito Gallo<sup>2</sup>, Dr. Antonio Rizzuti<sup>2</sup>, Dr. Juan Antonio Fernández Pierna<sup>3</sup>, Dr. Vincent Baeten<sup>3</sup>

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Over the last years, food traceability has earned the attention of the international scientific community, as well as the interest of the consumers, which are more and more concerned about the origin, integrity and safety of the food. Instead of searching for a specific contaminant or anomaly, untargeted analytical methods aim to describe specific food “fingerprints” characterizing a particular food product. Within the activities of the European Project “Food Integrity”, Work Package 18 aims at producing a consensus document (guidelines) on good practices and methodological procedures for the application and validation of untargeted analysis applied to food traceability. The Units involved in WP18 worked on different models using different untargeted analytical techniques, finally generating several data sets to be statistically processed. In November 2016 the USP “Guidance on developing and validating non-targeted methods for adulteration detection” has been released, with the aim of providing guidance on how to develop and implement one-class, non-targeted classification methods for the detection of economically-motivated adulteration (EMA)-related adulterants in food. Here we present the critical discussion of the results of a model study inspired by the indications reported in this guidance. For this, the processing through advanced mathematical tools of data generated using NMR and mid-infrared attenuated total reflectance (MIR-ATR) spectroscopy on pure and artificially adulterated saffron samples is implemented. Saffron is one of the most expensive spices throughout the world, and because of its limited production it is considered within the major candidates for economically motivated frauds.

## A decision framework for using analytical testing for food fraud reduction

**Prof. Francis Butler**<sup>1</sup>, Mr Niels Lucas Luijckx<sup>2</sup>, Dr Hans J.P. Marvin<sup>3</sup>, Dr Yamine Bouzembrak<sup>3</sup>, Dr Vahid Mojtahad<sup>4</sup>

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Many analytical technologies exist to detect fraud. However, expense often deters their adoption and routine use by the food industry. This work developed several aspects relating to the decision to use analytical testing for detecting food fraud. A review of available databases detailing fraud occurrences indicated that frequently, analytical testing was not the initial action to detect fraud. A web based survey among industry stakeholders showed that 86% of respondents already have fraud monitoring as part of their quality assurance programme. For those undertaking fraud monitoring, 67% used both documentation control and analytical measurements. 26% used documentation only control, whereas 7% reported using only analytical measurements. The total economic risk of food fraud comprises of a combination of direct or indirect and tangible or intangible damages that needs to be avoided. The economic risks of food fraud can therefore be categorized into four quadrants based on whether the damages are tangible/intangible or direct/indirect. This work developed a framework to assess the risk of fraud based on a simple decision tree. Based on a combination of production volume and value, a priority estimate of fraud occurrence can be made for ingredients, raw materials or products present in the company. Fraud risks can also be prioritised based on a probability assessment, which uses several factors including price volatility and supply chain factors. Together these simple tools can guide companies to set the right priorities in relation to preventing fraud, make benefit-cost analyses and allocate budget and resources appropriately.

## What impact will blockchain technology have on food traceability and authenticity?

**Dr. Petter Olsen**<sup>1</sup>

<sup>1</sup>*Nofima, Tromsøe, Norway*

Blockchain technology is taking the business world by storm; it is described as a disrupting technology and by some considered to be the biggest digital revolution since the internet itself. The most well-known application of blockchain technology is the Bitcoin digital currency, but it is also being used to facilitate smart contracts, crowd-funding, electronic voting, and more. There are obvious applications of blockchain technology in product supply chains where it can be used to support persistent, incorruptible records of statements and transactions.

This presentation briefly describes what blockchain technology is, and what functionality it offers. It analyses what impact blockchain technology will or might have on food traceability and authenticity, and it highlights what challenges the technology can solve, and what challenges it cannot solve.

## Food authenticity and food fraud – How to define terms and concepts?

**Dr. Petter Olsen**<sup>1</sup>

<sup>1</sup>*Nofima, Tromsøe, Norway*

Food authenticity and food fraud are multi-disciplinary research fields without well-established definitions for the related terms and concepts. For the research in this area to progress, it is important to arrive at a common understanding of what various terms and concepts mean. Various general standards exist, and various specialized standardization efforts are underway.

This presentation focuses on the consensus-based European standard "CEN WS/86 - Authenticity in the feed and food chain – General principles and basic requirements" that was delivered in spring 2018, and highlights the discussions and decisions that were made during the standardization process, and what the final results and recommendations were.

## DNA-based methods to (co-)verify food integrity

**Dr. Esther J. Van Leeuwe - Kok**<sup>1</sup>

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DNA-based methods to (co-)verify food integrity

Esther J. Kok

It is increasingly evident that authenticity issues will often require a multidisciplinary approach. So far most attention has focused on the potential of analytical chemistry methods. In recent years, other methods have been developed, and especially DNA-based tools have shown their value in a range of food composition and authenticity issues. Available DNA methods have expanded from basic DNA amplification methods, such as (q)PCR, to a range of isothermal amplification methods, allowing on-site applications, to the enabling DNA massive parallel sequencing strategies. These developments have been boosted by a series of European methods including, amongst others, the TRACE project and more recently the Decathlon project. In this presentation an overview will be provided on recent advances in DNA-based traceability, including results from the European Decathlon project, as well as other recent developments in DNA-based methodology. The focus will be on different applications of DNA-sequence-based traceability, using conventional massive parallel sequencing platforms, as well as third generation sequencing, such as the MinION sequencing device. Results will be present of applications in complex products to analyse for the underlying ingredients as well as for the potential presence of authorised as well as unauthorised genetically modified organisms (GMOs).

## Identified food integrity fraud in the beef industry (1997-2017)

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The European Horsemeat Scandal of 2013 highlighted vulnerability within the beef supply chain. Widespread media coverage of the event contributed to damage consumer trust, product recalls, serious effects on the sale of ground beef, and huge economic losses for defrauded companies. By reviewing previous incidents documented in Horizon Scan and the Rapid Alert System for Food and Feed (RASFF) and using foresight methodologies industry can identify vulnerable points along the food chain and learn to protect itself and its consumers from future fraud. By using the above databases this research identified 499 integrity issues associated with the beef industry in the past 20 years. Data was categorised by incident type and incident area, and mapped to visually display the occurrence of food integrity fraud in the beef supply chain and analysed. Results showed that the most common type of fraud in the beef industry is adulteration at 51% of all incidents, specifically adulterations with illegal veterinary medicine, antibiotics or growth promoters or legal substances found above MRL's encompassed 31% of all incidents. When incidents were classified into 13 different areas along the beef supply chain, 33% of all cases occurred during primary processing, making it the most vulnerable area. Of primary processing cases 93% are considered counterfeit as they are produced without proper inspection, in unapproved premises or given fraudulent health certificates. This mapping of vulnerabilities along the supply chain will help to identify prevention and detection methods that are needed in order to protect products, companies and the consumer.

## Application of stable carbon isotope ratio analysis to detect edible alcohol in Chinese Baijiu

**Doctor Qiding Zhong<sup>1</sup>**, Dr. Daobing Wang, Ms. Hongwei Yue

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Chinese Baijiu is one of the world's oldest distilled alcoholic beverages that produced from cereal fermentation, distillation and months or years ageing, it its illegal to added edible alcohol into Chinese Baijiu. Measurements of  $\delta^{13}\text{C}$  of ethanol has been perfromed on kinds of products. The results showed that the edible maize alcohol gives  $\delta^{13}\text{C}$  value of  $-10.92 \pm 0.15\text{‰}$ , while the ethanol  $\delta^{13}\text{C}$  value of Chinese Baijiu ranges from  $-28.58\text{‰}$  to  $-11.21\text{‰}$ . As sensory style of Chinese Baijiu corresponding to the composition of fermentation material, the kinds and amounts of cereal used for production are generally fixed for specific products, such as Chi-flavor product, only rice can be used and the  $\delta^{13}\text{C}$  value range from  $-28.58\text{‰}$  to  $-26.4\text{‰}$ , and sorghum: wheat: rice: maize= 55:10:30:5 for Strong-flavor production with  $\delta^{13}\text{C}$  value range from  $-23.6\text{‰}$  to  $-16.58\text{‰}$ . On base of the carbon isotope databank of Chinese Baijiu, the stable carbon isotope analysis can be adopted to detect maize alcohol in Chinese Baijiu.

## Factors influencing consumer intentions to purchase traceable minced beef and beef steak

**Dr. Michelle Spence**<sup>1</sup>, Dr. Violeta Stancu<sup>2</sup>, Prof. Christopher Elliott<sup>1</sup>, Prof. Moira Dean<sup>1</sup>

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Recently, traceability labels with a quick response code have been printed on product packaging to help consumers easily access traceability information through their smartphones. However, relatively little is known about consumers' purchase intentions toward traceable food or the main psychosocial antecedents of these. We analysed consumer (n=616) attitudes and purchase intentions towards traceable minced beef/beef steak in England, and identified psychosocial determinants of their purchase using the theory of planned behaviour (TPB). Respondents in each sub-group held a general favourable attitude with positive behavioural beliefs and high trust towards the traceable product; with the majority indicating that they would be willing to pay a 5-30% price premium (over the price of the conventional product). In the TPB model, attitude was the main determinant of intention to purchase each traceable product, followed by subjective norm and perceived behavioural control (PBC). The predictive power of the model increased significantly for each sub-group when it was extended with habits, trust, and frequency of purchase. In the TPB-extended minced beef model, PBC was no longer a significant driver, and trust replaced subjective norm as the second most important predictor. In the TPB-extended beef steak model, attitude, subjective norm and PBC were all still significant drivers of intention, however, in order of importance, production process habits and origin habits were more important than PBC. These findings have importance for those involved in beef production and marketing.

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Targeted and Nontargeted Chinese Baijiu Analysis by  $^1\text{H}$  NMR  
Spectroscopy Combined with Multivariate Statistical Analysis.

**Doctor Qiding Zhong**<sup>1</sup>, Mr. Shuangxi Fan, Mr. Tonghui Yang, Ms. YINUO LIU

<sup>1</sup>China National Research Institute Of Food & Fermentation Industries, Beijing, China

Nuclear magnetic resonance (NMR) spectroscopy is evaluated as an efficient analytical technique for simultaneous identification and quantitation of multiple compounds in Chinese Baijiu (spirit). The simple and easy-to-operate sample preparation required only 900  $\mu\text{L}$  of Chinese liquor, 100  $\mu\text{L}$  of phosphate buffer (pH = 2.0) including 0.1 % TSP-d<sub>4</sub> in D<sub>2</sub>O. Targeted simultaneous quantitative analysis of compounds in  $^1\text{H}$  NMR spectra, such as esters, organic acids, alcohols (except ethanol), were achieved based on the PULCON (pulse length based concentration determination) approach. A segment-wise peak alignment was employed to handle peak misalignments of recorded  $^1\text{H}$  NMR spectra. Binning of the aligned  $^1\text{H}$  NMR spectra was performed for data reduction. The resulting bins were employed as input variables for the subsequent multivariate analysis. A significant separation of bottle storage Chinese Baijiu in different ages and flavor types was achieved using non-targeted  $^1\text{H}$  NMR fingerprinting technique combined with multivariate data analysis. The results demonstrate that  $^1\text{H}$  NMR spectroscopy has a great potential to verify the authenticity of Chinese Baijiu and is of great importance tool for Chinese Baijiu quality control and market supervision.

## Laboratory-based characterization and traceability of an outbreak of necrotising enterocolitis in China

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**Objective:** Laboratory-based characterization and traceability were performed on an outbreak of necrotising enterocolitis (NEC) in a maternal and child health care hospital in China.

**Methods:** Thirty-seven samples were collected from 3 NEC cases, wherein the clinical manifestations included bloody stools. *Clostridium* spp. isolation and identification were carried out on stool, breast milk, milk-based infant powder and environmental swab samples collected during the NEC outbreak. Meanwhile, twenty-four swab samples were also taken from the hospital ward environmental, the hands of staffs as well as articles used by neonates daily all were tested for *Clostridium* spp. after disinfection following outbreak. Pulsed-field gel electrophoresis (PFGE) analysis was performed on all *Clostridium* strains obtained. **Results:** 45.95% (17/37) of these samples were positive for a presumptive *Clostridium* spp. One type of *Clostridium* spp. was cultured from 10 samples, and was identified as *C. butyricum*. Another *Clostridium* spp. isolate was cultured from 2 samples, and it was identified as *C. sporogens*. Both of these two types were cultured from 5 samples. PFGE analysis showed that all 15 *C. butyricum* and 7 *C. sporogens* isolated from the samples mentioned above produced indistinguishable pulsotypes respectively. No NEC cases were found after disinfection following the outbreak and all samples collected after outbreak were negative for *Clostridium* spp.

**Conclusion:** The outbreak of NEC was highly related to *C. butyricum* contamination within the hospital.

**Key words:** NEC; outbreak; nosocomial infection; *Clostridium butyricum*

## High pressure vs low pressure MSM: a standardized microscopical approach to prevent food fraud

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Aim of the study was to develop a standardized microscopic method to distinguish non-MSM (mechanically separated meat) from low and high pressure MSM. The distinction is needed because, as underlined by EFSA, the risk of microbial growth increases with muscle fibre degradation and pressure separation. Sixty swine reference samples (20 high pressure, 20 low pressure MSM, 20 fresh minced meat) were prepared for histological examination, stained with HE and Von Kossa and observed by two histopathologists. 10 fields (10X) from each sample were randomly selected and automatically analyzed by NIS-Elements. Area and number of fields occupied by calcium fragments, medium number of fragments/field, medium area/fragment, medium area/field were calculated; data were statistically analyzed.

All samples stained with HE were correctly recognized as MSM or non-MSM on the basis of presence/absence of bone and cartilaginous tissues; in all MSM samples stained with Von Kossa calcium deposits were highlighted. The most statistically significant parameter for the differentiation between low and high pressure MSM was the number of fields occupied by calcium fragments (cutpoint  $\geq 4$ ; Se=100%, Sp=100%). The number of fragments for sample was also a promising criterium (Se 95%, Sp 90%, cutpoint  $\geq 10$ ).

Our approach resulted successful in distinguishing MSM from non-MSM, representing a powerful tool to disclose frauds due to unauthorised or non-declared use of MSM. Further studies will be implemented in order to validate these preliminary data to set up a reliable tool allowing the correct distinction between high and low pressure MSM through an economic and standardizable analysis.

## Food industry stakeholders' perspectives on sharing information to prevent and detect food integrity issues (FoodIntegrity)

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One of the biggest challenges facing the food industry is assuring food integrity (FI). Dealing with complex FI-issues requires a multi-dimensional approach. Preventive actions and early reactive responses are key for the food supply chain. Information sharing could facilitate the identification and prevention of FI-issues. This study investigates attitudes towards a food integrity information sharing system (FIISS) among industry stakeholders in the European food supply chain. Insights into stakeholders' interest to participate and their conditions for joining a FIISS are assessed.

A total of 119 food industry stakeholders (46% SMEs) – covering all major food sectors susceptible to FI-issues – participated in an online quantitative survey between November 2017 and February 2018 as the first round of a Delphi study. The second round of the study consists of presenting the findings to industry and other stakeholders in an online qualitative feedback survey.

Three distinct groups of stakeholders were identified based on reported frequency of occurrence of and likelihood of detecting FI-issues. Food industry stakeholders strongly support the concept of a FIISS with an attitude score of 4.49 (S.D.=0.57) on a 5-point scale; and their willingness to participate is high (81%). Consensus exists regarding the advantages a FIISS can yield towards prevention and detection. A food safety authority (74%) or a newly established organisation (84%) were believed to be the most suitable third parties to organise a FIISS. Reactions diverged concerning the required level of transparency and the type of data stakeholders might be willing to share in a FIISS.

## Food supply chain traceability based on Blockchain technology

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As an emergent technology that enables the timestamp and immutable storage of verified data, Blockchain is a shared, distributed, traceable and transparent ledger for record-keeping, inherently resistant to modification of the data. Over the past few years, it has increasingly attracted the attention of different industries. As for food industry, to secure food safety, food chain records are needed and required to be traced and audited all the way, because the outbreak of food safety risk is unprecedented in any part of food supply chain. Blockchain is very likely a silver bullet for food supply chain traceability system to guarantee the integrity of food supply chain data from field to table.

To realize the Food supply chain traceability based on Blockchain technology, ICT such as Cloud computing, IOT, Blockchain should be utilized and applied to food supply chain. Here the report will analyze the advantages and disadvantages of three applications of blockchain technology: Public blockchain, Private blockchain, and Consortium blockchain in applying the food supply chain traceability system, and briefly introduce our research and application of ICT on food safety.

## Metabolite Profiles of Emerging Anabolic Agents in Sport and Food Producing Animals

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Selective androgen receptor modulators (SARMs) are a novel class of compounds that bind to the androgen receptor, displaying tissue selective enhanced anabolic and reduced androgenic activities relative to conventional steroids. SARMs, like other anabolics, are known to affect muscle mass and may be used within food systems to increase animal productivity, and in sports to enhance performance. Many SARM compounds, such as S-22, LGD-4033 and RAD140, have been investigated for clinical therapeutic applications, and consequently can be easily sourced, with positive analytical findings reported in both human and equine sports. In this study, the profiles of SARM metabolism within equine, bovine, porcine and murine *in vitro* systems is reported and compared. Liver subcellular fractions (microsomes and S9) were isolated from different species and characterised (protein concentration, P450 content, cytochrome b5 content, cytochrome c reductase activity). Microsomal fractions were incubated with respective SARMs to generate phase I metabolites, with phase II SARM metabolites formed by inclusion of S9 preparations. Generated phase I and II metabolites were analysed by ultra-high performance liquid chromatography coupled to drift tube ion mobility quadrupole time-of-flight mass spectrometry. Collision cross section (CCS) values were calculated from acquired drift times facilitating the identification of isomeric metabolites. Considerable interspecies differences were observed within identified metabolite profiles, highlighting the need to study metabolism of emerging anabolic agents such as SARMs specifically within the species of interest. On-going work will seek to incorporate these metabolite profiles into targeted mass spectrometry techniques to screen for SARM misuse within food producing animals.

## Rapid isotope analysis of non-exchangeable hydrogen in sugar molecules derivatised with MBTFA, using GC-chromium/high-temperature-conversion-IRMS

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An rapid procedure for the isotope analysis of the non-exchangeable hydrogen in mono and disaccharides has been developed to demonstrate the feasibility of detecting added C3 and C4 sugar products in foods and beverages susceptible to intentional economically motivated adulteration and fraud. The procedure utilizes a simple one-step reaction, with the derivatising agent N-methyl-bis-trifluoroacetamide, to substitute the exchangeable hydroxyl-hydrogen with trifluoroacetate derivatives that are sufficiently volatile to be separated and measured by gas chromatography coupled to isotope ratio mass spectrometry. The conversion of the derivatised sugars into the measuring gas is achieved using a high temperature chromium reactor that retains carbon, oxygen and fluorine whilst releasing hydrogen gas for stable isotope measurement. The new procedure has advantages over methods using nitro-sugar derivatives and degradation products, such as hexamethylenetetramine and calcium formate, in terms of ease of use, analysis time and sensitivity. The differences between the  $\delta^2\text{H}$  values of the non-exchangeable hydrogen in sugars from fruit juices and honey and those from beet and cane sugars/syrups permits the presence of these potential adulterants to be rapidly detected.

## Beef, glorious beef – where do you come from?

**Katharina Heinrich**

Consumers in the UK are increasingly interested in regional foods. The reasons for this vary from (a) patriotism, (b) decreased confidence in the quality and safety of food produced outside their local region or country, (c) characteristic organoleptic or culinary qualities or (d) concerns about 'food miles'.

The EU introduced beef labelling legislation in two stages, firstly on the 1st September 2000 (Regulation (EC) No 1760/2000 of the European Parliament) to initiate a system providing correct, complete and transparent information; designed to enable consumers to make an informed choice about retail beef in the marketplace and also to enable enforcement agencies to trace back retail beef to where it originated. The second stage came into force on the 1st January 2002 and required the inclusion of more precise information relating to where the source animal of retail beef had been born and reared.

In view of the above, the first British Beef Origin Project# (BBOP 1, FA0205) developed a database for verification of beef origin using multi-isotope and multi-element analysis. After completion of this project and review of the analysed samples, a lack of stable isotope data of authentic beef samples from certain regions, within the UK, were identified. The following projects – BBOP 2 (FA0152) with sampling in East Anglia, the Midlands and the South East of England; Scottish Beef Origin Project (SBOP, FS515009) with sampling in Scotland and Northern Ireland - were funded to address this issue.

The sampling strategy, analytical data and their statistical evaluation, in form of a web tool for monitoring routinely Scottish beef origin claims, will be presented.

#<http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=18069>

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## Carbon Isotope Abundances in the Authentication of Vinegar and Detection of Synthetic Acetic Acid Adulteration

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Carbon Isotope Abundances in the Authentication of Vinegar and Detection of Synthetic Acetic Acid Adulteration

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Fraudulent adulteration and or misrepresentation have been a problem for commercial vinegar in the Philippines. In this study, authentic vinegar samples from: sugar cane (acetator and conventional type of fermentation), coconut sap ("tuba"), rice, pineapple juice, and mango juice and commercial vinegar were obtained and calcium acetate was produced from each samples. Portions of the calcium acetate were reacted with orthophosphoric acid to recover glacial acetic acid (GAA) and the C14 activities (disintegrations per minute per gram carbon or dpm/g C) of GAAs were measured in a Tri-Carb 3180 TR/SL Liquid Scintillation Counter. Biogenic samples exhibited 12-15 dpm/gC activities, while synthetic samples showed 0-2 dpm/gC activities. The remaining portions of the calcium acetate were used to measure the <sup>13</sup>C/<sup>12</sup>C ratios (standardized against the International Pee Dee Belemnite Standard). Delta values ( $\delta^{13}\text{C}$ ) of acetic acid obtained from C4 plants including sugar cane, and pineapple were between (-12.2) to (-15.9) per mil; mango, a C3 plant, gave (-21.1) per mil; coconut sap (-23.3) and rice (- 26.3). Synthetic/petroleum derived vinegars exhibited delta values beyond (-30) per mil. Isotope ratio mass spectrometry and liquid scintillation counting are promising tools for revealing the botanical origin and method of production, and detection of synthetic acid adulteration in vinegar samples.

## Exploring Chinese consumers' perceptions of food fraud and traceability within the EU FOODINTEGRITY PROJECT

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The aim of this study was to gain insights into consumers' cognitions with regard to the authenticity, traceability and safety of foods that have been subject to fraud in China. In-depth one-to-one interviews were held during January 2015 with 20 middle/upper class Chinese consumers of mixed gender, educated to university level, and aged between 25-43 years. Interviewers defined food authenticity/inauthenticity before using a two-step questioning technique. Participants first considered nine food products in triads, arranged them in descending order in terms of their ease to be made inauthentic, and named all the ways (i.e. attributes) in which they could be made inauthentic, before rating how hard/easy it would be to make each food product inauthentic in this way. Secondly, participants took part in a laddering exercise to uncover how they linked food product attributes to consequences and their own underlying life values. Information collected during the interviews was content analysed, aggregated and interpreted through a hierarchical value map. "Imitation/fake products" was the most frequently laddered attribute (i.e. way that food could be made inauthentic), followed by "mixing/substitution" and "improper/absent certifications", then "adding chemicals/growth hormones", and "changes related to packaging". The most important consequence which participants linked to four of the attributes was "health", which in turn was linked to "well-being/quality of life" and "helps to manage everyday life", both of which were linked to "relationships with others (including family)", and finally "happiness". Findings have important implications for the development and marketing of authentic foods.

## Proteomic tools in campaign against fraud in infant formula

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The melamine scandal exploded in 2008 was a catastrophe to Chinese local dairy industry. It revealed public awareness toward food safety and fraud issues that were never faced before, also it has become the first priority of our government ever since. Melamine was a nitrogen rich compound aiming to cheat GB standard for infant formula protein content based on single Kjeldahl nitrogen method at the time. With ten years' series of effort, current situation has improved. Meanwhile, new terms of adulterations appear in infant formula including replacing whey proteins by using cheap bovine milk caseins, using plant sourced (Soy proteins) or digested protein (Not for special group's clinical use) instead of animal sourced proteins, potential risk from unknown nitrogen rich melamine-like compounds. With the development of modern analytical technologies, targeted proteomic tools based on LC-MS made it possible for scientist to precisely measure protein contents in milk qualitatively and quantitatively. By introducing stable isotope labeled signature peptide, system error generated from ionization caused by matrix effect could be minimized. So far, we have already established quantification methods for bovine caseins and whey proteins consisting  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and Lactoferrin. Our ultimate goal is to develop LC-MS strategies for all milk fraud detection, we hope we can share our experience via research project Horizon 2020 with our partners in E.U. or U.S. and eventually reach a mutual recognized global standard.

## Drivers of (Un)ethical Behaviour in Agricultural Value Chains: Evidence from Uganda

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Synopsis: We present findings from an on-going 3 year research project titled “Strengthening Agribusiness Ethics, Quality Standards & ICT Usage in Uganda’s value Chains” (AGRIQUEST). Evidence was gathered through exploratory field research carried out in the rice and cassava value chains in Uganda. The study aimed at understanding the drivers and unfolding processes of unethical behaviour in agricultural value chains. Impact: Our research shows that competing self-interests by farmers, low degrees of collaboration, ease to avoid sanctions and a short term-orientation in business relationships are the key drivers of unethical behaviour in agricultural value chains. To mitigate these problems, we find however that in such informal smallholder contexts characterised by institutional voids, farmer networks can emerge as supplementary informal institutions. We propose a framework for contextualisation of Western-based Corporate Social Responsibility (CSR) practices through “local voices” that facilitates integration in economic development plans, addressing societal root causes and providing on-site support. By teasing out the drivers of unethical behaviour and elucidating its socio-economic and cultural context, we provide a premise for more effective programming and greater efficiency of both local and international food value chains. Fit: The selected theme aims to understand the growing threat to global food system integrity and formulate a call for action to build robust global food defence systems. As we examine potential threats “at their source”, our findings and recommendations have a direct impact on the global food system into which most food produced in developing countries is exported.

## Facing ongoing challenges in the vanilla flavor authentication by improved isotopic ratio analysis

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Vanilla aroma is one of the most expensive, popular and widespread flavors in the food industry. The price level in 2017 reached 500\$ per kg vanilla pods or even higher for premium quality. Vanillin, the most characteristic compound in vanilla aroma can be produced by much cheaper methods like chemical and biotechnological synthesis. The price for the latter ones is 16-20 \$/kg and 500-1000 \$/kg for pure vanillin, respectively. The willingness of the consumer to pay a higher price for natural products in combination with the disparity of prices has led to many cases of economically motivated fraud. For more than 30 years efforts have been made to authenticate the origin of vanillin, whereas the assessment of the stable isotope carbon ratio is widely used. However, there are several cases where compound specific analysis shows restrictions. Synthetic vanillin can be enriched in Delta13C and thereby produce synthetic material with the same Delta13C values as natural vanillin. Moreover Delta13C values for biosynthetic and synthetic vanillin can overlap depending on the used precursor. An attempt to determine the traceability of different vanilla pods by compound stable isotope analysis was furthermore limited. More important information is available by the additional determination of carbon and hydrogen stable isotope values of the vanillin methoxyl group. In this study, this position specific isotope distribution of hydrogen and carbon for a dataset of vanilla pods from 14 different geographical origins, reflecting natural heterogeneity, has been analysed by IRMS to face current challenges for the vanilla authentication.

## Authentication of cow milk using stable isotopes analysis to ensure consumer safety in Sri Lanka

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The dairy sector contributes to the economy of Sri Lanka by way of employment generation and reducing nutritional poverty. Cow milk is vulnerable to a range of food safety hazards that may arise at any stage from production to marketing. Previous studies have shown that stable isotope ratios in food commodities can be used to verify the authenticity (geographic origin and adulteration). The objective of the present study was to establish baseline stable isotope data for authentic milk samples from four different milk production zones (namely dry zone, coconut triangle, mid country and upcountry) to determine the geographic origin. A total number of 135 milk samples was collected from the above milk production zones. Milk fat, casein and whey fraction were separated from each sample and freeze-dried separately. The stable isotope ratios of carbon and nitrogen and percentage of carbon and nitrogen were assessed for whole milk, milk fat, casein and whey by Isotope Ratio Mass Spectrometry (IRMS). Linear-Discriminant Analysis (LDA) was used to separate the four milk production zones. A model generated by LDA classified 96 % of upcountry samples, 92 % of dry zone samples, 78 % of mid-country samples and 75 % of coconut triangle samples correctly. There are ongoing efforts to develop this model to determine adulteration in cow milk. The authors acknowledge the financial and technical support received from the International Atomic Energy Agency, Vienna.

## Authentication of apple and strawberry aroma compounds using stable isotope approach

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The present work deals with the authenticity of natural flavourings, which are commonly falsified by knowingly providing a false declaration of origin. Initial results show that gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) analysis of key volatile compounds using headspace solid phase microextraction (HS-SPME) is an appropriate tool for authenticating apple and strawberry recovery aromas. The developed procedure contains several steps, including sample and standard selection, sample preparation, compound identification,  $\delta^{13}\text{C}$  measurements, data processing, database creation and authenticity assessment. Since many different compounds in various concentrations are present in a single sample, the selection of reference material and an appropriate method for processing data and interpreting the results is crucial. Analysis of commercial recovery aromas, labelled as natural, revealed that the  $\delta^{13}\text{C}$  value of the majority of the compounds present was within the expected authentic range. The data also revealed some possible falsifications of mainly strawberry samples. To gain more confidence in the data collected, an extensive database, which is under construction, is required. Our data also reveals some differences in  $\delta^{13}\text{C}$  value between natural apple and strawberry recovery aromas, which requires further investigation. Although the method was developed to differentiate between natural and synthetic apple and strawberry aroma compounds it can be easily transferred also to other commodities.

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## A Selective LC-MS/MS Method for Multiple Meat Speciation and Authenticity Detection in One Injection

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In 2013, horse and pig DNA were identified in beef products sold in several supermarket chains. This type of contamination not only misleads the consumer, but has health, religious, and ethical implications. Hence, it is imperative that analytical methods are sensitive and accurate enough to screen for the presence of meat adulteration in food products.

Traditionally, PCR and ELISA are used for meat speciation. Although sensitive, PCR is limited by the degradation of DNA in processed meat. ELISA is susceptible to cross-reactivity which can lead to false positive or false negative results, and lacks of multiplexing capability. Hence, LC-MS/MS provides an excellent alternative to these methodologies to identify and confirm different meat species with more accuracy and reliability.

In this work, we present a sensitive and robust LC-MS/MS-based meat speciation workflow for detecting pork, beef, lamb, chicken, duck and horse. The optimized sample preparation procedure is easy to follow and can be used for analyzing raw, cooked and processed meat products. Signature marker peptides unique to each species were identified and verified to ensure that they don't present any cross-species reactivity. Currently, the method is able to reliably detect each meat species at a detection limit of 1% on the QTRAP<sup>®</sup> 4500 system.



## Sensitive and Specific Allergen Screening Analysis Using LC-MS/MS

**Dr. Ashley Sage<sup>1</sup>**, Dr Jianru Stahl-Zeng<sup>1</sup>

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Allergens in food can result in severe or fatal reactions. At present, there is no known cure for an allergenic reaction and the only thing a person can do is to avoid the potential cause eg nuts, milk etc. Food allergens are of increasing interest due to food allergy recalls that have doubled in recent years. To help safeguard consumers from food related allergies, warning labels on packaging and in restaurants are a must these days to allow people to make a decision on what they eat. However, food testing is also important and as a result, it is vital to have a robust and specific analytical method to reliably identify and quantitate allergens that may be present. This work presents data from a method that has been highly characterized and verified to determine several different types of allergens using a tryptic digest and LC-MS/MS analysis to measure allergen peptides with a high degree of flexibility, specificity but also with high sensitivity

Samples were trypticly digested and the resulting extracts analyzed using LC-MS/MS. Peptides that were identified to be associated with a specific allergen were measured using electrospray ionization and scheduled MRM data acquisition. A comparison between traditional methods and the LC-MS/MS will be described. Important considerations for method development will be discussed. The ability of the method to identify allergens and to quantify them in food samples will be presented and discussed. Results from several different food matrices will be presented to demonstrate the potential of this method.

## STUDY OF STABLE ISOTOPES AND TRACE ELEMENTS COMPOSITION OF POLISH DAIRY PRODUCTS

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The aim of this study was to demonstrate the differences in regional and seasonal variations of isotopic and trace elements composition of Polish milk and dairy products. The samples of fresh and commercial milk were gained from main regions of milk production in Poland and measured by the use the isotope ratio mass spectrometer. The mass spectrometry technique (IRMS) is the main method for measurement of the stable isotopes content in food and beverages. Oxygen isotope ratio was determined in water samples by Gasbench instrument connected with a mass spectrometer. For the determination of C, N and S in solid materials Elemental Analyzer coupled with a mass spectrometer was used. The measurements of trace elements concentration were performed on ICP-MS. The work is continued for the bigger population of dairy products samples. The final aim of the study is to construct the database of stable isotopes and trace elements compositions of Polish dairy products. By the use the database for authenticity control of dairy product, the isotopic method will be implemented for everyday practice.

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# The threat from pathogens to the food system

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## DNA analysis of micromycetes producing patulin and ochratoxin A in food

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Food can be infected with micromycetes during its production. Micromycetes not only spoil food, but many strains can produce toxins. Patulin and ochratoxin A (OTA) are hepatotoxic, immunosuppressive and potentially carcinogenic mycotoxins. They are mainly produced by micromycetes of family *Aspergillus* and *Penicillium*. Mycotoxins are a big threat in food industry, therefore, it is necessary to find effective methods for their detection. Named micromycetes can be detected also by molecular-biological methods. This work is focused on Polymerase chain reaction (PCR) and Loop-Mediated Isothermal Amplification (LAMP). The aim of this study was to design PCR and LAMP protocols that allow to safely distinguish whether micromycetes are capable of producing patulin or OTA. Since a synthesis of mycotoxins is typically a multi-step process, key enzymes of the biosynthetic pathway of both mycotoxins, namely polyketide synthase (PKS) for OTA and isoeopoxydone dehydrogenase (IDH) for patulin, were selected. The DNA obtained from micromycetes UCT collection was isolated. A protocol for PCR amplification and then a LAMP protocol for the detection of the genes encoding the key enzymes of the biosynthetic pathways of both mycotoxins were designed and experimentally verified. Results of this study show that DNA analysis has a huge potential to be useful for pathogens detection in food.

## Limits of enumeration using EMA/PMA-qPCR

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Although quantitative real-time PCR (qPCR) is a very effective tool for identification and quantification of diverse microorganisms posing a threat to public health, one of its key drawback is the inability to distinguish between signals originating from dead, live or VBNC cells. This is significant limitation when qPCR is used to quantify live infective agents in direct food control and food processing industry, and it can easily result in false positive outcome or overestimation of live cells.

Pre-treatment of the analysed sample with DNA intercalating dyes, such as ethidium (EMA) and propidium monoazide (PMA), is widely used solution to reduce this issue. However, the efficacy of such approach is among others based on the ability of EMA/PMA to penetrate through cell membrane, which in this case defines cell viability. It is obvious that there may be some deviations in quantification caused when different killing conditions are applied. Therefore in this study, a killing assay was performed, evaluating the impact of several cell inactivation techniques with various modes of action on qPCR results.

As a model bacteria were chosen main thermotolerant *Campylobacter* spp. (*C. jejuni*, *C. coli*, *C. lari*), which are recognized as a leading human food-borne pathogens worldwide. They were inactivated in mixed suspension by a panel of eight lethal and one sub-lethal procedures, which bacteria can encounter during their life span and then quantified using previously designed EMA/PMA-qPCR. It was shown that not only various inactivation mechanisms affect the efficacy of qPCR but species-specific differences were apparent as well.

## Multi-species biofilms of significant bacterial pathogens

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Bacteria during its existence invented a variety of ways to adapt in the environment. Permanent microbial modification gave the emergence of formation called biofilm, whose composition and structure provides advantages manifested by changes in the phenotype and physiology of microbial populations, high stability and resistance. In the environment the prevalent form of life are multi-species biofilms, which are compared to mono-species biofilms more complex, virulent, stable and resistant. Coexistence of the various microbes in this type of biofilm is accompanied by a number of interactions that affect the overall distribution, biomass and contribute to the increased resistance. These multi-species communities can be a beneficial part of the human microflora. In the industry bacterial biofilm can contribute for example to processes of bioremediation, the production of biofuels or preserving food. However, the ability of the formation of a biofilm has also some pathogenic microorganisms, such as *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*, which can become a source of human diseases. Biofilms of these bacteria were detected in the dairy industry, the beef plants, factories with seafood and fish or on the medical equipment. The characteristic biofilm stability, the inability of the detection by classical cultivation techniques and resistance to the antimicrobials has become a major problem in many branches. In this work classical microbiologic method, molecular-biologic method and confocal scanning microscopy was used for study of multi-species biofilms of foodborne bacterial pathogens.

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## Analysis of noroviruses in food and water by PCR with fluorescence detection

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Noroviruses are the group of non-enveloped single-stranded RNA viruses and are considered to be significant agents of gastroenteritis. The number of people infected by noroviruses increases every year. Infection is characterized by diarrhea, vomiting, and stomach pain. Noroviruses are transmitted primarily through the fecal-oral route, either by direct person-to-person spread or through contaminated food or water.

Therefore, it is necessary to find an effective method for noroviruses detection. The determination of noroviruses in food and feed is described in the methodology in ČSN P CEN ISO/TS 15216. This methodology is based on polymerase chain reaction with fluorescence detection in real time (qPCR), which became a very often used molecular-biological method in many fields. This qPCR method precedes the isolation of RNA and transcript of isolated RNA using reverse transcription into cDNA. Whole methodology is a very time and money demanding. In our study, we modified and experimentally verified this methodology. Samples of strawberries and mussels from market network of the Czech Republic were analysed by optimized methodology. Noroviruses of genogroup GI or GII were not detected in any tested samples.

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## Formulation and evaluation of a phage based 'cocktail' as control tool for antimicrobials in vegetables

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Destructive soft rot Enterobacteriaceae, including Pectobacterium, Dickeya and Pantoea, affect a number of plants including vegetables and fruits, causing high economic losses for producers and a potential health hazard through the enhanced proliferation of pathogens dangerous to human health such as E. coli and Salmonella. There is currently no treatment for soft rot Enterobacteriaceae, and control is largely based on the use of sanitary growing practices. The increasing number of epidemics in recent years caused by soft rot pathogens in Europe indicated a need for the formulation of commercially available and effective biocontrol measures to counteract these pathogens. Highly specific bacterial viruses known as bacteriophages have been investigated by researchers as biocontrol tools to treat bacterial diseases. Scientists could utilize novel bacteriophages with unique properties to formulate effective control tools for growing bacterial resistance. Bacteriophages isolated from processing water have been identified using transmission electron microscopy (TEM) and Next-Generation Sequencing (NGS) and tested for antimicrobial activity. Bacteriophage 'cocktails' against soft rot Enterobacteriaceae occurring in potato and onion have been developed and assessed through bioassays and field trials. It has been shown that the phage 'cocktails' decreased soft rot symptoms and increased yields in potatoes and onions in vivo compared with the positive control. This work shows that phage-based formulations could be developed as an effective future biocontrol to prevent bacterial diseases in plants or food.

## Early detection of plant diseases using Remote Sensing

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Plant pathology is increasingly important in changing climates to ensure a sustainable food supply system. Novel surveillance systems enabling early detection for plant pathogens that cause damage or disease are an area of interest in the era of precision farming. Certain pathogen infections in plants are reported to alter the water content and chemical composition of the plant. Research has demonstrated that alterations such as these can cause shifts in the reflected light within ranges which are observable through Thermal Infrared imaging. It is postulated that these changes occur before visible symptoms of disease arise whereby infrared imagery could be utilised as a potential early detection method for plant diseases.

This aim of this project is to evaluate the feasibility of infrared imagery as an early detection system using model plant species, with a view to linking the technology with unmanned aerial vehicles (i.e. drones) coupled with geospatial analysis enabling efficient remote management operations. Three different model plant species, as control and treated groups, were evaluated under either conditions of physical damage or infection with the plant pathogen *Phytophthora ramorum* to evaluate the theory of the use of infrared imagery to detect changes in plant health. The infrared images were collated over a defined time period and the data generated was compared to visible observation of plant health status. The current literature will be discussed to suggest a technical road map that could be created for the application of this technology in offering a novel remote tool for surveillance.



## Isothermal amplification of nucleic acids for rapid and multiplexed detection of antibiotic-resistance in *Campylobacter jejuni*

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*Campylobacter jejuni* is a food-borne pathogen commonly found in poultry industry, and has been the main cause for gastroenteritis, incurring high medical cost and childhood morbidity worldwide. Due to the heavy use of antibiotics in the past, antibiotic-resistance in *Campylobacter jejuni* has been developed in many countries such as Spain, United States, Egypt, Hong Kong etc. In 2017, WHO has categorised antibiotic-resistant *Campylobacter* as high priority pathogens list. It is important to detect the emergence of antibiotic-resistance in countries where the resistance have yet developed. In addition, this would help better prescription by medical practitioner prior to treatment. In this study, recombinase polymerase amplification was developed to detect *C. jejuni*, and to identify if the pathogen is resistant to antibiotics. Particular interest was initially placed in the amplification of tetracycline-resistant (TetO) and ciprofloxacin-resistant (*gyrA*) genes in *C. jejuni* clinical isolates obtained in Spain. The results show that the amplification can be carried out in 10 min under isothermal condition below 40°C, and optimisation of multiplex amplification, sensitivity and specificity test against other bacterial strains such as *E. coli* and *L. monocytogenes* were carried out. Future work includes the transfer of the assay onto a paper-based analytical device that results in a portable device that is suitable for low resource settings.

## Northern Ireland's Largest E. coli O157 Outbreak, Belfast, 2012

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The largest outbreak of Escherichia coli O157 to date in Northern Ireland occurred in October 2012 with 141 clinically confirmed cases. E. coli O157 is a highly infectious bacterium that can cause illness in humans including potentially fatal complications. There were no deaths but nineteen people were hospitalised. The outbreak was associated with Flicks Restaurant which closed voluntarily and this successfully controlled the outbreak.

Descriptive and epidemiological studies were undertaken. The case control study found exposure to chopped parsley was significantly associated with illness, although there were significant caveats. Analysis by Food Handler likely to have garnished the food showed a statistically significant association between being a case and having a meal garnished by 'Food Handler 1', although there were again significant caveats. Typing of isolates from patients confirmed the outbreak strain was very different from the other sporadic NI strains.

Although no E. coli O157 was found in any of the 155 samples there was evidence to suggest faecal contamination in three foods including the ready-to-eat parsley. EHOs observed food hygiene contraventions and the Food Business Operator pleaded guilty to 11 offences in Belfast Crown Court (fined £110,000).

It was not possible to definitively determine the source of the outbreak but WGS results suggested a greater likelihood that contaminated food was the source than a colonised food handler. Food was the vehicle of spread within the restaurant with some evidence to implicate parsley.

The Outbreak Control Team made several recommendations and reinforced existing advice relating to E. coli O157.

## Next-generation sequencing in microbial food risk assessment: are we there yet?

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Despite the ever increase in rigorous control and monitoring measures to assure safe food along the entire farm-to-fork chain, the past decade also witnessed an increase in microbial food alerts. Hence, research on food safety and quality remain of utmost importance. Complementary, at least as important, is the necessity to be able to assess the potential microbial risks along the food chain.

Risk assessment relies on sound scientific data. Unfortunately, often, quality data is limited if not lacking. High-throughput tools such as next-generation sequencing (NGS) potentially could fill this gap. NGS applications are not new in the field of food microbiology with applications ranging from pathogen detection along the food chain, food epidemiology studies, whole genome analysis of food-associated micro-organisms up to describing complete food microbiomes. Yet, its application in the area of microbial risk assessment is still largely unexplored and faces important challenges.

The possibilities of NGS for risk assessment are ample, but so are the questions on the subject that need to be addressed. In this work, we present a state-of-the-art review on the application of NGS in relation to microbial food risk assessment. One of the major strengths of NGS lies in its capacity to generate a lot of data, but to what extent can this wealth be of use in the framework of hazard identification, hazard characterization and exposure assessment to perform a sound risk characterization which in turn will allow to make evidence based risk management decisions.

## DNA sequence-based re-assessment of archived *Cronobacter sakazakii* isolated from dairy imported into China between 2005-2006

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*Cronobacter* species (previously called as *Enterobacter sakazakii*) are associated with severe foodborne infections in neonates and infants, with particular pathovars associated with specific clinical presentations. This study re-analyzed, using multi-locus sequence typing (MLST) and whole genome sequence with single nucleotide polymorphism analysis (WGS-SNP), 52 strains which had been identified as *Enterobacter sakazakii*. These strains had been isolated from dairy imports into China from different countries between 2005-6. Bioinformatic analysis was then used to analyze the relatedness and global dissemination of these strains. *FusA* allele sequencing revealed that 49/52 strains were *Cronobacter sakazakii*, while the remaining 3 strains were proved to be false positive. The *C. sakazakii* strains comprised of 8 sequence types. The predominant sequence type was ST13. WGS-SNP analysis of the 32 *C. sakazakii* ST13 strains revealed 5 clusters and 5 unique strains. The mis-identification of 3 isolates as *Cronobacter* spp. reinforces the need to apply reliable methods to reduce the incidence of false positive and false negative results which may be of clinical significance. The WGS-SNP analysis demonstrated that indistinguishable *Cronobacter* strains within a sequence type can be unrelated and may originate from multiple sources.

## Uncovering the strategies within the decision-making framework of food risk analysis in the Nordics

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Our paper addresses issues surrounding public health risks and how different management regimes can contribute to food safety. We compare the Norwegian food safety governance system with Sweden's which is mainly based on EU rules, while Norway is committed to follow these rules through the EEA Agreement. In doing so we consider variations of risk management strategies both across national borders (thus taking into consideration differences in regulations, policies and culture) as well as variations in daily routines (i.e., no outbreak present) vs. situations of crisis (i.e., an outbreak is recognized). We focus specifically on the area of meat hygiene and study how risk analysis frameworks are designed and how they perform, as well as on how outbreaks of food-borne illnesses have been managed. The analysis follows the qualitative paradigm with in-depth extensive interviews with key stakeholders in Norway and Sweden, covering several key areas in food safety governance and politics, including: the design of adequate risk analysis frameworks, identifying key factors involved in decision-making and how to shape these designs (economic, legal, political-cultural etc.), identifying actors involved in developing the strategic risk assessment protocols and possible interplay among formal/informal governance structures, external vs. internal risks in the organizations (e.g., systemic risks, emerging risk areas, other external factors like regulations), risk monitoring and reporting activities, information and communication (e.g., sharing information, communicating the strategic risk profile and risk management plan, communication across the organization, communication with stakeholders – i.e., regulators, agencies, shareholders etc.).

## Control of nematode parasites in livestock under climate change

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Parasites impact negatively on growth rates and milk production in grazing livestock, and their control relies heavily on anthelmintic drugs. Many parasite species have free-living stages outside the host whose development, survival and availability are strongly influenced by climatic factors. As infection patterns change and become more variable under climate warming, tools to predict and avoid high risk situations are needed in order to better target treatment and maintain disease control. Predictive models were developed for the spring scour worm of lambs, *Nematodirus battus*, and used successfully to advise farmers on optimum treatment times. Models for other nematode species are more difficult to translate directly into decision support tools, but give insights into altered seasonal patterns under climate change and more efficient control strategies. For example, predicted future species distribution and key times of transmission risk can inform priorities for the development of alternative control approaches including vaccines and breeding for parasite resistance. Outcomes will be reduced chemical use in grazing livestock and delayed development of anthelmintic resistance, which threatens to limit control options in future.

## A case study in using NGS to track microbial pathogens in dairy manufacturing facilities

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Next generation sequencing is increasingly being used as a tool to characterise microbial pathogens present in the food chain. *Cronobacter* spp. is an opportunistic pathogen widely associated with powdered infant formula. More recently, other dairy manufacturing facilities producing dairy ingredients have become concerned with *Cronobacter* spp. as their products may be included as ingredients in infant formula. This work describes the outcomes of surveillance programmes for *Cronobacter* spp. in two large scale dairy ingredient manufacturing facilities. Environmental swabs and product samples were taken frequently from the facilities over a period of several months. These samples were initially tested for *Cronobacter* spp. using the ISO 22964 (2017) method and positive samples were confirmed using RT-PCR. These samples were then sequenced on an Illumina HiSeq platform. Initial MLST analysis based on the sequence data typically indicated more than one sequence type present in the facilities during the surveillance time frame. Preliminary core genome analysis of samples from within the same sequence type using ROARY indicated a low level of genetic diversity. Provisionally, this indicates a degree of persistence of the strains within the process facility over the surveillance period. On several occasions, the sequence data combined with other metadata successfully pinpointed the source of contamination within the process facility. The studies demonstrated that combining the NGS data with the detailed temporal and spatial metadata associated with the samples proved a valuable tool in identifying the source / transmission and persistence of microbial pathogens in the process environment.

## A 16S rDNA-based Sequencing Study on the Microbiome in Irish Powdered Infant Formula Production Sites

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**Aim:** Microbial safety is influenced by the microbial composition of raw materials and the production environment. This influences the quality and safety of the final powdered infant formula (PIF) product. Up to now no research has emerged that describes the environmental microbiome of a PIF factory production site. The aim of this study was to describe the environmental microbiome of two local PIF production sites based on geographical and temporal differences, and generate data to support risk control measures.

**Method:** Two PIF factories producing the same PIF brand located in Ireland were chosen for environment sampling, with sampling areas selected to include the low- (LC), medium- (MC) and high-care (HC) areas within these factories. Genomic DNA was extracted from environment samples collected and then sequenced using a paired-end, 300 × 2 bp 16S rDNA sequencing protocol on a MiSeq platform.

**Results:** A total of 60 samples were collected from the two factories during the two-year environment sampling, and the sequence data generated demonstrated microbial composition differences in the care areas sampled. Genera including *Acinetobacter*, *Pseudomonas* and other unclassified *Pseudomonadaceae* were common across the factory. *Halomonas* were found in high prevalence in the LC area, *Pseudoxanthomonas* and *Stenotrophomonas* accounted for high percentages in the MC area, whilst the HC area has a high abundance in *Streptococcus*, *Lactococcus* and *Staphylococcus*.

**Conclusion and Prospects:** 16S rDNA-based sequencing provided an overall description of the bacterial population colonizing in the PIF production environment, and provided information that may assist in pathogen control.



## Proteolytic and Lipolytic Activities of Pseudomonas spp. Isolated From Raw Milk in Mekong Delta, Vietnam

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Pseudomonas spp. plays an important role in milk spoilage. During the storage of raw milk they produce many thermo-tolerant lipolytic and proteolytic enzymes that reduce both the quality and shelf life of processed milk. This study focused on bacteria isolation and characterization of biochemical properties, then determined proteolytic and lipolytic activities of Pseudomonas spp. and identified the strongest proteolytic and lipolytic activities of Pseudomonas spp. by 16S rRNA genes sequencing. Fourteen bacterial strains belonging to Pseudomonas spp. were isolated from ten raw milk samples from the cow-milk stations and cow-milk farms from TienGiang, CanTho, SocTrang provinces in Mekong Delta, Vietnam. Among 14 isolates, nine isolates had proteolytic activity, while ten isolates had lipolytic activity and eight isolates had both. ST3 isolate had the strongest proteolytic activity and was 100% of identify with Pseudomonas putida strain Seab04 16S rDNA. CT3 isolate had the strongest lipolytic activity and was 100% of identify with Pseudomonas putida strain BASUP8716S rDNA.

Keywords: Pseudomonas, proteolytic, lipolytic, spoilage, contamination, raw milk

## Characteristics of carbapenem and colistin-resistant Enterobacteriaceae from diarrheic patients and the food chain in China

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Foodborne disease caused by the consumption of food contaminated with microbes is a serious and constant threat to public health. The contamination of food with antimicrobial resistant bacteria such as carbapenem- and mcr-resistant Enterobacteriaceae is a potential risk for consumers and food handlers. This study was conducted to investigate the prevalence and genetic characteristics of CRE- and MCR-producing isolates from diarrheic patients and food chain in China. Carbapenem- and mcr-resistant Enterobacteriaceae were screened by standard cultural methods. Isolated strains were further characterized by molecular based methods and whole genome sequence (WGS). The diversity of resistance encoding genes to carbapenem was identified in the isolates from diarrheic patients and the food chain. The most common type found was blaNDM-5 followed by blaNDM-1, blaNDM-7, blaNDM-9 and blaKCP-1. Only mcr-1 and mcr-3 genes were detected and six isolates harbored both and originated from pig slaughterhouses. Interestingly, ~40% isolates were co-resistant to carbapenem and colistin and all were cultured from the food chain. Most of these antibiotic-resistant genes were located on mobile genetic elements being identified by conjugation and transformation. This study provides further evidence that meat must be considered an important source of carbapenem- and MCR-producing Enterobacteriaceae and whole genome sequence of the isolates showed some clones appeared to have existed for several years and had been disseminating between humans and food-producing animals. Their subsequent evaluation will provide the basis for scientifically sound risk assessment. Ongoing surveillance by regulatory agencies is required to identify routes of transmission.

## Weissella Cibaria NN20 Isolates Sequesters Afb1 Invitro, Ferment Milk with Good Viscosity as yogurt

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Fermented foods prove many benefits for human health. The purpose of this study was to determine if these strains sequestered aflatoxin B1 (AFB1) invitro. We isolated 300 gram positive lactic acid bacteria (LAB) from Kimere a fermented food product from Eastern Kenya. Sixteen strains were identified for further investigation. The maximum survival in gastric juice, captured by measuring optic densities spectrophotometrically at 600nm was for Weissella cibaria NN20 isolates in comparison to positive control probiotic Lactobacillus rhamnosus GR1 and negative control Escherichia coli GR12 (1.515±0.132, 1.459±0.085, 1.442±0.047 respectively). There was similar survival of Weissella cibaria NN20 compared to Escherichia coli GR12. Binding with AFB1 was found to be slightly better for Weissella cibaria NN20 (43.7±2.3 %) than Lactobacillus rhamnosus GR1. The final pH, viscosity and general organoleptic acceptance compared to fermented milk with traditional yogurt starter cultures (P>0.05).

Keywords: Weissella cibaria NN20; yogurt; Kimere; AFB1

## Antimicrobial resistance among bacterial agents from food animals in a developing country: the Nigerian experience

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According to the World Health Organisation, antimicrobial resistance (AMR) is an increasingly serious threat to public health globally and requires action across all government and society. This threat results from the fact that new resistance mechanisms emerge and are spread globally leading to the emergence of untreatable infectious diseases, prolonged illnesses, increase health care cost and increase mortality. Although AMR develops naturally over time, usually via gene mutations, inappropriate use of antimicrobial agents has contributed in accelerating the evolution of AMR. Antimicrobial use in food animals, particularly poultry, swine and cattle, has been reported to play significant role in the development of AMR in food-borne commensal and pathogenic bacteria, with eventual spread of these resistant bacteria to humans via consumption of contaminated meat/meat products or handling of animals or meat/meat products. My research group at the University of Nigeria, Nsukka, Nigeria, in collaboration with the research group of Prof. Carmen Torres at the University of La Rioja, Logrono, Spain have been working on the AMR in bacteria of food animal origin for a couple of years. Our experience has shown that a very high percentage of enteric bacteria from these food animals are resistant to ampicillin, tetracycline, streptomycin, and sulphamethoxazole-trimethoprim. Isolation of fluoroquinolone- and cephalosporin-resistant bacteria is on the increase. Multidrug-resistant Gram positive (particularly staphylococci) and Gram negative (particularly *Escherichia coli*, *Klebsiella* and *Ochrobactrum* species) bacteria are a common occurrence in food animals in Nigeria. We have elucidated the molecular mechanisms of resistance in these bacteria.

## Scanning and remote sensing technologies for biosecurity

**Dr. Francisca Aba Ansah<sup>1</sup>**

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Being a self-motivated researcher, entrepreneur and development worker, I have worked with farmers, small to medium scale food processors and large-scale Agri-processing industries in various capacities. As part of my doctoral research in the Management of Innovations in Agriculture and Food systems, I expanded my experience portfolio working with Agri- industries in Italy and USA to determine the impact of the technologies used and processing steps on quality (including microbial quality) of fresh-cut leafy vegetables. Prior to my Ph.D., I also worked as an Agricultural Livelihoods' and Food Security Coordinator at Concern Universal, where I led among others, the development of market-access and postharvest services project which established a business model for supply chain actors to enhance the delivery of quality (mould-free and insect-free) maize to consumers and agro-processing industries. Working in the capacity of a farmer trainer and consultant, I adopted a participatory analysis tool to develop value-chains for new product development and for fresh produce export.

From my experiences, produce handling along various value chains, particularly in fresh produce and ready to eat food sector pose serious health risks to humans, both at the country and intercontinental level. Though several quality testing and management systems are available, technologies for rapid detections of pathogens, their resistance strains and geographical alerts systems along the value chain is limited. To ensure biosecurity, global monitoring systems that combine scanning, geodata and remote sensing to identify, quantify, prevent the spread of pathogens as well as determine early mitigation strategies are highly recommended.

## South Africa listeriosis outbreak: are developing countries the biggest developing threat to the food system?

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The South Africa listeriosis outbreak (2017-2018) is one of the world's biggest recorded outbreaks according to the World Health Organisation. The source of the specific sequence type 6 (ST6) *Listeria monocytogenes* strain: ready-to-eat processed meat products. Deaths tolled at least 180, with half being children. The population groups most affected were low-income consumers as processed meat products are cheaper and therefore, more affordable for them. However, processed meat products are also known to be vulnerable to fraudulent practises. Consequently, other issues that have surfaced concern the use of cheap Brazilian mechanically deboned meat (MDM) in the products. Conveniently, following (and possibly coinciding?) with Brazil's rotten meat scandal, whilst listeriosis is also not a compulsorily notifiable disease in Brazil. Although MDM and other meat products are legally imported and used, it is vulnerable to fraud through the falsification of laboratory test results and mislabelling/repacking. Consequently, serious food safety issues can arise from such illegal practices. Yet, finger-pointing does not solve the crisis as preventative control measures should have been in place. Given its strict regulatory framework, this crisis has revealed that the South African meat/food industry may not be as highly regulated and enforced as anticipated. The ongoing contamination through unhygienic practices is a major concern and questions the execution of preventative controls by leading food manufacturers as set by quality assurance certification schemes. The lack of effective monitoring and cooperation from meat companies with investigators indicates negligence for the human health threat to the food system.

## A Critical Review of Farmers' Perceptions and Understanding of Antimicrobial Resistance

**Miss. Hollie Bradford**<sup>1</sup>

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Antimicrobial Resistance (AMR) occurs when micro-organisms develop the ability to counteract antimicrobial drugs through previous exposure. The past decade has seen an increased prevalence of AMR bacteria due to the widespread use of antimicrobials. AMR reportedly kills more than 700,000 people globally and if no action is taken, an estimated 10 million people will die every year by 2050. A substantial share of antimicrobial consumption is attributed to animal production, particularly within the pig industry as it is recognised to be a high user of antimicrobials. Considerable research has focused on the scientific mechanisms of AMR and antimicrobial misuse in human medicine; however, limited literature exists regarding the perception of pig farmers towards AMR.

Three databases; PubMed, Web of Science and Veterinary Record were used to search keywords 'AMR', 'farmers', 'perceptions' and 'pigs' in order to identify relevant papers (n=11). Papers that included the words 'attitudes', 'perceptions', 'beliefs', 'knowledge' or 'opinions' in the title were included in the study whilst journals that did not were excluded.

Results suggest that farmers perceive antimicrobials as useful for the prevention and treatment of diseases in pigs; reporting high levels of benefits from their use. They had limited knowledge surrounding the risk of antimicrobials to human health and were less worried about AMR than about financial and legal issues related to pig farming.

To combat the overuse of antimicrobials within the pig sector, it is necessary to gain an understanding of farmers' perceptions and practices and therefore more research needs to be undertaken.

## Molecular characterisation of antimicrobial resistance of Escherichia coli isolates obtained from meat in South Africa

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This study aimed to characterise antibiotics resistance of Escherichia coli isolates from the formal (FMS) and informal (INMS) meat sectors. A total of 162 and 102 E. coli isolates from the FMS, and INMS respectively were isolated by standard culture-based, and biochemical reactions. The isolates were further confirmed by polymerase chain reaction (PCR). The disc diffusion method was used to screen for antimicrobial susceptibility against 19 different antibiotics. The presence of class 1-2 integrons in each E. coli isolates was assessed using 3-CS and 5-CS regions specific primers. Among the 19 antimicrobials selected from 9 antibiotic classes, resistance among tetracyclines, aminoglycosides, cephalosporins, and nitrofurans were found to be more frequent than carbapenems and phenicol with a noticeable increase in the number of multi-drug resistance ranging from three to ten antimicrobials. A total of 20 resistance determinants were assessed with their prevalence and distributions obtained as follows for FMS and INMS respectively; [aminoglycosides: aadA (40.6%; 31.9%), aacC2 (21.4%; 31%), aphA1 (20.8%; 15.1%), aphA2 (37.7%; 18.9%) and strA (6.5%; 9.4%)], [ $\beta$ -lactams: ampC (20%; 45%), blaTEM, (4.4%; 13.3), and blaZ (8.9%; 2.2%)], [Chloramphenicol: catI (1.7%; 1.7%), and cmIA1 (1.7%; 1.7%)] and [tetracyclines: tetA (7.7%; 15.4%), tetB (11.5%; 24%), and tetM, (1.9%; 8.7%)], and [sulfonamides: sul1 (22.2%; 26.7%), sul2 (17.8%; 6.7%)]. Multiple antibiotic resistance (MAR) indexes ranged from 0.2 to 0.5. The results reveal a high prevalence of multidrug-resistant E. coli isolates and resistance determinants suggesting that consumers of such meat are at risk of contracting antibiotic resistant E. coli-related foodborne disease



## Implementing an antibiotic reduction policy across the global broiler supply chain of a UK retailer

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Against a background of increasing concern about the emergence of antimicrobial resistance in agriculture, Tesco Stores (UK) Ltd have published their antibiotic commitment plan. This emphasizes the need for a reduction in both total use and the highest priority Critically Important Antibiotics; publication of progress against targets, and the embedding of an underpinning education programme across all own brand species sector supply chains globally. These commitments are supported by an associated programme of independent on-farm inspections and the analysis of monthly submitted Outcome Measure data, addressing key welfare indicators including antibiotic use. This process is most developed in the broiler supply chain where 3 years of data have been tracked and trended by: production type, processing site and geography (to include UK, EU and non EU companies). To date this programme, supported by individual and whole sector comparative reviews, has demonstrated the capacity for a supply chain as a whole to make significant ( $P < 0.05$ ) reductions in antibiotic use over a 3 year period. Over this time frame the supply base has moved from a binomial distribution with discrete 'low' (4.5 +/- SEM 4.2 mg/kg.) and 'high' (21.7 +/- SEM 7.2 mg/kg) user populations, to a single population with an average use of 10.2 mg/kg. Geography is still the biggest single determinant of use but all companies show ongoing or sustained reduction. The work to date illustrates the capacity for retailers to drive integrated antibiotic reduction strategies within commercial frameworks and provide reassurance to the consumer.

## Meat Handling in Abattoirs in Cross River: A Major Health Risk. What Data Exit?

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A case study of meat handling in major abattoir and data availability was undertaken in Calabar, Cross River State - Nigeria. Each day 18 - 40 cattle are slaughtered in government and private-owned abattoir across the capital city, the procedure of inspection by the veterinary officers do not reflect the status of the cattle's health as they often lack basic tools and infrastructure for standard inspection therefore relying only on their physical examinations capabilities for the job. Most often than not, there is little or no documentations of their findings. In addition, the slaughtering, processing and marketing of meat and meat products from the abattoir are carried out in crude and unhygienic manner. Personal observations were made; buttressed by photographs on the operations at these abattoirs. This practice poses high risk of contamination of meat and meat products with harmful viral, bacterial, parasitic, fungal and spilled chemical agents that can cause severe or even fatal disease in humans in addition to the treats of serious zoonotic diseases in humans. The near total lack of documentation and proper data keeping may be responsible for inadequate decision making process and thus inadequate food safety and health policies with the resultant poor health, overall low economic and social development of the State in particular and the country as a whole.

We strongly recommend that adequate measures be taken by all stake holders to both fund and enforce existing legislations bordering on issues of food hygiene and community health. Provide Open Data integration platforms.

## Review of rapid diagnostics for antibiotic residue analysis

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Antibiotic residues found in food products as a result of veterinary treatment have proven to be problematic for the food industry. The presence of antibiotics in food can have a long term knock-on impact on the consumer, for example from bacterial antibiotic resistance (1). Intensification of food production involving elevated use of antibiotics has led to several types of bacteria being identified as antibiotic-resistant (2). The use of antibiotics and growing consumer demand has led to increased concern for downstream commercialised products. Antibiotics are either banned or maximum residue limits (MRLs) are set by the EU in order to prevent their overuse, with the aim being to safeguard the quantity of antibiotics entering the food chain (3). Current detection methods ensure there is a number of food types free from antibiotics. However, commercial screening methods are often limited to one group of antibiotics, with some exceptions in multiplexing. Furthermore, confirmatory methods such as LC-MS are relatively more expensive and require trained personnel. Biosensors have become of interest in the last two decades as a potential screening method to detect several different analytes in one test (4). Several studies have highlighted the use of biosensors in detecting antibiotic residues in food products such as milk, honey and poultry (5-7). Portable diagnostics are of increasing interest because of their use as a field-based assay (8). Here, we will review the key methods of analysis for the detection of identified problematic antibiotics associated with food production, and their utilisation across different food areas.

## Nanodiagnosics for Detection of Foodborne Pathogens using Phage-Derived Proteins

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The high mortality rate associated with *Listeria monocytogenes* as well as its ability to adapt to the harsh conditions employed in food processing make this pathogen a significant concern in the ready-to-eat food industry. The monitoring of *L. monocytogenes* is therefore, vital to ensure the safety of our food supply. To date, rapid detection methods display either poor sensitivity (10<sup>2</sup>-10<sup>4</sup> cfu/ml) or low specificity for *L. monocytogenes*. This research aims to combine two independently successful technologies, the rapid portable nature of biosensors based on planar waveguide technology and the extraordinary specificity of bacteriophage-derived affinity proteins for pathogen detection. Phages have co-evolved with their bacterial hosts to recognise and infect their target cells with extraordinary specificity that may be harnessed for the purpose of bacterial detection. The recognition of the host by the phage occurs primarily through the receptor-binding proteins, or RBPs, which is generally located in the phage tail. In this work to date, phages against *L. monocytogenes* strains of the 4b serotype were isolated from mushroom compost. The genome sequences of two of these phages, phage vB\_Lmo\_188 and phage vB\_Lmo\_293, were elucidated and through a series of mutational analysis experiments, the RBPs were identified. Recombinant production of these proteins is currently on-going. These proteins will form the basis of the biorecognition of *L. monocytogenes* in a rapid real-time test for the online monitoring of *L. monocytogenes* in food matrices with improved sensitivity (10<sup>2</sup> cfu/ml) over current methods.

## Atmospheric Cold Plasma for degradation of antibiotic contaminants: Transformation products and their residual antibacterial effects

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Antibiotics, such as ofloxacin (OFX) and ciprofloxacin (CFX) are frequently contaminants in wastewater, presenting a potential risk to non-target organisms and to human health. We studied the efficacy of atmospheric air plasma for the degradation of antibiotics in water and meat processing effluent, the antibacterial activity of samples submitted to the plasma process and the by-products formed. Cold plasma treatment successfully degraded antibiotics, with efficacies of 88% ( $k=0.054 \text{ min}^{-1}$ ) for CFX and 91% ( $k=0.092 \text{ min}^{-1}$ ) for OFX, respectively. Attack by hydroxyl radical and ozone were the main degradation processes. The safe degradation of antibiotics should be accompanied with loss of biological function. For both treated antibiotics, the antibacterial activity was reduced using disk diffusion assay, however, using a micro-broth dilution assay, there was an increased antibacterial activity of plasma treated CFX. This pointed to the generation of compounds more toxic to cells due to antibiotic exposure to cold plasma, as the effects were not attributable to plasma treated water alone. Because of the demonstrated increase in antimicrobial potency of ACP treated CFX, a study on bacterial adaptation to CFX was conducted. For *E. coli*, the tendency of bacterial adaptation after repeated exposure to untreated CFX was observed with 8-fold increase in MIC recorded. Importantly, no such adaptation was apparent for ACP treated antibiotic. It is recommended that cold plasma as an AOP for effluents that may contain antibiotics or residues is optimised for complete loss of biological activity or is combined with a separate process to ensure life cycle safety.

# Human exposure to chemical cocktails present in foods

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## Detection of human exposures to natural toxins

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Development of diagnostic assays to confirm and monitor human exposures to environmental toxins are necessary to improve treatment strategies. Human exposures to toxins occur despite food and water monitoring programs. Our lab has developed a panel of methods to measure several toxins, including paralytic shellfish poisoning toxins (PSPs), amanitins, and microcystins in human biological matrices. Numerous cases of human PSP exposures have been confirmed using these clinical methods coupled with food remnant analysis. Information from human exposure samples in conjunction with food and water analysis is especially powerful in supporting public health efforts to identify, monitor, and prevent human exposures to natural toxins.

## Persistent Organic Pollutant (POPs) mixtures inhibit the transactivation activities of the rat AhR in vitro

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All living organisms are exposed to persistent organic pollutants (POPs) not as individual compounds but as mixtures of chemicals. However, to assess the toxicity of POPs, scientific studies usually focus on the effect of one single compound at a time and do not address the mixture effect. This study aims to determine, in vitro, the effect of a mixture of POPs at the level of the rat Aryl hydrocarbon Receptor (rAhR) function. In this study, luciferase reporter Dioxin responsive rat hepatoma cell lines (DR-H4IIE) were used to screen both rAhR agonistic and antagonistic activities of 29 compounds listed as POPs under the 2001 Stockholm Convention. Their total mixture, prepared according to their concentrations found in human blood, was also tested for the same activities. The results showed that only 5 out of the 29 compounds (PBDE 99, PBDE 153, PBDE 154, PCB 138, and PCB 118) showed rAhR agonistic activities. However, 20 of them showed rAhR antagonistic activities. Not surprisingly, the mixture also displayed an rAhR antagonistic activity. The inhibition of the rAhR transactivation activity was recorded with concentrations of the POP mixture corresponding to 75 times the blood level and above, which could be plausibly reached in human after a food contamination incident, for example. The calculated IC<sub>50</sub> of the total mixture, using an additive model, was 16.8 μM, which is 3 times higher than the measured IC<sub>50</sub> (5.07 ± 2.02 μM). Submixtures will be tested to reveal the mechanism beneath the mixture effects (additive, antagonistic or synergic).

## Decontamination of antibiotic residues with cold atmospheric pressure plasma

**Dr. Ewa Wielogorska<sup>1,2</sup>**, Dr Julie Meneely<sup>1</sup>, Prof. Brendan F. Gilmore<sup>2</sup>, Prof. William G. Graham<sup>3</sup>, Prof. Christopher T. Elliott<sup>1</sup>

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Progressing climate change raises concerns regarding both food safety and security worldwide. Increased efforts are directed towards increasing agricultural productivity and more efficient management of current resources while assuring consumers' safety by continuous monitoring of variety of man-made and natural chemical residues in food commodities and the environment itself. In order to limit food wastage through rejection of non-compliant produce, novel technologies enabling food detoxification are being currently explored. The present study aims at evaluating the efficiency of cold atmospheric plasma technology in removal of antibiotic residues in milk. The decomposition efficiently and its optimisation for fifteen B-lactam antibiotics, including penicillins and cephalosporins in both solvent and food systems has been evaluated. Possible decontamination by-products and their persistence have been assessed, as well as the influence of plasma treatment on small molecular mass analytes composition in the milk matrix. Results in solvent based systems show that significant degradation of all compounds can be achieved within first 5 minutes of treatment and was shown to be heavily dependent on plasma composition and analytes' structure. The process proved to be repeatable and reproducible between the exposure days. Transfer to milk presents analytes' decomposition efficiency dependence on the complexity of the matrix. Also, limited changes have been observed in small molecular compounds composition of the matrix with no increased toxicity in multiple endpoint liver cell high content bioassay (HepG2 cell line). Nevertheless, encouraging degradation observed for the majority of residues assessed suggests possible alternative applications such as water or sewage treatment.



## Detoxification of mycotoxins in maize using cold atmospheric plasma technology

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Mycotoxins are toxic secondary metabolites mainly produced by different fungal species such as *Aspergillus*, *Penicillium* and *Fusarium* that contaminate a wide range of agricultural products including cereals, fruits, nuts, and spices. Mycotoxin contamination is an increasing food safety issue with an estimated 25% of the world's grains contaminated. Additionally, an increasing presence of mycotoxins in agricultural commodities have been linked to climate change where factors such as droughts, elevated temperatures, moisture levels and plant stress-related responses are associated with fungal growth, consequently, production of mycotoxins. Therefore, mycotoxin-decontamination strategies that are economically viable, environmentally benign, not adversely affecting food quality will play an important role in tackling current and future food safety and security challenges. The current study aims at assessing Cold Atmospheric Pressure Plasma (CAPP) technology for the purpose of mycotoxin decontamination of maize which is a staple cereal worldwide. The results show that for the majority of the toxins assessed, including aflatoxin B1, fumonisin B1, ochratoxin A, zearalenone and enniatin B, a 50% degradation in a solvent system can be achieved within 12min of treatment with CAPP with limited by-products formation. In the sample matrix, over 60% decontamination was demonstrated within 10min of treatment for aflatoxin B1 and fumonisin B1, two of the world's most important mycotoxins in terms of adverse effects. Additionally, assessment of small molecular compounds composition changes in the matrix after CAPP treatment demonstrated significant changes in chemical profiles, however, no toxicity in multiple endpoint liver cell high content bioassay (HepG2 cell line) has been recorded.

## Rapid Detection of Tetrodotoxin in shellfish using an immunosensor

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Tetrodotoxin (TTX) is a potent neurotoxin emerging in European waters with suggestions that it may be linked to increasing ocean temperatures but may also be due to increased targeted surveillance. It has been determined in mussels and oysters in England in 2013 and 2014 and Greece and Holland in 2015. Its detection in seafood was previously performed by a mouse bioassay (MBA) for paralytic shellfish poisoning (PSP) toxins as TTX is not monitored independently in Europe. As an AOAC-accredited high-performance liquid chromatography (HPLC) method was accepted by the European Union as a first action screening method for PSP toxins a separate method of analysis was required for TTX or else this toxin would go undetected in seafood produce posing potentially serious health consequences. In this study, a nanoarray planar waveguide biosensor was developed and validated for the detection of tetrodotoxin. This technique offers the in situ analysis of TTX in shellfish to meet the required EFSA recommendations of 44 µg TTX/kg of shellfish meat with an LOQ between 0.1 and 25 µg TTX/kg. The applicability to natural samples was investigated, limited matrix effects were observed, with recovery at the IC50 value of 20 µg/kg greater than 90 % and % CV less than 20 %. The comparison of the method with LC-MS for contaminated mussel and oyster samples has shown the compatibility and feasibility of this immunosensor to be able to support monitoring programs and research activities for evaluation of the toxin occurrences and levels of exposure.

## Variation in elemental nutrient composition of grains, cereals and food plants using rapid X-ray fluorescence.

**Mr. Manus Carey<sup>1</sup>**

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XRF is a potentially rapid and non-destructive analytical tool that could be applied to grain nutrition. It will be demonstrated that having matrix matched certified reference materials (CRMs) is imperative in obtaining reliable, accurate and robust measurements across the elemental range for XRF. These CRMs can also be used in post-analytical corrective calculations to adjust for analytical bias for certain important elements. In the rice matrix; iron Fe, copper Cu and magnesium Mg are significantly over estimated but with appropriate correction, all these elements will subsequently read back closer to the expected value and with a high degree of reproducibility.

The XRF performance was compared with that from inductive coupled plasma mass spectrometry, highlighting the advantages and limitations of both approaches. An example of this is phosphorous P which gave reproducible results by both methods but there is a 10% difference (under-estimation) by ICP/MS. XRF has a much higher limit of detection, LOD, as compared to ICP-MS so we examined the effect of sample ashing to pre-concentrate the solid for XRF analysis. An example of this was bromine Br in Kidney Beans which was not detected in the original reference material but then returned a result at 106% of the certified value after ashing.

## Human exposure to cyanotoxins: Exploring their in vitro detoxification using atmospheric cold plasma treatment

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Recently, there have been increases in freshwater Harmful Algal Blooms (HABs) globally. HABs can produce cyanotoxins, many of which are hepatotoxic such as the microcystins (MCs). Cyanotoxins have been reported in freshwater systems across the world, with their health effects including; promotion of various cancers, neurotoxicity, genotoxicity and potential carcinogenicity. Human exposure to these occurs through numerous pathways, including the ingestion of contaminated water and recreational use of water bodies contaminated with HABs. Current methods utilised by water treatment facilities to remove cyanotoxins from drinking water can be successful if tailored to individual toxins. However, with certain cyanobacterial species capable of producing more than one class of cyanotoxin, as well as producing numerous congeners, their removal could become more problematic, posing a risk to consumers. Therefore, there is a need to try and effectively detoxify drinking water contaminated with cyanotoxins to safeguard human health by use of new and novel techniques, such as atmospheric cold plasma treatment. To investigate this, six MC congeners, nodularin (NOD), cylindrospermopsin (CYN) and anatoxin-A (ATX-A) were subjected to atmospheric cold plasma treatment, comparing the use of helium gas alone against a helium/oxygen gas admixture. The results showed effective degradation of the MCs and NOD in comparison to the cyanotoxins CYN and ATX-A which showed relative stability, with the technique found to be more effective when using helium gas only. This study demonstrates that atmospheric cold plasma treatment could be an effective tool for the degradation of cyanotoxins such as the MCs in water.

## Occurrence of multiple contaminants in large sets of tea and coffee samples

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Tea and coffee, major representatives of caffeine containing botanicals, belong among the the most traded commodities worldwide. Next to a number of health-promoting compounds with antioxidant, antibacterial and potentially anti-carcinogenic effects, both of these stimulants may contain also a variety of contaminants represented by residues of agrochemicals, mycotoxins and process contaminants. As regards their determination, it is a rather challenging task due to the considerable matrix complexity and high amount of commonly co-extracted caffeine.

Within this study, internal validated analytical approaches based on chromatographic methods and mass spectrometric detection were used for determination of a wide spectrum of pesticide residues (n = 357) and mycotoxins (n = 57) in 120 samples of black, oolong and green tea collected in southeast Asia. Another set of 119 samples of green, roasted and instant coffee from the EU market was examined for the presence of predominant mycotoxin ochratoxin A. Process contaminants acrylamide and furan were also analyzed in the selected coffee samples (n = 48).

The results showed extensive occurrence of pesticide residues in the vast majority of tea samples, the most contaminated sample was positive for 16 residues. Altogether, 37 % of teas would not comply with EU legislation. The coffee showed rather lower contamination, only one sample of instant coffee exceeded the maximum EU limit for ochratoxin A and two samples of instant coffee for acrylamide. Interestingly, the majority of the samples positive for ochratoxin A (14-(S)-ochratoxin A) were also positive for its degradation product - stereoisomer 14-(R)-ochratoxin A.

## Measuring the combined exposure to aflatoxin B1 and microcystin-LR using High Content Analysis

**Dr. Julie Meneely**<sup>1</sup>, Prof. Christopher Elliott<sup>1</sup>

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As human co-exposure to natural toxins through food is inevitable, it is incumbent on the appropriate authorities to ensure that risk assessments are “fit for purpose” to safeguard health and maintain international trade.

Aflatoxin B1 (AFB1), a frequent contaminant in many crops and Microcystin-LR (MC-LR), produced in freshwater by cyanobacteria are naturally occurring potent hepatotoxins that threaten human health. Populations in the poorest regions of the world may suffer repeated simultaneous exposure to these contaminants at or above maximum regulatory limits.

By evaluating the combined effects of these toxins at relevant concentrations, additive, antagonistic or synergistic effects revealed will improve risk assessments. Using High Content Analysis, multiple cytotoxicity endpoints, cell number (CN), nuclear area (NA), nuclear intensity (NI), mitochondrial mass (MM) and Mitochondrial membrane potential (MMP) were measured for the individual toxins as well as mixtures in various cell lines.

Results highlighted that significant cytotoxic effects were observed for AFB1 above the regulatory limit in all cell lines while no cytotoxic effects were observed for MC-LR in any cell lines. With the mixture of AFB1 500ng/ml and MC-LR 250ng/ml, greater cytotoxicity was observed in HepG2 cells and synergy was evident for NI. Antagonistic effects were observed with this mixture for NA in both HepG2 and MDBK cells while antagonism was observed for all mixtures tested, including below regulatory limits for CN in HepG2 cells.

The antagonism demonstrated between exposure to AFB1 and MC-LR is clear evidence of the complexity around trying to regulate for human exposure to multiple contaminants.

## PROTECTION against Endocrine Disruptors; Detection, mixtures, health effects, risk assessment and communication

**Dr. Lisa Connolly**<sup>1</sup>

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PROTECTED will develop expertise and protective capabilities against Endocrine Disruptors (EDs). EDs and their mixtures are a modern day health concern leading to failing ecological systems, poor agricultural production and health effects such as obesity, cancer and infertility. While analytical methods have advanced enormously, focus has been mainly on synthetic chemicals, overlooking emerging EDs and real-life multiple substance exposure. Creative, entrepreneurial and innovative early-stage-researchers equipped with skills to assess and understand the real-life risk of EDs and trained to convert resulting knowledge and ideas into accessible tools and services for the long-term control of potential ED risk are urgently needed.

The PROTECTED Innovative-Training-Network [ITN] will provide 15 individual research projects with exposure to scientific, innovative and entrepreneurial training across the ITN. The intersectoral network is comprised of 12 training sites at academia, research centres, a bioassay technology SME, a QSAR technology SME, water provider, and animal feed supplier. Together they cover multiple disciplines including analytical science of food, feed, and environment, epidemiology, risk assessment, social science and toxicology.

This combined expertise enables a highly focused program for developing novel tools and training for the detection, analysis and improved risk assessment of EDs and mixture effects. Methodology will include emerging technologies; multiplexed analysis, mixture modelling, mechanistic and exposure studies, explants and cell or whole organism bioassays. The project will provide a unique and high level of training for a new generation of specialists to aid the efficient development of future control strategies for improved health.

For more information see: <http://protected.eu.com/>

## Food Fortress: The Development and Implementation of a Globally Recognised Sampling/Testing Scheme in Northern Ireland.

**Dr. Simon Haughey**<sup>1</sup>, Ms Holly Montgomery<sup>1</sup>, Mr Robin Irvine<sup>2</sup>, Prof. Chris Elliott<sup>1</sup>

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Crises involving chemical contamination, including dioxins/PCBs, aflatoxins, heavy metals, of animal feedstuffs can have a detrimental effect on the whole food supply chain. This was the driver which led to the leading animal feed companies in Northern Ireland as part of the Northern Ireland Grain Trade Association and the Institute for Global Food Security to developing a system to rank the risks associated with animal feed and feed ingredients and implementing an unique industry wide testing scheme to help mitigate the risks.

The risk model was developed using the information available in the EU RASFF portal and a scoring system related to the hazards identified. The model is updated on a regular basis and a report sent to the industrial partners.

An industry-wide program of strategic sampling and testing, co-operation to reduce the risk from the principal contaminants which threaten this supply chain has been implemented. The program, called Food Fortress, is the most advanced of its kind anywhere in the world and is supported by academia and the local food industry as well as the regulators, FSA and DAERA. Its members include all of the feed industry in Northern Ireland with additional companies from the Republic of Ireland and Great Britain. Currently, the red meat and dairy sectors are actively pursuing projects associated with the implementation of the Food Fortress within their industries. A full update will be presented on the unique scheme that is putting Northern Ireland on the global map in relation to food safety and security.



## The relationship between Product Information Transparency and Consumer Trust: Benefits and Challenges

**Mr. John Keogh<sup>1</sup>**

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Conventional wisdom suggests that transparency is an incontrovertible practice in public and private sector governance. Moreover, transparency is viewed as foundational for the efficient functioning of markets and provides a substratum layer for trust to function as a social lubricant by reducing information asymmetry.

Today, consumers worry about the safety and authenticity of foods they buy in retail and consume in food service. This results from the near constant media amplification of food-related crises, scares and scandals which has heightened their awareness of personal health and safety as well as the increased risk of food fraud and unsafe practices. Subsequently, consumers are sensitized to deceptive practices, ethical lapses as well as opportunistic and immoral behavior by bad actors in global, regional and local food supply chains. As a consequence, doubt is raised on whom to trust, and what information to trust. Moreover, consumers lack the know-how and expertise to determine food quality and safety and cannot verify the authenticity of food and credence claims such as organic, non-GMO, 100% beef, halal etc.

Consumers want increased transparency about where and how their foods were grown and processed. Their information demands extend beyond personal health and safety to growing societal concerns related to child labour, slavery and sustainable practices.

Transparency in the food supply chain is a nascent area of research and the relationship to consumer trust lacks empirical evidence. The briefing provides a critical update and is grounded in John's academic research and practical advisory to governments, industry and solution providers.

## Citizen science drivers in food testing: lab exit?

**Prof. Michel Nielen<sup>1,2</sup>**

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Imagine how many random and suspect samples are being taken for food quality and safety testing within the European Union (EU): millions each year again and again. Typically, all these samples are taken on-site at farms, slaughterhouses, border inspection points, retail shops, etc., documented, transported to a control laboratory, screened for target substances such as food contaminants and drug residues, and finally the few suspects from screening methods must be confirmed by validated instrumental methods in order to declare the sample non-compliant or compliant. Despite all these efforts, we are still facing frequent food incidents and fraud issues. So a paradigm shift in food quality and safety testing is needed in order to free resources for an intensified combat against fraud in the food chain. Consumers are not amused and their trust in food quality is decreasing, industry and retailers are facing claims, officials show limited communication skills.... In this situation simplified solutions emerge in academia and industry, promising simple yes/no answers to consumer food quality and safety testing needs. Soon, citizen science power may dominate the testing agenda and support (or undermine) consumer trust. Questions pop-up related to quality aspects of 'simplified solutions', ownership and communication of data, the future role of laboratories and the education of professionals. Scientific training in novel smartphone-based technologies is expected to have a major impact on future EU monitoring practices and, moreover, pave the road for Citizen Science. Evidence-based sensing will be the key.

[www.foodsmartphone.eu](http://www.foodsmartphone.eu) ; [www.foodsmartphone.blog](http://www.foodsmartphone.blog) ;

Twitter: @foodsmartphone ; YouTube IXceX3TITzs

## Co-occurrence of mycotoxins in animal feeds and effects on growth performance of broiler chickens

**Mr. Oluwatobi Kolawole<sup>1</sup>**, Prof. Christopher Elliott<sup>1</sup>, Dr Lisa Connolly<sup>1</sup>, Dr. Ewa Wielogorska<sup>1</sup>

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Mycotoxins are toxic secondary metabolites produced by filamentous fungi (mold). There are about 500 known mycotoxins, and many are still being discovered. Co-occurrence of mycotoxins is likely to arise because most fungi can simultaneously produce many mycotoxins, and feed ingredients can be contaminated by several fungi. In this study, twenty-five finished feed samples intended for broilers were collected in three batches [B1 (n=8), B2 (n=9), B3(n=8)] from poultry performance houses in Northern Ireland. A QuEChERS-based LC-MS method was applied to investigate the co-occurrence of mycotoxins in the feed samples. All samples were found to be co-contaminated with sixteen mycotoxins including: Deoxynivalenol, Zearalenone, Fumonisin (B, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>), Enniatins (A, A<sub>1</sub>, B, B<sub>1</sub>), Beauvericin, Apicidin, Aurofurasin, Tentoxin, Meleagrins, Equisetin and Mycophenolic acid. In terms of mean values, significant levels of deoxynivalenol were found in samples of B1 (980 µg/kg) and B2 (740 µg/kg) compared to samples of B3 (260 µg/kg). However, samples of B1 and B2 were below the European Union guidance values for deoxynivalenol and zearalenone. Data on growth performance of broilers fed the naturally contaminated feeds, indicate that birds on B1 feeds had increased average daily gain, feed efficiency and reduced mortality compared to birds that fed on feeds of B3 and B2 (p<0.05). There is a worldwide prevalence of regulated, unregulated and emerging mycotoxins in animal feeds. Low levels of these mycotoxin cocktails could have a negative impact on animal health, and a possible carry-over to animal derived products leading to mycotoxins intake by humans.

## Aspergillus Species and Aflatoxin Levels in Sorghum Stored For Different Period and Storage System

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### Abstract

Sorghum serves as staple food for over 100 million people in Sub-Saharan African countries. It is the most important nutritional security crop and ranks third among major cereal crops in terms of area and production next to teff and maize in Ethiopia. However, sorghum is susceptible to contamination by molds that produces Aflatoxins that causes hepatotoxic and carcinogenic effects on humans and animal. This study was conducted to assess Aspergillus species and Aflatoxins level in sorghum (*sorghum bicolor* L.) stored under different storage system for different storage period. Thirty samples were analyzed for Aflatoxins contamination using HPLC equipped with fluorescent detector and Aspergillus species were isolated and identified using phenotypic features in Potato Dextrose Agar culture media. About 56.7%, 16.7%, and 23.3% of the sorghum samples were found to be contaminated with *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus parasiticus*, respectively. The level of total Aflatoxin, AFB1, AFB2, AFG1, and AFG2 were in the range of 11.44 to 344.26 $\mu$ g/kg, 3.95 to 153.72 $\mu$ g/kg, 1.17 to 91.82 $\mu$ g/kg, 9.87 to 139.64 $\mu$ g/kg, and 3.22 to 52.02 $\mu$ g/kg, respectively. The concentration of Aflatoxins in all sorghum samples surpassed the maximum level set by the European commission and therefore, deserves attention to control them across the sorghum value-chain.

Keywords: Sorghum, Aflatoxins, Aspergillus spp., Storage Period, Storage System

## A systems biology approach to elucidate the effect of chronic microcystin-LR exposure

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The growing worldwide presence of cyanobacteria in freshwater bodies is an increasing concern. Fueled by eutrophication, cyanobacteria proliferate and form a threat to human and animal health by producing toxic secondary metabolites. These include microcystins, the most toxic variant of which is microcystin-LR (MC-LR).

The main route of exposure to microcystins is through consumption of contaminated drinking water. They have also been shown to bioaccumulate in fish, snails, crustacean and crops irrigated with contaminated water.

Once ingested, MC-LR is transported to the liver and inhibits protein phosphatases. Effects of MC-LR toxicity include cytoskeletal disorganization, apoptosis, genotoxicity and possibly carcinogenicity. While the World Health Organization has set a provisional daily intake (TDI) for MC-LR, data from epidemiology studies implicate hepatotoxicity in humans chronically exposed to MC-LR amounts similar to the TDI.

The effect of MC-LR exposure has been investigated in the proteome, metabolome and transcriptome in rodents and fish. It is difficult to translate the results into a human scenario because of the experimental designs applied. Indeed, most studies administered MC-LR via intraperitoneal injection or used high toxin concentrations.

We applied a systems biology approach to elucidate the effect of low-dose MC-LR exposure by integrating data from different biological systems. Pigs were chronically exposed to low MC-LR concentrations by oral gavage to mimic “real-life” human exposure conditions. Preliminary results suggest that low MC-LR concentrations have little effect on the liver metabolome and blood proteome and metabolome. Further analyses will reveal whether other –omes and organs are affected.

## Divinylbenzene samplers as surrogate tool for biological monitoring of (micro)pollutants in the marine environment

**Mr. Steve Huysman**<sup>1</sup>, Mr. Francis Vanryckeghem<sup>1</sup>, Dr. Foppe Smedes<sup>2</sup>, Prof. Herman Van Langenhove<sup>1</sup>, Prof. Kristof Demeestere<sup>1</sup>, Prof. Lynn Vanhaecke<sup>1</sup>

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A plethora of indicators have been used as monitoring tools to evaluate the impact of emerging contaminants on human and ocean health. Nevertheless, the analysis of bio-indicators results in some disadvantages, i.e. lack of cost-efficiency, labor intensive and depending on the metabolization of the organism. Therefore, this study presents a novel surrogate-biomonitoring technique, based on a divinylbenzene (DVB) sorbent, for mimicking the bioaccumulation of aquatic organisms. Static exposure designs were used for investigating the kinetic parameters of 186 emerging contaminants (i.e. 28 pesticides, 7 personal care products, 52 pharmaceuticals, 70 hormones and 27 plasticizers). The latter were successfully calculated and validated by a model that was used for evaluating the bioaccumulation (Greenwood et al, 2007). During the validation, the models displayed significant ( $p < 0.05$ ) non-linear correlations, which resulted in the determination of kinetic and equilibrium parameters. The uptake rates and partition water coefficients ranged respectively between 5 to 40 L.d<sup>-1</sup> and 5 to 10. Additionally, the DVB was extensively calibrated for using this novel technique as a quantitative surrogate of aquatic organisms. Finally as a proof of principle, DVB samplers were directly applied in marine waters and accumulated a broad polarity range of compounds (Log P from 2 to 10). Moreover, the uptake rates obtained by the laboratory pseudo-static exposure design were 100-fold lower than the field due to the enclosing glass fiber of the DVB. In conclusion, this unique technique may lead to quantify the risk of emerging contaminants that can be accumulated by aquatic organisms.

## Regulatory Roles of Histone Deacetylases 1 and 2 in Pb-induced Neurotoxicity

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Lead (Pb) prevails among the environmental hazards against human health. Although increasing evidence highlights the epigenetic roles underlying the Pb-induced neurotoxicity, the exact mechanisms concerning histone acetylation and its causative agents are still at its infancy. In the present study, the roles of histone deacetylases 1 and 2 (HDAC1/2), as well as histone H3 Lys9 acetylation (Ac-H3K9), in Pb-induced neurotoxicity were investigated. Pb was administered to PC12 cells at 10  $\mu$ M for 24 hours. And Sprague-Dawley (SD) rats were exposed to Pb from parturition to 2 months. It indicates that HDAC2 was up-regulated accompanied by Ac-H3K9 down-regulation. Meanwhile, chromatin immunoprecipitation (ChIP) assay revealed that the changes of HDAC2 were attributed to histone H3 Lys27 trimethylation (H3K27me3) occupancy on its promoter. Blockade of HDAC2 with either Trichostatin A (TSA) or HDAC2-knocking down construct (shHDAC2) resulted in amelioration of neurite outgrowth deficits via increasing Ac-H3K9 levels. It implies that HDAC2 plays essential regulatory roles in Pb-induced neurotoxicity. And, co-immunoprecipitation (CO-IP) trials revealed that HDAC2 co-localized with HDAC1, forming a so-called HDAC1/2 complex. Subsequently, it was shown that HDAC1/2 repression could markedly prevent neurite outgrowth impairment and rescue the spatial memory deficits caused by Pb exposure, unequivocally implicating this complex in the studied toxicological process. Furthermore, Notch2 maybe the functional target of the HDAC1/2 & Ac-H3K9 alterations. Our study provided insight into the precise roles of HDAC1/2 in Pb-induced neurotoxicity, and thereby provided a promising molecular target for medical intervention of neurological disorders with environmental etiology.

## The potential of Persistent Organic Pollutants (POPs) mixtures induce cytotoxicity in enteroendocrine pGIP/neo: STC-1 cells

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Rates of type 2 diabetes (T2D) and obesity are increasing globally and “non-traditional” factors could play an important role such as human exposure to environmental contaminants which can interact with micronutrients or disrupt the gut microbiome.

High content analysis (HCA) was used to investigate persistent organic pollutants (POP) mixtures for cytotoxic effects on EE cells and endocrine disruption via (ant)agonism of the glucagon-like peptide 1 receptor (GLP-1R). The POPs concentrations tested reflected the actual levels measured in Scandinavian human blood (total mix). A perfluorinated (PFAAs) mixture, a brominated (Br) mixture, a chlorinated (Cl) mixture containing polychlorinated biphenyls and also hexachlorobenzene, p,p'-dichlorodiphenyldichloroethylene, three hexachlorocyclohexanes, three chlordanes, and dieldrin were tested.

No (ant)agonism of the GLP-1R was observed following exposure to any of the POP mixtures. Cytotoxic analysis of EE cells in a 3 h exposure presented significant reductions in cell number at the higher concentrations of the Br, PFAAs + Br and PFAAs + Cl mixtures. Additionally, mitochondrial membrane potential was significantly reduced by the PFAAs and Br mixtures at the highest concentration. A 24 h exposure resulted in significant decreases in cell number (all POP mixtures) and nuclear area and mitochondrial membrane potential (PFAAs, Br and PFAAs + Br mixtures). The observed effects suggest that apoptosis may be occurring. Therefore, while the POP mixtures did not disrupt GLP-1 hormone receptor interaction, the toxic impact on the (EE) cells, could result in reduced function of gut hormone signalling such as GLP-1, potentially increasing obesity and diabetes risk.



## Statistical modeling predicts risk associated with microbiome of food manufacturing plants

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Food manufacturing plants dealing with dairy or meat products are constantly challenged by the risk of pathogenic outbreaks unless proper control measures are taken. In addition to storage and refrigeration, the daily cleaning and sanitizing procedures of the environment at the processing facilities might lead to the adaptation of certain microbes and can lead to persistent contamination. Here, we illustrate the power of 16S rRNA gene sequencing and statistical modeling in predicting risk in processing plants.

In this study, routine samples from the processing plant environment, raw materials, and the end products were taken through two years. The samples were analyzed by high-throughput sequencing of 16S rRNA gene amplicons containing the V3-V4 region. The microbiome was analysed using Mothur. Using rigorous statistical modeling approaches, the risk associated with each sample was analysed as a function of the respective microbiome.

We found that the overall microbiome differed between a dairy plant and a meat plant, possibly due to the innate bacterial community preferences in milk and meat samples and their effects on the whole plant in general. Statistical modeling showed that risk in a dairy plant could be associated with certain genera (Ex: *Butyrivibrio* and *Brucella*) while risk in a meat plant could be attributed to genera such as *Weissella* and *Tatumella*. We conclude that statistical modeling on microbiome of food manufacturing plants can predict risk and any changes in certain bacterial genera can adversely affect the food quality.

## Analysis of the Chemical Safety of Insect Consumption as Alternative for the Increasing Food Requirements

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The continuously growing world population is expected to more than double the demand for meat products by 2050, creating an urgent need for alternative and safe food supplies from sustainable sources, such as the consumption of insects, or entomophagy. However, health hazards including (organic) chemical contaminants are not sufficiently identified nor monitored to guarantee health-safe end products. As such, the development of a unique, broad-range extraction and detection technique, specific for insect tissues was pursued. Assisted by a fractional factorial design, generic extraction techniques and UHPLC-Q-Orbitrap™-HRMS detection parameters were meticulously optimized for 4 insect species (yellow mealworm, grasshopper, house cricket and black soldier fly) for a large spectrum of pesticides (n=25), (veterinary) drugs (n=29), and mycotoxins (n=25). To prove the method 'fit-for-purpose', a successful validation was performed, both qualitatively, by determining the screening detection limit (SDL), selectivity and specificity, as well as semi-quantitatively, by assessing the repeatability (relative standard deviation (RSD)) and recovery. For both the mealworm and grasshopper, 60 compounds were detected at the respective SDL level, compared to 56 and 46 compounds in the cricket and black soldier fly, with RSD-values in line with European regulations (CD 2009/90/EC). Only 7, 6, 10 and 19 compounds were partially detected, while the remaining respective 3, 4, 3 and 5 compounds could not be detected following extraction of spiked insect tissues. Eventually, the effectivity of the screening methodology was demonstrated on real insect samples, reared under different conditions, and revealed only small traces for a minority of the contaminants of interest.

## Novel insights in the mitigation of deoxynivalenol through baking under real industry conditions

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Mycotoxins are toxic secondary metabolites produced by fungi which commonly occur in food and pose a health risk to the consumer. To protect the consumer from harmful effects, the European Commission set maximum levels for certain mycotoxins in food. Deoxynivalenol (DON), a type B trichotecene, is the most prevalent mycotoxin in cereal commodities. Its toxic effects include acute temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness and fever.

The effect of the baking process on the DON content is controversial. Although most studies reported a reduction of the DON content, the reduction rates were highly variable. isoDON, norDON A, norDON B and norDON C were identified as degradation products of DON in food matrices. As norDON A, B, C were shown to be less toxic than DON, DON might be partially detoxified during baking. However, it is not clear to which extent DON is converted into its less toxic norDON derivatives and whether all DON degradation products have been identified so far.

We hypothesized that i) DON is degraded during food processing and ii) the degradation products exhibit different toxicity than the parent compound. The objectives of this study were to i) identify and quantify all degradation products of DON that are formed during food processing under real industry conditions and ii) to assess their toxicity. In this study, the complete fate of DON during baking under real industry conditions was elucidated, which is the first step to a risk assessment of DON including all its degradation products.

## Biofluid and tissue metabolic profiling by 1D-1H-NMR spectroscopy highlighting exposure to emerging drugs of abuse.

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Keywords: NMR metabolomics, SARM, food safety

Selective androgen receptor modulators (SARMs) have been investigated within drug discovery fields due to their tissue selectivity potential. This property provides for the possibility for these chemical agents to be misused to artificially enhance animal performance both in livestock production and in sports. One approach to detect SARM misuse focuses on the use of metabolomics biomarker discovery to identify metabolites highlighting biological effects of drug administration which subsequently can be applied in screening detection analyses. An in vivo exposure study within a rodent model was used to extrapolate biological effects in mammalian species of three different SARMs of particular concern. Animals were randomly allocated to four study groups (n=8 per cohort) and over a 17 day period treated as indicated via oral gavage - control (vehicle), Ostarine (3 mg/kg bodyweight), LGD-4033 (3 mg/kg) and RAD140 (3 mg/kg). Biofluid and tissues obtained both during and at the end of the treatment period were subjected to metabolomic profiling analysis by 1D 1H NMR. Acquired data was processed using Bayesil, and multivariate data analysis techniques applied to build descriptive and predictive models, enabling putative exposure biomarkers to be selected and confirmed through online database searches. Ongoing work is focused on the identification and biological interpretation of selected metabolite markers with a view to developing analytical methods to widen the currently limited window for SARM detection in food producing animals.

## Effects of endocrine disruptors on estrogen signaling in zebrafish larvae.

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Among the toxic environmental chemicals that are resistant to environmental degradation, per-fluorinated, -chlorinated and -brominated compounds are the most persistent organic pollutant (POPs). Due to their lipophilic nature, they enter the food chain and bioaccumulate from simple to complex organisms. They are widely detected in human blood and various tissues, and have been associated with various pathological conditions including endocrine disruption (ED).

Zebrafish will be used as model organism due to their fast development, transparent embryos, low cost, high offspring rate and small size. We investigate the mechanism behind the detrimental effect of EDs specifically on the estrogen receptors in developing zebrafish larvae by studying their modulatory effect on morphology, survival, and gene expression. We will test both pure compounds and mixtures (POPs) found in human blood and mother's milk in Norway. Studying mixtures will make our study more realistic as real exposure to such chemicals is mostly in a mixture scenario. Furthermore, we will test the function of each of the estrogen receptors ER ( $\alpha$ ,  $\beta$ , GPER) in the observed effects using specific agonists and antagonists, and using mutant lines for ERs obtained through CRISPR/Cas-9 system. Using tissue-specific fluorescent zebrafish lines, we will isolate specific cell types by FAC sorting to analyze modulation of gene expression by estrogenic compounds in these tissues.

We will show preliminary results testing pure compounds, mixtures, and mixtures with antagonists on zebrafish larvae. Although primarily addressing basic research questions, our results will be relevant for human health and diseases such as cancer.

## Intestinal toxicity of mycotoxin mixtures

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Mycotoxins are toxic secondary metabolites of fungi that colonize various agricultural products. These toxins are, therefore, natural food contaminants. Complex mixtures of mycotoxins are naturally present in food, since the same food commodity can be colonized by different fungal species, and because one fungal species can produce several mycotoxins. The toxicity of mixtures cannot always be predicted based upon their individual toxicities and mycotoxin mixture can result in antagonistic, additive or synergistic effects. In our lab, we investigated the toxic effect of mixtures of trichothecene in the intestine using both epithelial cells and jejunal explants. The interactions between the mycotoxins were analyzed using the isobologram - combination index method. In intestinal epithelial cells from human and porcine origin, we observed that the cytotoxic effect of several mycotoxin mixtures was synergistic, especially at low doses. The synergy was further confirmed in pig jejunal explants, where the combined presence of deoxynivalenol and nivalenol promoted a synergic inflammatory response as measured by the level of mRNA encoding for IL-1 $\alpha$ , IL-1 $\beta$ , IL-8, IL-17A and IL-22. Taken together, these results indicate that the simultaneous presence of mycotoxins in food commodities and diet may be more toxic than predicted from the mycotoxins alone. This synergy should be taken into account in the risk assessment of mycotoxins.

## PRESENCE OF PYRROLIZIDINE ALKALOIDS IN TEAS AND HERBAL INFUSIONS: PRELIMINARY MONITORING AND RISK CHARACTERIZATION

**Ms. Elisabetta Caprai<sup>1</sup>**, Ms. Emanuela Ammoni<sup>2</sup>, Mr. Giuseppe Polonini<sup>1</sup>, Ms. Maria Grazia Caschetto<sup>1</sup>, Ms. Stefania Bonan<sup>1</sup>

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Pyrrolizidine alkaloids (PAs) are secondary metabolites produced by over 6000 plant species worldwide, mainly from the botanical families Boraginaceae, Asteraceae and Fabaceae. In animal studies, some PAs have proven to be genotoxic carcinogens.

An analytical method for the determination of PAs in teas and herbal infusions was developed in-house and allowed to detect up to 28 PAs. Analysis consist of an acidic/organic extraction and SPE clean up.

Identification and determination of PAs are performed by liquid chromatographic separation and mass spectrometric detection. LOQs values for all PAs were 3 µg/kg.

For a preliminary monitoring of PAs in teas and herbal infusions, 50 samples were collected within the Italian market. Samples included teas and herbs for hot infusion drinks. PAs content of each sample was calculated as the sum of the concentrations of all detected analytes.

Concentrations of PAs in individual samples ranged from 3 µg/kg to over 1000 µg PAs/kg of dried matter.

Most contaminated samples were black tea and miscellaneous herbal infusions. The main average contributors to the total PAs concentrations in samples were retrorsine, lycopsamine, senecionine isomers and their N-oxide forms.

Occurrence resulting from analysis was used to calculate a provisional chronic exposure assessment due to the consumption of PAs containing teas and infusions. Assessment was carried out assuming a daily ingestion of PAs deriving from 2g of tea/infusions. Average and worst case scenarios were assessed for adults and children for a preliminary risk characterization using the MOE approach and assuming equal toxicological potency for all alkaloids.

## Neonatal exposure to soy phytoestrogens decreases fertility in mice.

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Soy food is rich in phytoestrogens, especially genistein (GEN), which can interfere with the endocrine system causing beneficial or detrimental effects according to doses, sex and exposure age. During developmental critical periods (as in the first postnatal week), it may disrupt the formation of specific steroid-sensitive neuronal circuits (e.g. NOS and arginine-vasopressin system), causing irreversible damages, even at low doses. Since those circuits interact with catecholamines, we investigated whether early postnatal GEN treatment, with a dose comparable to the exposure levels in babies fed with soy-based formulas, may affect tyrosine hydroxylase (TH, a marker for catecholaminergic neurons) expression in mice. GEN treatment had no effect on TH+ cell density in mesencephalic neurons, modulating motor control and rewarding behavior, nor in some hypothalamic areas as paraventricular nucleus, in which nNOS and arginine-vasopressin neurons were affected. However, in females, it caused a permanent change in a subpopulation of hypothalamic TH+ neurons in the anteroventral periventricular region, which is part of the hypothalamic- pituitary –gonadal axis. Interestingly, this effect was not mimicked by estradiol treatment, indicating that it may not be mediated by the binding to an estrogen receptor.

Moreover, we analyzed reproductive peripheral data: gonadal hormonal levels, affected by GEN treatment in both sexes. Moreover, females had irregular estrous cycles and higher density of tertiary branches in the mammary glands, a hallmark of diestrous.

Present and past results indicate that GEN exposure in early postnatal life may result in permanent alteration of several widely diffused neurotransmitters' systems, which may decrease fertility.



## In vitro endocrine disrupting effect of Persistent Organic Pollutants (POPs) on the estrogen receptor (ER).

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Persistent organic pollutants (POPs) are chemicals that persist in the environment over long periods of time, with the potential to biomagnify in the food chain and bioaccumulate in the body. POPs such as organochlorine pesticides and PCBs are long suspected carcinogens. Various studies have shown a correlation between POPs and estrogen related tumours, particularly breast cancer in women (Arrebola et al. 2016). In the past, researchers have focused on the investigation of individual chemicals or POP's in relation to their adverse health effects. In real life however, humans are exposed to a cocktail of chemicals that can have synergistic cellular effects, even at sub lethal concentrations (Rainey et al. 2017). These chemicals, including halogenated compounds, may potentially act as endocrine disruptors, mimicking endogenous hormones through binding to their receptors and either blocking them (antagonism) or adding to the effect of endogenous hormones (agonism).

This study aims to determine the in vitro effect of an environmentally relevant mixture of POPs, modelled from the Scandinavian population (Berntsen et al. 2017). Our group has previously shown the interaction of these POPs and their mixtures on the glucocorticoid receptor (GR) (Wilson et al. 2016). In this study, effects at the level of the estrogen receptor will be investigated. The mixture, containing 29 POPs and prepared according to concentrations found in human blood, will be dose dependent tested within a range including above and below blood levels by mammalian reporter gene assay for agonism and antagonism of the estrogen receptor. Cytotoxicity will also be assessed.

## Charactering Exposome of Food Contamination and Chinese Total Diet Study

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The project of Charactering Exposome of Food Contamination and Chinese Total Diet Study under China National Key R&D Programms was just granted by Ministry of Science and Technology of P.R. China. In this 4 years project, some novel measurement and modeling approaches for characterizing humen exposure to food contaminants, along the source-to-outcome pathway, will be developed by 6 tasks in this project.

In task 1, the 6th China total diet study will be conducted in 24 provinces of China. Based on the results of this study, the dietary exposure assessment of the traditional and emerging pollutions of Chinese main foods will be updated.

In task 2, a national biomonitoring will be conducted to measure these food pollutants or their metabolites in biological specimens of the populations from the provinces involving in the total diet study. The biomonitoring will focus on the early life exposures.

In task 3, the bioaccessibility of POPs, heavy metals and their forms and perchlorate in food will be investigated to make a more accurate dietary exposure assessment.

In task 4, the PBTK modelling platforms and parameters of 25 typical food pollutants will be established to investigate the link between dietary exposure and internal exposure levels.

In task 5, the exposure biomarkers and effect biomarkers for dioxins, cadmium, perchlorate, BFRs and PFASs will be explored using metabolomics approaches.

In task 6, the dose-effect relationship for dioxins, cadmium, perchlorate, BFRs and PFASs will be explored based on the environmental epidemiological study to propose benchmark doses for these pollutants.

## The role of environmental contamination in the production of veterinary drug residue non-compliant animals

**Dr. Terence Fodey<sup>1</sup>**, Dr. Steven Crooks<sup>1</sup>, Mr Wesley Smyth<sup>1</sup>, Mr Paul Barnes<sup>1</sup>

<sup>1</sup>*Agri-Food and Biosciences Institute, Belfast, United Kingdom*

Previously, using the non-steroidal anti-inflammatory drug phenylbutazone as a model, it has been shown that untreated cattle can easily become contaminated with non-compliant levels of veterinary drug residues. Although European Union legislation prohibits the use of phenylbutazone in food producing animals, 28% of bovine non-compliances for non-steroidal anti-inflammatory drugs between 2008 and 2014 were attributed to phenylbutazone.

A range of contamination sources such as feeding vessels, contact with treated animals and grazing on pasture previously grazed by treated animals has been identified as potential causes of non-compliance. In this study the source of contaminated pasture was investigated further but with incorporation of normal farming practices. The study demonstrated that the common practice of storing animal waste over winter months before spreading on farm pasture in the spring can cause subsequent grazing cattle (untreated) to present as non-compliant if the waste has been produced by treated animals. It was also determined that this contamination, which can persist over a significant period, may be due to the ingestion of as little as 30 µg of phenylbutazone by a 500 kg bullock. Ultra-high-performance liquid chromatography coupled with mass-spectrometric detection was employed to analyse bovine plasma samples for the presence of detectable residues ( $CC\alpha = 0.28 \text{ ng ml}^{-1}$ ) of phenylbutazone.

## The potential of an untreated cow presenting non-compliant florfenicol residues in milk

**Dr Terence Fodey<sup>1</sup>**, Dr. Steven Crooks<sup>1</sup>, Mr Dermot Faulkner<sup>1</sup>, Mr Colin Thompson<sup>1</sup>, Mr Paul Barnes<sup>1</sup>

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Florfenicol, is a synthetic antibiotic and a fluorinated, methyl-sulfonyl derivative of the antibiotic chloramphenicol. While chloramphenicol is prohibited from use in food producing animals in the European Union, florfenicol may be used in accordance with a range of established maximum residue levels that are related to various species and tissue types. However, florfenicol is not permitted for use in animals producing milk or eggs for human consumption and for these sample types any detectable concentration of the drug would represent a non-compliance with European Union legislation. Regulatory laboratories within the European Union occasionally detect residues of unauthorised veterinary drugs, possibly through illegal use or human error. However, it has also been shown previously that animals which have not been treated with a drug may produce non-compliant samples through association with treated animals. The objective of this study was to determine if housing an untreated milk producing animal with other cattle treated with florfenicol could give rise to detectable levels of the drug in milk and therefore non-compliant samples. Using a commercial product, three male bovines were treated with a therapeutic dose of florfenicol and housed with a milk producing cow known to be free from florfenicol residues. Milk samples were collected twice daily and analysed for the presence of the drug (florfenicol and its metabolites measured as florfenicol amine) using an immunochemical screening method and confirmed using a MRM3 UPLC-MS method with a CC $\alpha$  of 0.2  $\mu\text{g kg}^{-1}$ . Results and recommendations will be presented at the conference.

## Creation of a virtual reference laboratory to support an EU-China laboratory network for food safety

**Ms. Susan MacDonald**<sup>1</sup>, Mr Sean Panton<sup>1</sup>, Dr Martin Rose<sup>1</sup>

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This poster will describe the plan for the development of a virtual 'Reference Laboratory 2010' (RL2020) involving EU and Chinese scientists and technicians to be developed within the Horizon 2020 project 'EU China-Safe' (Grant 727864). This will use web-based communication and a common IT platform to allow analytical data to be accessed and processed remotely by any member of the network. It will also be a shared resource with details of best practice with standard operating procedures; validation documents; quality control measures and food and feed safety regulations from the EU and China. It will be used as a learning resource hub allowing best practice from both China and the EU to be shared. Staff will be able to work on the same analytical processes, it is envisioned that data outputs generated will be converted to allow transfer between laboratories, meaning outputs from one continent can be processed in another. This will result in an ability to track commodities traded in both directions and view their compliance status, allowing for the first time, true mutual recognition of control measures for food safety and reducing barriers to trade.

The poster impact will be to showcase the potential practical value that will be obtained from EU China Safe project, demonstrating co-operation and collaboration to produce a joint improved mechanism for food safety controls. It fits with Theme 3 as RL2010 will be used to measure and control chemical contaminants in food, thus reducing the risk to consumers in 2 continents.

## A SEMI-QUANTITATIVE UHPLC-MS/MS ASSAY TO DETECT SARMS IN URINE SAMPLES

**Emiliano Ventura**

A SEMI-QUANTITATIVE UHPLC-MS/MS ASSAY TO DETECT SARMS IN URINE SAMPLES

E. VENTURA\*<sup>1</sup>, A. GADAJ<sup>2</sup>, GAIL MONTEITH<sup>1</sup>, ALEXIS RIPOCHE<sup>1</sup>, TOM BUCKLEY<sup>2</sup> AND M. H. MOONEY<sup>1</sup>

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Selective androgen receptor modulators (SARMs) are an emerging class of performance-enhancing chemicals, many of which are in drug development phases of investigation as potential therapeutics for a variety of clinical indications. SARMs, in comparison to anabolic androgenic steroids (AAS), exhibit higher tissue-selectivity for the androgen receptor, lack of undesirable androgenic-side effects, and are orally bioavailable. These properties together with associated anabolic effects including increases in mass density of bone and muscle underline why SARMs have gained recent popularity as drugs of abuse. As a consequence of the possible use of SARMs in illicit practises, analytical strategies are required that can be applied both in animal sports to monitor for doping practices, and in farm livestock to ensure food is free from related contaminating residues. In this study, a semi-quantitative UHPLC-MS/MS method was developed to monitor the misuse of 15 SARM compounds belonging to eight different families, in bovine, equine and canine urine, respectively, following liquid-liquid extraction with TBME. The method was validated according to the Community Reference Laboratories Residues (CRLs) 20/1/2010 guidelines with CC $\beta$  values determined at 1 ng mL<sup>-1</sup>, excluding andarine (2 ng mL<sup>-1</sup>) and BMS-564929 (5 ng mL<sup>-1</sup>), in three different species. This rapid, simple and cost effective semi-quantitative assay is currently being employed for screening of bovine, equine and canine urine to determine the potential level of SARMs abuse in stock farming and competition animals, ensuring consumer safety and fair play in animal performance sports. Acknowledgement: The research was supported by funding from the European Union Horizon 2020 Research and Innovation Action under Grant Agreement No. 642380.

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## Assessment of Microbiological and Residual Antibiotics Status in Milk Sold in Abeokuta, Ogun State

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Antibiotics are used extensively in the dairy sector to enhance health and productivity of food animals. Presence of antibiotic residues above maximum limit is of serious public health concern. There is paucity of data on antibiotic residues in milk sold in Nigeria. This study investigated the prevalence and concentration of antibiotic residues in twenty brands of milk. Isolation and identification of bacteria were done using aerobic plate count and 16S rRNA gene sequencing method. Antibiotic sensitivity, plasmid profile and antibiotic residues were done using disc diffusion, alkaline lysis and HPLC with ultraviolet detection at 355nm respectively. Aerobic plate counts ranged from  $2.5 \times 10^2$ -  $6.5 \times 10^2$  CFU/mL for evaporated milk and  $2.5 \times 10^1$  -  $6.0 \times 10^1$  CFU/mL for powdered milk. Ten bacterial species were identified as *Bacillus subtilis* (25 %), *Lactobacillus fermentum* (20 %), *Bacillus mycoides* (12.5 %), *Lactobacillus plantarum* (10 %), *Bacillus megaterium* (10 %), *Lactobacillus acidophilus* (10 %) *Lactobacillus brevis* (5 %), *Lactobacillus alimentarius* (2.5 %), *Lactobacillus delbrueckii* (2.5 %) and *Micrococcus luteus* (2.5 %). Isolates were sensitive to erythromycin, cefuroxime, and gentamycin at 95 % and resistant to tetracycline and ampicillin (12.5 %), ofloxacin (10 %), doxycycline and penicillin (7.5 %). Drug resistant isolates were subjected to plasmid profiling. Antibiotic residues were detected in 10 milk brands analysed. Residual levels of tetracycline, oxytetracycline and chlortetracycline ranged between 5ng/kg – 1569ng/kg. Presence of antibiotic residues in evaporated and powdered milk is an indication that the public is exposed to the chronic effects of the residues.

## After-effect of Agriculture Chemicals on humans.

**Mr. Segun Michael<sup>1</sup>**

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The use of chemicals in preventing and curing the outbreak of plant/crop disease in agriculture is inevitable. Chemicals used in this regard penetrate crops and soil where it is applied. Humans consumption of chemically-treated crops are exposed to adverse effects.

The recent outbreak of maize 'armyworm' disease in Nigeria, my home country and other countries in Africa last year was a huge challenge to farmers who experienced great loss during planting and harvest season.

The farmers had to use pesticides on their farmland to minimize loss as a result of the armyworm disease, as a last resort.

The pesticide would penetrate the maize and soil in which it is applied and pose a health risk for consumers of the maize even when it is properly cooked.

Maize is a staple food in many Nigerian homes due to economic recession and inability of many financially-disadvantaged families to afford decent and balanced diet meals due to the fact that those families live on an income of less than a dollar per day.

The armyworm disease that affect maize have been curtailed though the after effect of the pesticides used in the process cannot be overemphasized.

Chemicals are also used in animal husbandry sector of agriculture to eradicate swine flu that affects pigs in Nigeria.

I am of the opinion that safe chemicals should be made use of in agriculture to eradicate its after-effect of harmful disease to humans especially cancer disease which result in loss of lives of hundreds of people per day.



## Production of chemical-free crops: Organic Fertilizer Use among Garden Egg Farmers in Enugu State, Nigeria

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Food is getting the blame as the cause of many illnesses these days. Scientists and public health advocates believe that a number of increasingly common problems that afflict children are linked to exposure to toxic chemicals and pesticides use in cultivation. Research shows that organic food can be more nutritious for humans. The use of organic fertilizer ensures that garden egg (*Solanum melongena*), a fruit rich in vitamins and minerals is free of harmful chemicals. The end consumers who eat this organic product are less prone to diseases such as cancer, strokes and skin disorders among others. I have had the experience of working closely with garden egg farmers who use organic fertilizer in their farms. I observed farmers used more of farmyard manure because it was more accessible and less time consuming. Although the quantity of organic fertilizer applied was not up to the recommended rate which made production low, farmers preferred using it because, the garden egg produced is more tasteful and keeps longer at room temperature. However, farmers encountered constraints in the use of organic fertilizer like: lack of skill, time consuming, difficulty in collection and handling of organic fertilizer. It was noticed that farmers have been using organic fertilizer in an average of six years at the point of field experience (2017). Efforts are made to encourage farmers in the use of organic fertilizer not only in garden egg production but also in other crops through the Department of Agricultural Extension outreach programme where I lecture.

## Consumer Perceptions of Endocrine-Disrupting Chemicals in the Food Chain and the Environment: A Critical Review.

**Ms. Alana Cutliffe<sup>1</sup>**, Dr. Lisa Connolly<sup>1</sup>, Prof. Moira Dean<sup>1</sup>

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### Background:

Endocrine-disrupting chemicals (EDCs) are those with the potential to interfere with our hormones. Found in certain plastics, pesticides, heavy metals and industrial pollutants, they often come in contact with our food. An increasing amount of research links EDC-exposure with adverse health-effects; there is, however, limited research exploring consumer understanding of EDC's.

### Aim:

This study critically reviewed past studies assessing "Consumer Perceptions of EDCs".

### Methods:

31 papers (published from 2000 onward, in English and available in full-text) were identified through searching six databases (i.e. "PubMed"; "Web of Science"; "PsychINFO"; "EMBASE"; "MEDLINE"; and "Maternity and Infant Care") using keywords such as: "endocrine-disrupting chemicals"; "environmental contaminants"; "perceptions"; "consumer"; "safety"; "food"; and "pregnant".

### Results:

Consumers were unfamiliar with EDCs, and few were aware of the putative health effects of these chemicals; misconceptions were also common. While the media / the Internet were common EDC-related information-sources, consumers trusted information from clinicians and scientists more. Consumers thought EDCs were dangerous and a significant proportion was willing to engage in risk-reduction behaviours. Key factors predicting consumer engagement were: perceived normative pressure; perceived severity; perceived susceptibility; pre-existing beliefs; trust in information-source; and food-shopping habits. The main barriers were: low prioritization of EDCs; difficulties in assessing the risk associated with these chemicals; and self-efficacy.

### Conclusions:

Results highlight a general lack-of-knowledge about the putative health effects of EDCs and their sources-of-exposure. Significant proportions of consumers are motivated to engage in risk-reduction behaviours. Strategies to overcome barriers to appropriate risk-reduction behaviours are required; these warrant further investigation.

## Mycotoxycological safety assessment of complementary foods consumed by infants and young children in Nigeria

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This study assessed the mycotoxycological risks associated with consumption of complementary foods by infants and young children (IYC) in Nigeria. Moulds and mycotoxins in 137 industrially- and household-processed complementary food samples from voluntary households in Lagos and Ogun states of Nigeria were determined by molecular and LC-MS/MS methods, respectively. IYC exposures to the mycotoxins were estimated by the deterministic approach. Diverse potentially toxigenic Aspergilli were identified in the foods. Twenty-nine major mycotoxins and derivatives, in addition to other 112 microbial metabolites occurred in the samples. Aflatoxins and fumonisins were more frequently quantified in the foods, at mean concentrations exceeding the EU limits 0.1 and 200 µg/kg set for processed baby foods. About 20% of commercial milk-based foods contained beauvericin and chloramphenicol (a bacterial metabolite). T-2+HT-2 toxins were found only in infant formula and at levels that exceeded the 15 µg/kg limit set by the EU. The household-processed complementary foods contained significantly ( $p < 0.05$ ) higher toxin levels than the industrially-processed foods. The recommended tolerable daily intake (TDI) values for the mycotoxins were exceeded by the estimates from this study. The application of good pre- and post-harvest practices as regards processing of the raw grains, routine surveillance and monitoring of processed foods, country-level mycotoxin legislation are urgently required to forestall outbreaks.

**Keywords:** aflatoxins; complementary foods; exposure assessment; fumonisins; infant nutrition; multiple mycotoxins.

**Response:** I have been working on mycotoxins for more than 10 years, and I have given oral talks on mycotoxin-exposure related topics at the World Mycotoxin Forum (2014 and 2018).

## Correlating urinary mycotoxin concentrations with dietary intake

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Cereal-based foodstuffs for human consumption are often contaminated with diverse patterns and amounts of mycotoxins that may exert mild to severe health implications. This paper presents the association between multiple urinary mycotoxin biomarker levels assessed from two consistent days as first morning urine and corresponding toxin concentrations in maize-fufu consumed the evening before in Bamunka village, Cameroon. Urinary and food mycotoxin levels were measured using innovative LC-MS/MS based methods. Twelve mycotoxins including aflatoxin M1 (AFM1), fumonisin B1 (FB1), deoxynivalenol, nivalenol, citrinine, and zearalenone including the key metabolites  $\alpha$ - and  $\beta$ -zearalenol were quantified in urine. AFM1 levels in urine (day 1: mean 0.026, range 0.004-0.09  $\mu\text{g L}^{-1}$ ; day 2: mean 0.21, range 0.008-0.5  $\mu\text{g L}^{-1}$ ) reflected presence of AFB1 in fufu (mean 0.6, range n.d-0.6  $\mu\text{g kg}^{-1}$ ). Likewise, urinary FB1 amounts (day 1: mean 0.005, range 0.0001-0.02  $\mu\text{g L}^{-1}$ ; day 2: mean 0.02, range 0.004-0.07  $\mu\text{g L}^{-1}$ ) reflected levels in fufu (mean: 140, range 48-709  $\mu\text{g kg}^{-1}$ ). The levels of quantified toxins in urine correlated with the amounts in food, with progressive excretion of each metabolite in urine from day 1 to day 2.

Keyword: food safety, exposure assessment, mycotoxin mixtures, human urine, SSA, excretion kinetics

## Effects of Defined Human-Exposure-based Mixtures of Persistent Organic Pollutants on Androgen Receptor Translocation and Transactivation.

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Endocrine disrupting chemicals (EDCs) are exogenous substances/mixtures that alter endocrine function causing adverse health effects. Persistent organic pollutants (POPs), a class of EDCs, are highly resistant to degradation and remain in the environment for long periods of time. Our aim is to study the effects on androgen receptor (AR) translocation and transactivation of a defined POP mixture that consists of 29 compounds modelled from human exposure levels based on a Scandinavian population.

The study assessed a total mixture and 6 sub-mixtures; i) perfluorinated (PFC) mix, ii) brominated (Br) mix, iii) chlorinated (Cl) mix, iv) Br-Cl mix, v) PFC-Br mix, and vi) PFC-Cl mix, and 6 individual PFCs, ranging from 1/10x to 500x exposure levels relative to blood level. High content screening (HCS) analysed translocation activity of the AR in fluorescently-tagged AR U2OS cell lines. Reporter gene assay (RGA) analysed transactivity of luciferase-tagged AR TARM-Luc cell lines. Cytotoxic analysis was performed concomitantly with each assay for sample control.

No agonism was detected. Antagonism was detected for AR translocation upon exposure to Cl mix 1/10x, 1x, 50x, and PFC-Br mix at 1/10x, 1x, and 50x. PFOS 500x and Br-Cl mix 100x and 500x significantly antagonised androgen receptor transactivation as determined by RGA. Interestingly, some compounds/mixtures worked synergistically with testosterone and upregulated translocation (PFOS 1/10x, 100x, and 500x; PFOA 1/10x; and PFNA 1x and 500x) and transactivation (PFC mix 1x and PFDA 500x).

This study concludes sub-mixtures and individual compounds of a defined human-exposure-based POP mixture interact with the AR thus warranting future investigation.

## boosting female farmers 'income from poultry production in Nigeria through bio-control of aflatoxins in maize

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Aflatoxins are secondary metabolites (toxins) produced by toxigenic 'Aspergillus flavus' and 'A. parasiticus' that contaminate crops in the field during harvesting and post-harvest storage leading to post-harvest losses and reduction in daily income of farmers in Nigeria. The presence of aflatoxins in foodstuffs is an important concern for human and animal health. Aflatoxins are also suspected of causing a variety of human diseases, including some forms of cancer. It has been estimated that aflatoxin-contaminated grains cost grain handlers several hundred million dollars annually. Hence, our desire to contribute to the reduction of such losses in Nigeria where humans and animals are highly susceptible to aflatoxicosis. Therefore, controlling aflatoxins in maize will boost maize production for food security and wealth creation in Nigeria. In the specific research studies, Bio-control agents was used to control the aflatoxin using the dried under-utilized medicinal plant 'Lemon grass' (*Cymbopogon citratus*) which was used in lining the packaging material to protect the crop from post-harvest losses due to Aflatoxin, because Lemon grass has been discovered as a good aflatoxin binder

## A novel approach to reduction of toxigenic *Aspergillus flavus* level in maize

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The Influence of different drying technique on the reduction or elimination of toxigenic *Aspergillus flavus* level of maize seeds collected from Ondo State, Nigeria were investigated. Different drying method were used such as the use of constructed wooden solar dryer lined with aluminium foil, constructed wooden solar dryer lined with black polythene, calabash (*Crescentia cujete*), tray lined with plantain (*Musa paradisiaca*) leaf, tray lined with *Thaumatococcus daniellii* leaf. Sterile maize seeds were inoculated with spore suspension of toxigenic *Aspergillus flavus* for three weeks. After three weeks of drying, inoculated maize grains dried with calabash was found to have the lowest spore levels of *Aspergillus flavus* with the aid of counting chamber (950,000 sfu/ml) as compared to the value of spores counted from the other drying method used and inoculated maize dried with tray lined with *Thaumatococcus daniellii* leaf had the highest spores counted (2,350,000 sfu/ml). There was continuous decrease in the values of spores counted from inoculated maize grain dried with plantain (*Musa paradisiaca*) leaf and maize grain dried with calabash. Slight fluctuation occurs in the sporulation level of the maize from other drying method. Solar dryer lined with aluminium foil was more effective in reducing the sporulating ability of *A. flavus* than solar dryer lined with black polythene (3,600,000, 5,300,000, 1,550,000 and 7,000,000, 8,100,000, 2,000,000 sfu/ml respectively). From the research, it can be concluded that the use of traditional calabash and solar dryer lined with aluminium foil were very effective in reducing toxigenic *Aspergillus flavus* level in maize.

## Designing a roadmap for mycotoxin prevention in food and feed.

**Mrs. Lynsey Finlay<sup>1</sup>**

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Mycotoxins are a diverse group of biologically active toxic secondary metabolites produced mainly by filamentous fungi. The key toxins are identified in families which include aflatoxins ochratoxins, fumonisins, trichothecenes, zearalenones and ergot alkaloids. Currently there are over 300 known mycotoxins and these may co-occur in feed or food. The main aim of this study was to conduct a systematic review of the methods of analysis for mycotoxin determination whereby co-occurrence can be addressed. The concept was to rank these methods in suitability for detection to limit exposure but also to rank for use in defined fields based on suitability for the end user as a prevention and mitigation tool. A systematic literature review was performed from various databases, internet searches and through the examination of policy papers to identify the different mycotoxins and mycotoxin mixtures affecting food and feed on a global scale. The literature has identified numerous methods of analysis that can be used for mycotoxin detection incorporating physicochemical, immunological and molecular techniques. The methods of analysis were ranked and a road map developed for their suitability in use for the detection of multiple mycotoxins. The current lab state of the art technique is liquid chromatography coupled to tandem MS (LC-MS/MS) though this method is not field deployable and affordable in all regulatory environments. One (bio)analytical method may not be suitable for all circumstances and therefore a roadmap of the current methods of analysis available would enable strategies for analysis to be implemented in different scenarios.



## The endocrine disrupting potential of Bisphenol-A and its structural analogues using glucocorticoid and progesterone receptors.

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The most widely used plasticiser to date has been Bisphenol-A (BPA). With BPA now being recognised as an endocrine disruptor<sup>1</sup>, analogues such as Bisphenol-F (BPF) and Bisphenol-S (BPS) are being introduced as alternatives<sup>2</sup>. However, whether these analogues are safer remains to be fully investigated. Studies into their potential to act as endocrine disruptors have mainly focused on the estrogen, androgen and AhR receptors. This study aims to assess their interactions with the glucocorticoid (GR) and progesterone (PR) receptors.

BPA, BPF and BPS (at concentrations ranging from 0.02-200  $\mu$ M) were assessed for (ant)agonism of the GR and PR receptors by mammalian reporter gene assay (RGA), using the glucocorticoid responsive TGRM-Luc and progesterone responsive TM-Luc cell lines, respectively. Cytotoxic analysis was performed in parallel using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) cell viability assay.

Agonism was not detected in the GR or PR receptor at any of the concentrations tested. However, a decreased transactivation response was detected in the GR and PR receptor for all compounds (BPA, BPF and BPS) at their highest test concentration of 200 $\mu$ M. However, this reduced transactivation is most likely attributed to reduced cell viability as determined by the MTT assay.

This study concludes that BPA, BPF and BPS do not antagonise the GR or PR receptor and are cytotoxic to the GR and PR cell lines at the highest concentration tested. BPA alternatives should be investigated further for endocrine disrupting potential of other target pathways.

## Rapid discrimination of Bitter Almonds based on their amygdalyn content by using Near Infrared Spectroscopy

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Amygdalyn is a cyanogenic diglucoside found in bitter almonds (*Prunus amygdalus*) which can be hydrolyzed after consumption into hydrogen cyanide. Because of the potential health hazard associated with the ingestion of this compound, the food industry is looking for methodologies which allow differentiation between sweet and bitter almonds. This study aims to use NIRs to discriminate both types of almonds. HPLC-DAD analysis was used to quantify the levels of amygdalin and provide almond classification.

A set of 150 almonds were analysed by using a FOOS NIRS-System6500 spectrometer equipped with a Direct Contact Analyser. Whole almonds were placed individually on the DCA with a exposure diameter of 1cm. Two spectra were recorded for each almond in reflectance mode from 408 to 2492 nm, every 8 nm. To determine amygdalin content, almonds were processed individually and analysed by HPLC-DAD following a protocol previously described<sup>1</sup>. Partial Least Squares Discriminant Analysis (PLSDA2) was applied to the set of 30 bitter almonds with high levels of amygdalin (ranging from 29.1 to 77.6 mg/kg), and another with sweet ones, which included 120 almonds with very low (only 6 almonds with < 5mg/kg) or even no quantities of amygdalin. Data were processed by using WIN ISI III software.

The PLSDA model showed good parameters obtaining a classification accuracy of 100%. The preliminary results demonstrated that NIRs can provide valuable information on the almond bitterness. Furthermore, more work is in process to enlarge the libraries and to properly validate the models and to test other classification algorithms.

## NANOARRAY DIAGNOSTICS: TOOLS FOR PREVENTION AND MITIGATION STRATEGIES TO COMBAT EXPOSURE TO CHEMICAL COCKTAILS

**Dr. Katrina Campbell**<sup>1</sup>

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Contaminant monitoring from microbiological, chemical and fraudulent sources in agri-food production is an important yet complex issue. A huge investment in time and effort is placed on these activities by regulatory and industrial laboratories. Although sophisticated techniques such as chromatography and spectrometry provide accurate and conclusive results, screening tests allow a much higher low cost throughput of samples with less operator training. Biosensors combine a biological recognition element with a transducer to produce a measurable signal proportional to the extent of interaction between the recognition element and contaminant. Different uses of biosensing instrumentation available are extremely varied, with agri-food analysis an emerging and growing application. The advantages offered by biosensors over traditional immunoassay screening methods with respect to food analysis, include automation, improved reproducibility, speed and real time analysis. The miniaturisation of immunoassays and biosensors towards nanosensing offers

not only enhanced sensitivity but portability and multiplexing capabilities. As fresh demands from consumers and regulators grow to improve the integrity of food the need for improved smart nano-technologies has never been greater.

Progress has been made to the development and validation of nanoarrays that can detect both single and multiple contaminants in food samples to offer a holistic approach to agri-food safety. ELISA spot and planar waveguide nanoarray technologies have been developed for the rapid and multiplex analysis of contaminants to prevent and mitigate against exposure to cocktails of contaminants to be compatible with yet enhance food control procedures being applicable in portable field based analysis.

## Review of methods of analysis for Marine Biotoxins

**Dr. Rebecca Kyle<sup>1</sup>**, Dr. Katrina Campbell<sup>1</sup>

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Marine biotoxins are produced by naturally occurring phytoplankton which may contain these highly toxic chemicals. The biotoxins can accumulate in shellfish causing illnesses ranging from headaches, vomiting and diarrhoea to neurological problems and even death in humans once ingested. European-wide legislation has been produced to ensure safe levels of these toxins in seafood leading to the development of different testing methods. Currently a number of methods have been employed to test for biotoxins including animal bioassays, HPLC, LC-MS/MS, ELISAs, immunoassays and functional assays, however some of these tests require the use of specialist laboratories and personnel. This poster will compare the methods of analysis currently in use and examine the emerging technologies and approaches for their suitability and use for the detection and monitoring of the key regulated biotoxins: diarrhetic shellfish poisons; OA and dinophysistoxins, azaspiracids (AZA) and analogues, amnesic shellfish poisons: domoic acid (DA) and paralytic shellfish poisons: saxitoxins (STX).

# Delivering the nutritional needs for the 21st century global population

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## Occurrence and risks associated with allergens in processed foods with precautionary labeling

**Mrs. Emilie Manny**<sup>1,2</sup>, PhD Virginie Barrere<sup>1,2</sup>, PhD Jérémie Théolier<sup>1,2</sup>, Mrs. Stéphanie Parent<sup>1</sup>, PhD Samuel Godefroy<sup>1,2</sup>

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The use of precautionary statements on food allergens continue to increase, reducing the spectrum of products available to allergic people. Foods bearing allergen precautionary labeling should be avoided by allergic consumers as they are supposed to have undergone a risk assessment that could not rule out the presence of the allergen. However, studies from other countries revealed that products bearing advisory labeling did not necessarily correlate with the presence of the allergen. Furthermore, the increase of the use of such statements has resulted in consumers ignoring such mentions as a result of the limitations in their choice of nutritious foods. This is further supported by the fact that no symptoms are necessarily experienced as a result of the consumption of some of these products. This study aims to assess the prevalence of the occurrence of three major allergens (milk, eggs peanuts) in products sold in Quebec, with or without advisory labeling. The occurrence data generated will be used to carry out risk assessments to ascertain the level of risk potentially faced by consumers ignoring precautionary labeling in processed foods. It would also consider situations where such statements are not warranted. The poster fits theme 4 as it addresses considerations around food and nutrition security for allergic consumers as a result of labeling practices because it induces challenges in nutrition of allergic people.

## Testing of fibrous nanomaterials as a barrier for microorganisms

**Ing. Simona Lencová<sup>1</sup>**, Dr. Kamila Zdeňková<sup>1</sup>, Dr. Hana Stiborová<sup>1</sup>, Dr. Věra Jenčová<sup>2</sup>, Prof. Kateřina Demnerová<sup>1</sup>

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Nowadays, nanotechnologies are used in many branches including food industry and medicine. Especially the antimicrobial properties of fibrous nanomaterials given either by the nature of the material or by material modifications by an addition of for example silver particles, are utilized in these fields. Nanomaterials are used in food industry for an improvement of foods nutritional properties or for a production of food packages increasing food durability or providing a protection from undesirable effects. Nanomaterials could be a future of food industry and can help people suffering from food shortages. In medicine, nanomaterials are used for scaffolds development for tissue engineering. In this study, we focus on a microbiological analysis of different nanomaterials types made from polymers polycaprolactone, polylactic acid and polyamide characterized by fiber diameter, pore size distribution and mass area density. All of these characteristics play an important role in interactions between nanomaterials and microorganisms. Attention is focused on microorganisms such as bacteria (*Escherichia coli*, *Staphylococcus epidermidis*, *Bacillus subtilis*) and yeasts (*Candida albicans*, *Saccharomyces cerevisiae*). For all the nanomaterials, we observe and compare the permeability of microorganisms via microbial suspension filtration over a filter from a folded nanomaterial. A total count of passed microorganisms is determined from a gained filtrate. By these tests we can evaluate materials suitability for a use in food industry or medicine. The most convenient material should hold a majority of microorganisms and it could represent a reliable barrier between a sample and external environment. This study was supported by a grant GA17-15936S.

## Nutritional Sustainability - integration of nutrient composition from alternative protein sources into a sustainability concept

**Doctor Volker Lammers**<sup>1</sup>, Dr. Sabine Bornkessel<sup>1,2</sup>, Prof. Dr. Stefan Toepfl<sup>1,2</sup>, Dr. Volker Heinz<sup>1</sup>, Dr. Sergiy Smetana<sup>1</sup>

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The production of food (especially meat products) contributes to climate change. The development of meat substitutes as alternative protein sources is one possible approach to improve the environmental state. However, in this development, the nutrient composition should also be considered, as the need to feed growing population remains. Therefore, the aim of the research is to develop a concept for sustainability assessment that includes nutritional aspects for selected alternative protein sources.

For this purpose, alternative protein sources from plants, insects and microalgae are analyzed on the basis of their environmental impact (Life Cycle Assessment) and their nutrient composition. The energy consumption and the global warming potential of microalgae are higher than those for the production of plants or insects. However, there are hardly any differences in land use and water consumption. The nutrient profiles vary widely among the different varieties or species in their levels of macro and micronutrients. In summary, microalgae have high levels of unsaturated fatty acids; insects are rich in vitamins and have a high protein content with essential amino acid composition.

Specific alternative sources of protein provide different life cycle assessments as well as different nutrient composition qualities. Beneficial results of life cycle assessment are not necessarily linked to a good nutritional composition. Therefore, a rating should always consider both the environmental impact and the nutritional significance in both positive and negative spectra of influence. For this purpose, this concept must be further developed in order to enable a differentiated consideration of individual alternative protein sources.

## The challenges and opportunities facing the aquaculture industry and its role in meeting nutritional needs

**Miss. Michaela Fox**<sup>1</sup>, Dr. Heather Moore<sup>2</sup>, Dr. Matt Service<sup>2</sup>, Prof. Moira Dean<sup>1</sup>, Dr. Katrina Campbell<sup>1</sup>

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Aquatic food makes an important contribution to human health, particularly as an animal protein resource and is enjoyed by consumers for cultural and gastronomic reasons. It is sourced from the wild fisheries and aquaculture sectors and must be safe, sufficient and nutritious to fulfil the needs and wants of the consumer, whilst achieving environmental, social and economic sustainability for the long term. Aquaculture has become a vital source to achieve these requirements since the wild stocks are approaching their maximum sustainable potential. However, this sector is highly dependent on its natural environment and is threatened by current and emerging threats. This study analyses the literature and liaises with the industry to define the aquaculture industry in NI and determine the barriers and facilitators to production. The research identified 6 key categories which impacted the sector, namely; “Economic”, “Environmental”, “Technical”, “Welfare”, “Political” and “Consumer” factors. The most commonly cited themes within these categories included: Financial support is minimal and operational costs are high. Disease is a constant threat. The availability of sites is low. Climate change and other water users pose an uncontrollable and uncertain risk. Aquatic species are highly perishable and storage and transport networks are vulnerable to failures or delays. And the consumer and political environment is uncertain. Ultimately, NI has a comparative advantage for aquaculture in terms of Island location. But these factors, their interactions and solutions need communicated to the industry if production is to remain profitable and sustainable and allow consumer demands to be met.



## Linking Northern Irish milk iodine to farming landscape

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Iodine establishes the classic example of how a trace element can impact human health, iodine deficiency in the environment can result in various diseases collectively referred to as iodine deficiency disorders (IDD). Industrialised countries, including Northern Ireland, are experiencing an increase in the prevalence of iodine deficiency amongst sub-populations, particularly teenage girls and females of child bearing age. This is a major health concern, and research focus, within Northern Ireland. The aim of the research is to characterise the iodine concentration of milk samples, a major dietary source of iodine, in Northern Ireland to find the geochemical, topographical, bovine husbandry and climate regulation of milk iodine. Using ICP-MS analytical methodology, iodine concentrations were determined for samples (soil, silage, grass and milk), collected from 71 farms across Northern Ireland. Additional soil parameters including, soil pH and the organic content of soil were also analysed. Results of this study showed that the proximity to the sea coast was an influential factor in determining the iodine concentrations of soil ( $p < 0.0001$ ). Furthermore, statistically significant relationships between soil to grass iodine concentrations ( $p < 0.0001$ ), and grass to silage iodine concentrations ( $p = 0.0009$ ). These results suggest that there are strong iodine relationships in relation to the terrestrial environment. However, a disconnect is apparent in relation to the environmental and milk iodine concentrations, as milk demonstrated no statistically significant relationships with any environmental variables ( $p > 0.05$ ). Additionally, a 150-fold range was observed in individual milk samples and a 30-fold variation was found at farm level.

## Metabolomic fingerprinting / profiling in assessment of health risks associated with dietary lipids

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Although lipids represent one of the major and essential group of nutrients in human diet, associated health risks should be also be taken into consideration as well. Specifically, oxidised lipids known to be toxic in cell systems have become of concern since after absorption from the diet into lymph or blood, they may exert a wide range of adverse effects (as distinct from those formed in vivo). Also, some processing contaminants, such as fatty acids ester of MCPD, that may be contained in relatively high quantities in some refined oils, namely in palm oil, pose a health threat for consumers. In our study, we employed high resolution mass spectrometry (HRMS) based fingerprinting / profiling strategy to characterize the pool of oxidized lipids and chlorine containing organics occurring in various types of diets. The use of non-target metabolomic screening of oxidized lipids in human plasma will be presented too, the relevance of this approach for indication of diet related oxidation stress will be discussed.

## Critical assessment of lipid fraction in different types of diets

**Miss. Klara Navratilova<sup>1</sup>**, Mr. Vit Kosek<sup>1</sup>, Miss. Tereza Kucerova<sup>1</sup>, Prof. Jana Hajslova<sup>1</sup>

<sup>1</sup>*University of Chemistry and Technology, Prague, Czech Republic*

Dietary lipids represent an important nutrients playing many important roles in maintaining health and preventing diseases. On the other hand, the process of unsaturated lipids oxidation results in food quality losses (including sensorial), moreover, increased intake of those oxidized products can be associated with adverse health effects. The main object of this study was to analyse oxidized lipids in various types of diets and to correlate the results with other lipophilic compounds related to the oxidation process (e.g. fat soluble vitamins and other antioxidants). Samples originated from two main sources: i) commercially available weekly diet plans and ii) duplicates of daily Central European diets. Sample extracts were analysed using metabolomics approach based on ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS). The lipids content and their pattern was correlated with the extent of oxidization and a presence of other components with antioxidation potential occurring in diets.

## Waste NOT - turning food "waste" into healthy new food fibre ingredients

### **Mr. Ross Campbell**<sup>1</sup>

<sup>1</sup>*CyberColloids Ltd., Carrigaline, Ireland*

In the past, the production of food ingredients, including food texture agents, has led to the purposeful extraction, removal, substitution and throwing away of substantial amounts of biomass that often includes key functional and nutritional components. Whilst a few years ago, the sub-products of food processing constituted an economic and environmental problem, today they are considered promising sources of functional compounds with commercial value.

CyberColloids are food texture specialists with a particular interest in the upgrade of by-products and "waste" from food processing e.g. pomace, pulp and peels and also new or underutilised wholefood biomass resources e.g. produce that is out-graded or surplus to market requirements. We are working with a wide variety of materials, including citrus, sugarbeet, seaweed, carrot, potatoes, apple and cereals to develop new functionalised food fibres. Functionalised food fibres are typically minimally processed vegetable, fruit and cereal materials that contain both soluble and insoluble fibre fractions with different textural (and nutritional) functionalities. We target specific fibre components in order to promote their textural functionality and to produce new, label friendly, food ingredients with water binding, gelling and fat replacement properties. These new food fibres are valuable tools for use in delivering healthy food formulations.

We find that our approach provides real benefits to food processing companies: in delivering more natural & less processed food ingredients that consumers and industry are demanding; in turning potential "waste" streams into valuable resources and thus, making a valid contribution towards the wider issues of resource sustainability and nutrition security.

## Project Daire: Derry/Londonderry as the nexus city for food, education, trust and health

**Prof. Jayne Woodside**<sup>1</sup>, Dr Moira Dean<sup>1</sup>, Dr Michelle McKinley<sup>1</sup>, Dr Laura Dunne<sup>1</sup>, Dr Patrick McCole<sup>1</sup>, Dr Ruth Hunter<sup>1</sup>, Dr Niamh O'Connell<sup>1</sup>, Dr Danielle McCarthy<sup>2</sup>, Professor Chris Elliott<sup>1</sup>

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This project is designed to more effectively link the NI Agrifood sector, education and health. Its purpose is to build trust and encourage positive engagement between the community (with primary school children the initial target) and their local food supply chain, ultimately driving improvements in health, wellbeing and educational status.

A NI Food Ambassador programme will be developed, creating a network of those knowledgeable and passionate about the NI food sector, those key to local food provision and representatives from the local community. These ambassadors will create engagement material: experiential, fun and informative educational activities based around the NI Agrifood sector that can be integrated with the school curriculum (engage). In parallel, the opportunities to influence the quality of food offered within schools, and increased inclusion of NI-sourced food, will be explored (nourish). The impact of both the engage and nourish interventions, alone and in combination, on trust development, value perceptions, educational attainment, nutritional knowledge/intake, health and wellbeing will be assessed in Derry primary schools over one year, with accompanying detailed evaluation. These initial evaluations will be used to refine the interventions, followed by roll-out to secondary schools/the wider community.

The legacy of this project will be (i) a network of inspired, connected local people who trust and value their food supply chain, from soil to serve, and are ambassadors for NI food, and (ii) a region where schoolchildren have the opportunity to learn about and engage with their local food supply, influencing their longer-term food choices, wellbeing and performance.

## Encouraging adherence to a Mediterranean Diet in a non-Mediterranean population: the TEAM-MED project

**Prof. Jayne Woodside**<sup>1</sup>, Dr Claire McEvoy<sup>1</sup>, Dr Sarah Moore<sup>1</sup>, Ms Christina Erwin<sup>1</sup>, Dr Margaret Cupples<sup>1</sup>, Dr Katherine Appleton<sup>2</sup>, Professor Frank Kee<sup>1</sup>, Professor Chris Patterson<sup>1</sup>, Professor Ian Young<sup>1</sup>, Dr Laura McGowan<sup>1</sup>, Dr Roisin O'Neill<sup>1</sup>, Dr Michelle McKinley<sup>1</sup>

<sup>1</sup>Queen's University Belfast, Belfast, United Kingdom, <sup>2</sup>University of Bournemouth, Bournemouth, United Kingdom

Adoption of a Mediterranean Diet (MD) significantly reduces both primary and secondary cardiovascular disease (CVD) risk. However, interventions to achieve dietary change are often intensive and prohibitively expensive to scale up for public health. Peer support potentially offers an alternative, low cost approach to encourage dietary behaviour change. The Trial to Encourage Adoption and Maintenance of a Mediterranean Diet (TEAMMED) was a pilot randomised controlled trial developed using the Medical Research Council framework for design and evaluation of complex interventions. A total of 75 overweight adults who did not follow a MD (Mediterranean Diet Score (MDS)  $\leq 3$ ) and are at high CVD risk were randomly assigned to either: a minimal intervention (written MD educational material), a proven intensive intervention (personal dietetic motivational interviewing, quarterly group support, written educational materials and key MD foods) or the developed peer support intervention (group-based community programme delivered by lay peers) for 12-months. Diet and nutrient biomarkers were assessed at baseline, 3-, 6- and 12 months. The primary endpoint was change in MDS from baseline to 6 months. A process evaluation determined fidelity of implementation, feasibility and acceptability of the peer support MD intervention. MDS increased significantly in all intervention groups at 3-, 6- and 12 months, with analysis of nutritional biomarkers ongoing. The process evaluation revealed that group peer support was an acceptable intervention to encourage people at high CVD risk to consume a MD, but identified potential areas for modification in the TEAM-MED peer support intervention, prior to a full scale trial.

## Whole grain intakes in Irish Adults: findings from the National Adults Nutrition Survey (NANS)

**Dr. Anne Nugent**<sup>1,2</sup>, Dr Clare O'Donovan<sup>2</sup>, Ms Niamh Devlin<sup>2</sup>, Dr Maria Buffini<sup>2</sup>, Dr Janette Walton<sup>3,4</sup>, Prof. Albert Flynn<sup>3</sup>, Prof. Michael J Gibney<sup>2</sup>, Dr Breige McNulty<sup>2</sup>

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Observational studies link high whole grain intakes to reduced risk of many chronic diseases. This study quantified whole grain intakes in the Irish adult population and examined the major contributing sources. It also investigated potential dietary strategies to improve whole grain intakes. Whole grain intakes were calculated in a nationally representative sample of 1500 Irish adults using data from the most recent national food survey, the National Adult Nutrition Survey (NANS). Food consumption was assessed, at brand level where possible, using a 4-day semi-weighed food diary with whole grain content estimated from labels on a dry matter basis. Mean daily whole grain intakes were  $27.8 \pm 29.4$  g/d, with only 19% of the population meeting the quantity specific recommendation of 48g per day. Wheat was the highest contributor to whole grain intake at 66%, followed by oats at 26%. High whole grain intakes were associated with higher dietary intakes of fibre, magnesium, potassium, phosphorus and a higher alternative Mediterranean Diet Score. Whole grain foods were most frequently eaten at breakfast time. Regression analysis revealed that consumption of an additional 10g of whole grain containing 'ready to eat breakfast cereals', 'rice or pastas', or 'breads' each day would increase intake of whole grains by an extra 5g, 3.5g and 2.7g respectively. This study reveals low intakes of whole grains in Irish adults. Recommending cereals, breads and grains with higher whole grain content as part of public health campaigns could improve whole grain intakes.

## Discovery of metabolic food identity markers by machine learning technologies

**Dr. Joachim Kopka**<sup>1</sup>, Mr. Christoph Alt<sup>1</sup>, Prof. Daniel Wunderlin<sup>2</sup>, Mr. Alexander Erban<sup>1</sup>

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Verification of food identity is crucial to establish consumer trust in original food ingredients and in the labelling of components of processed food. Besides genetic markers, the metabolic composition of food ingredients enables the discovery of single chemicals or chemical patterns that are unique identifiers of original food ingredients that are marketed directly or used to create complex composite or processed food. Since the year 2000 methods of metabolite profiling gained importance for the global targeted and non-targeted metabolic profiling of biological material. These methods are now the basis of the metabolomics field of biological sciences. Metabolomic methods are now routinely used across all biological sciences, for health and medical applications and for food analysis. Typical experiments are fast and high-through-put. Large experiments generate big, reproducible, and information-rich data sets that are suitable for non-biased statistical analysis towards discovery novel marker molecules. We take statistical analysis one step further by applying bioinformatics machine learning technology to non-biased marker discovery. We demonstrate the concept by a test case of three seed ingredients that are typically used either in non-processed food, such as muesli, or processed foods, like crackers, cookies or pasta. We demonstrate a workflow of metabolic marker discovery that starts with multiplexed standardized chemical profiling of seed material from documented sources. We followed standardized protocols of metabolomic analysis and statistical data analysis. In particular and novel for this purpose, we applied machine learning namely decision tree analyses to select informative chemicals and define simple metabolite abundance-based rules for seed classification.



## Challenges improving Chinese consumers' confidence in the safety of infant formula

**Dr. Si Chen**<sup>1</sup>, Ms. Ting Lu<sup>2</sup>, Dr. Liran Shan<sup>2</sup>, Prof. Patrick Wall<sup>1,2</sup>

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Consumer confidence and trust in the safety and quality of food products are key ingredients if trade in food is to grow between the EU and China. As part of the EU Sino Safe project attempts are being made to identify and address consumer concerns that present barriers to trade. In China, consumer confidence in the safety of domestically produced infant formula was damaged by the 2008 Melamine crisis. This crisis triggered legislative reform of the oversight of the entire supply chain and in particular the infant formula sector. A more robust infrastructure for risk assessment, risk management and risk communication was created akin to the reforms that occurred in the EU as a result of BSE and the Belgian Dioxin crisis.

This paper will describe the qualitative and quantitative methodologies used to gain insights from Chinese Regulators, the food industry and consumers on how they view the safety of the food supply in China and in particular the safety of both domestically produced and imported infant formula. It will discuss the findings and how these will be used to inform measures to build trust and confidence. These measures will be trialed on subsets of the Chinese population in an attempt to restore trust and their efficacy will be evaluated. This research is part of the ongoing EU Sino Safe Project and this presentation will outline the most up to date results.

## Nondestructive techniques for food reassurance

**Prof. Xiaobo Zou**<sup>1</sup>

<sup>1</sup>*Food Science, Zhenjiang, China*

Recently, Consumers and governments are looking increasingly at the safety, authenticity and quality of food commodities. This has driven the attention towards the nondestructive sensing techniques used for rapid analyzing these commodities. The main food quality traits that can be assurance using Nondestructive sensing techniques are sensory characteristics, chemical composition, physicochemical properties, health-protecting properties, nutritional characteristics and safety. A wide range of nondestructive sensing techniques, from optical, acoustical, electrical, to nuclear magnetic, x-ray, microwave and Terahertz, are all based on physical principle. Some of these techniques are now in a period of transition between experimental and applied utilization and several sensors and instruments are reviewed. Faced with innovation and attention to key challenges, such nondestructive sensors are expected to open up new exciting avenues in the field of portable and wearable wireless sensing devices and connecting with mobile networks, and thus find considerable use in a wide range of food assurance applications. This comprehensive coverage will be useful for academic, scientific and industrial community in treating and applying the facts in developing/testing new devices for food assurance based on nondestructive sensing.

## Why Risk Communication often fails to deliver consumer confidence?

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Successfully engaging with the public to understand their perceptions of risk is never an easy task. Scientists and the public often hold different opinions as to whether something is risky or not. Communication scientific material in a comprehensible manner to the lay public is challenging. Furthermore, effective communication is not “one-way” information delivery but “two-way” dialogue. There is a need to identify consumer concerns and formulate suitable communication messages and deliver them via the appropriate communication channels to the different segments of the population. The EU-China Safe project aims to increase consumers’ confidence in food safety and facilitate trade between EU and China. This cannot be achieved if consumers lack confidence in specific products. Marketing strategies will be unsuccessful unless underlying consumer concerns whether based on genuine issues or miss information are addressed. This poster will present the findings and conclusions from an online quantitative survey of Chinese consumers undertaken in February 2018. A sample size of 3,000 was used and participants were asked for their opinions on aspects of food safety controls in China and their view on the safety of selected food categories. The survey attempts to elucidate the factors that contribute to the purchasing decisions of Chinese consumers and will examine the determinants of trust and confidence. The findings will be used to devise communication strategies that address consumer miss perceptions and align their view more closely with the scientific risk assessors. These communication strategies will be evaluated to assess their efficacy.

## The use of untargeted Near-Infrared (NIR) spectroscopy to predict quality of black tea during oxidation

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Black tea is a fragrant brew prepared from the tender shoots and leaves of the plant *Camellia sinensis*. In black tea manufacturing, one of the most significant processes which can influence tea quality is oxidation. Within this reaction, catechins react with enzymes to produce theaflavins and thearubigins. Quantities of these polyphenol compounds are associated with tea quality. The completion of the process is usually judged on a visual assessment of colour and aroma. The project aim is to introduce NIR spectroscopy as an online screening method to provide rapid information on changes in key catechin compounds during processing which are associated with black tea quality. Using a lab-scale method for black tea processing, the leaf samples will be macerated and oxidised in an incubation chamber and measurements made between 30 to 120 minutes. The spectra of samples will be measured using NIR reflectance in fresh and dry form. The profile of key catechins and caffeine will be assessed using a Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) method. The project will focus on linking NIR spectral signals to HPLC reference values to create a chemometric model to estimate oxidation status and predict quality potential at given time points during oxidation. The model will be optimised by including samples from scaled up processes.

## The Increasing Prevalence of Food Allergies Poses a Huge Threat to the World's Wellbeing

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In industrialized nations, food allergies are a growing epidemic and are considered a major threat to our wellbeing. Cow milk allergy is one of the first allergies to occur in early childhood and early life sensitization has been associated with an increased risk to develop the atopic march, including eczema, asthma and other food allergies later in life. As such, more research is urgently needed to gain more insights into this disease. To address this problem, this study evaluated a unique multi-matrix platform for polar metabolic fingerprinting of feces, plasma and urine, applying UHPLC-Q-Orbitrap™-HRMS, to determine the optimal matrix for future research on cow milk allergy in children. Plasma is popular for metabolomic analysis, but collection is problematic in young children, while feces and urine are readily available biofluids. All three fingerprinting approaches were proven 'fit-for-purpose' through extensive validation in which excellent linearity (coefficient of determination  $R^2 > 0.99$  or  $0.90$  respectively), recovery and precision (coefficient of variance (CV)  $< 15\%$  or  $30\%$  respectively) were observed. The effectivity of the platform was demonstrated by subjecting simultaneously collected fecal, urine and plasma samples from 10 healthy volunteers to metabolic profiling and fingerprinting, yielding respectively 9672, 9647, and 6122 components, with a substantial overlap of the plasma metabolome with fecal (69.48%) and urinary samples (76.79%). Orthogonal partial least-squares discriminant analysis (OPLS-DA) provided similar results for feces and plasma in discriminating according to gender ( $p$ -value = 0.036), suggesting feces as a promising alternative biofluid to plasma for food allergy research.

## CHEMICAL MARKERS TO IDENTIFY CHIA, FLAX AND SESAME FROM RAW SEEDS TO BAKERY PRODUCTS

**Professor Daniel Wunderlin<sup>1,2</sup>**, Mr Federico Brigante<sup>1,2</sup>, Mr Agustín Lucini<sup>1,2</sup>, Dr. Natalia Pigni<sup>1,2</sup>, Prof. Pablo Ribotta<sup>1</sup>, Prof. María Verónica Baroni<sup>1,2</sup>

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Chia, sesame and flax seeds are highly appreciated by their outstanding nutritional facts (e.g. reduction of serum cholesterol, antioxidant properties, etc.). Thus, the development of methods to verify the integrity of such seeds as well as processed foods containing them is of public interest.

We hypothesized that some chemical compounds, like polyphenols, can be used as markers of the integrity of both seeds and foods containing them. Thus, the main goal of this work was to verify if the polyphenol profile could afford suitable markers to identify chia, sesame and flax seeds, verifying if such markers remain stable in a baked food (sweet cookies).

The polyphenol profile was assessed using HPLC-ESI-qTOF (HRMS), finding 29 compounds in chia seeds (mainly hydroxycinnamic acids and flavonols), 28 in sesame (most of them lignans), and 10 in flax (mostly flavonoids). As it is expected, the number of polyphenols decreased in baked cookies, affording 11 compounds in chia cookies, 7 in sesame cookies and 2 in flax cookies. When cookies were prepared using a mix of three seeds (equal parts), we found 5 hydroxycinnamic acids characteristics from chia, 7 lignans present in sesame seeds and 2 flavonoids typical from flax.

Thus, each seed showed a unique polyphenol profile, helping with the identification of frauds. Although some polyphenols were lost during the baking process, remainder compounds were useful to identify the presence of seeds in baked cookies, even when containing a mix of seeds.

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## How to confirm your mayonnaise is a real mayonnaise?

**Mr Peter B. Hoos<sup>1</sup>**

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How to confirm your mayonnaise is a real mayonnaise?  
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Keywords: Mayonnaise, Egg yolk, Cholesterol, Phospholipids.

The definition of Real Mayonnaise is described in the EU code of practice and regulated per country on total fat content and egg yolk content. Fat quantification methods are controlled methods within all food laboratories. Cholesterol is the most straightforward egg yolk marker in mayonnaise with AOAC 994.10 and JAOAC 97 as typical reference methods. Unfortunately, due to the mayonnaise matrix, it is still difficult to analyse cholesterol with GC-FID. Butter is a suitable cholesterol reference material with comparable difficult matrix. Phospholipids can also be used as egg yolk markers but due to strong emulsifying properties of the different molecules in combination with the mayonnaise matrix, their analysis is even more challenging. For the quantification and confirmation <sup>31</sup>P-NMR with single phase extraction is a more obvious analytical technique to use for phospholipids quantification in mayonnaise. To conclude, Real Mayonnaise is a difficult analytical matrix.

## Handheld NIRS analyses for classification of Asturcelta autochthonous swine breed carcasses

**Dr. Begoña De la Roza-Delgado<sup>1</sup>**, Dr. Sagrario Modroño<sup>1</sup>, Dr Luis-José Royo<sup>1</sup>, Ms. María Cuevas-Valdés<sup>1</sup>  
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In the 21st century, the interest in recovering autochthonous pig breeds emerged. In the North of Spain, the recovery of the Asturian pig was renamed as Asturcelta porcine breed. Nowadays, its products have different characteristics in their fat composition and sensory attributes in base of feeding regimen. Food ingredient fraud and economically motivated adulteration are emerging risks and immediately a detection method does not currently exist. Nevertheless, over the last decade portable sensors Near Infrared Spectrophotometers have shown their potential for in-situ analysis. The aim of this work was to carry out preliminary studies for in-situ classification of individual Asturcelta carcasses by feeding regimen at slaughterhouse.

106 Asturcelta carcasses were slaughtered. 53 pigs fed under semiextensive system with compound feed. 53 pigs fed under extensive system with forest resources including wild acorns and chestnuts. A handheld PHAZIR™ ((PhIR, Phazir 1624, Polychromix Inc., USA) NIRS sensor working in reflectance mode was used for NIRS analysis. Ten spectra of three different places of carcasses were collected over left middle gluteus skin and fat and over the intact meat samples on the longissimus dorsi muscle. Chemometric calculations were performed using PLS2 discriminant analysis by WinISI II package v.1.50 (Infrasoft International, Port Matilda, PA, USA, 2000). After mathematical treatments (smooth, derivatives, etc,) 93.87% of semi-extensive gluteus fat, 90% of extensive gluteus skin, and 81.81% in extensive intact meat samples were correctly classified. External validation, carried out with 10 samples, gave us a 100% of appropriate classification.



## In situ authentication of “premium” Iberian Pig ham using Near infrared spectroscopy portable sensors

**Prof. Tom Fearn**<sup>1</sup>, Dr. Cecilia Riccioli<sup>2</sup>, Prof. Ana Garrido-Varo<sup>2</sup>, Professor Dolores Pérez-Marín<sup>2</sup>

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Iberian hams are labeled according to the pigs' diet and the percentage of the pigs' Iberian ancestry, with an acorn diet and pure-bred Iberians being most desirable.

Given that the “premium” Iberian ham has more unsaturated fatty acids than those fed with compound feeds, the authentication of the feeding regime was traditionally carried out using the tactile sensation, the melting point (slip point) or the iodine value.

Previous research undertaken by the authors has demonstrated the ability of NIRS not only for the prediction of the fatty acid profile, but also for using the spectral information for classification of carcasses according to the feeding regime or in other words, according to the commercial category, directly related with the price. A key step in the development of multivariate qualitative methods is the selection of a suitable chemometric treatment. In the framework of the FOODINTEGRITY project, the suitability of different classification strategies was studied.

The data set comprised 495 samples from 45 different producers. The samples were measured in two years: 66 were measured in 2016 and the remaining 429 in 2017. Of the 495 samples, 265 were premium grade and 230 were non-premium grade. Samples were measured using a handheld spectrometer (MicroNIR 1700, Viavi Solution). These spectra comprise 125 absorbances at wavelengths from 908 to 1676 nm. Results (up to 96% of samples corrected classified) show the feasibility of the use of spectral data to make a direct classification as premium or non-premium, without going via a quantitative prediction of the fatty acids.

## TOOL-KIT TO ASSESS FOOD INTEGRITY OF CHIA, FLAX AND SESAME IN RAW AND PROCESSED FOODS

**Professor Daniel Wunderlin**<sup>1,2</sup>, Prof. María Verónica Baroni<sup>1,2</sup>, Dr. Joachim Kopka<sup>3</sup>, Prof. Pilar Peral García<sup>4</sup>, Mr. Federico Brigante<sup>1,2</sup>, Mr. Agustín Lucini<sup>1,2</sup>, Dr. Natalia Pigni<sup>1,2</sup>, Prof. Pablo Ribotta<sup>1</sup>, Dr. Alexander Erban<sup>3</sup>, Dr. Christoph Alt<sup>3</sup>, Dr Diego Posik<sup>4</sup>, Ms María Eugenia Zappa<sup>4</sup>, Ms María Cecilia Bruno<sup>4</sup>, Ms. Victoria Lyall<sup>4</sup>, Dr. Guillermo Giovambattista<sup>4</sup>

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Outstanding nutritive seeds, including chia, sesame and flax, are highly appreciated by consumers looking for nutraceuticals. Thus, the development of methods to verify the integrity of such seeds as well as processed foods containing them is of public interest.

We hypothesized that a tool-kit, constructed with chemical, genetic and metabolomic markers could be used to verify the integrity of both seeds and foods containing them. Thus, the main goal of this work was constructing a tool-kit with markers from different methods to identify chia, sesame and flax seeds, verifying if such markers remain stable in processed foods.

Tool-kit development was based on both target and non-target methods to identify suitable metabolites to be included in a model. Additionally, genetic markers were also investigated as a third tool to be integrated in the kit. Preliminary results show that chemical markers can be obtained by a target analysis of polyphenols profile with the help of chemometrics, while non-target analysis, coupled to bioinformatics machine learning technology, lead to non-biased marker discovery. A robust DNA extraction method was developed, in addition to multi-specie PCR-real time assay, based on *rbcL* gene and real time melt curve plots, which provide with an accurate evaluation on the presence of nutritive seeds in both raw and processed foods.

Our current challenge is integrating three different tools in a unique, combined, tool-kit to full assess the integrity of chia, sesame and flax from seeds to complex foods containing them.

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## Total Elemental Analysis of Food Samples with ICP-OES and ICP-MS

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The measurement of toxic, essential and nutritional elements in food has, through regulatory drivers and today's health-conscious consumers become a routine part of food quality monitoring. Alongside regulatory compliance, it is necessary to monitor potentially toxic contaminants that could enter the food chain via a series of pathways including, but not limited to, industrial pollution or environmental contamination. For these reasons, it is essential to have a simple, robust, multi-elemental analysis method for major and minor concentrations of elements in food.

ICP-OES and ICP-MS are sensitive and rapid techniques with wide linear dynamic range and as such are ideal tools for the analysis of trace and major analytes in food in one analytical run. The accuracy of these techniques is demonstrated through the analysis of food based certified materials following microwave digestion.

The principal challenge for trace elemental ICP-based techniques are interferences that stem from the complex food matrix, the reagents used to prepare the sample and the plasma source. This poster reviews different strategies including collision/reaction cell (CRC) strategies in triple quadrupole ICP-MS for the accurate analysis of trace elements in more challenging matrices.

## Seaweed production in Northern Ireland for the pig industry

**Dr. Lauren Ford<sup>1</sup>**, Dr. Pamela Walsh<sup>1</sup>, Dr. Gary Sheldrake<sup>1</sup>, Dr. Katerina Theodoridou<sup>2</sup>, Prof. Jaimie Dick<sup>3</sup>  
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This poster will present using seaweed as a supplement to animal feed in order to increase the sustainability of producing feed to rear pigs. 20% of food production for pigs reared in Northern Ireland is sourced from outside of Europe. Sustainable practices utilising seaweed as a crop could help to bridge the gap between the supply chain and the demands placed on the agri-food industry. In a world where population is ever increasing it is important to keep supply chains short. Using seaweed grown in Northern Ireland can also help to increase the traceability of the food chain from feed to fork and reduce the carbon footprint associated with animal feed.

This poster will present the biochemical profiles of four brown seaweeds harvested in Northern Ireland. The changing biochemical profiles due to seasonality changes the properties of the seaweed as an animal feed supplement. Chemicals particularly prone to seasonal change are the phlorotannins (phenolic compounds specific to brown seaweed). These chemicals have important health benefits for both the animals being reared and therefore the quality of the meat as food for humans.

However high concentrations of these compounds can cause complexation to the proteins in the food and result in lower digestibility of food. Therefore in vitro data, testing the seasonal effects on the digestibility of seaweed supplemented food will be presented herein along with the biochemical profiles. This will give some clarity on the health benefits and potential of replacing the deficit feed with seaweed.

## From Anaerobic Digestate to Microalgal Animal Feeds

**Dr. Daniel McDowell<sup>1</sup>**, Dr Gary Sheldrake<sup>1</sup>, Dr Katerina Theodoridou<sup>2</sup>, Prof. Jaimie Dick<sup>3</sup>, Prof. Matt Julius<sup>4</sup>, Dr Pamela Walsh<sup>1</sup>

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An important factor in improving human nutrition is the enhancement of food products. The improvement of livestock products can be achieved by enriching the animal diet with various nutrient dense supplements. Microalgae have been identified as one such source which could be incorporated into livestock diets, to improve animal wellbeing and nutritional value. Microalgae have many beneficial nutritional qualities including fatty acids, proteins, minerals and antioxidants. A potentially sustainable source of nutrients to grow the microalgae is anaerobic digestate (AD). This research investigates the potential for growing microalgae from AD fertiliser. Thereafter, the fatty acid composition of the algae will be analysed with particular focus placed on polyunsaturated fatty acids. The microalgae species *Phaeodactylum tricornutum* has been chosen as the species of choice for this study because of its ability to accumulate fatty acids. This species will be grown in photobioreactors with various nutrient sources. These nutrient sources will be a range of AD concentrations and a synthetic nutrient source as a control. The microalgae will be harvested and tested for fatty acid composition and quantity using GC-FID. This will indicate the potential of the algae as a livestock nutrient supplement. The results will also indicate the potential of using AD as a microalgae nutrient source. Further work will investigate the pathogen profile of algae to assess its feasibility as an animal feed.

## An examination of the determinants of muscle mass and strength in older adults in Ireland

**Ms. Ellen Flanagan**<sup>1</sup>, Dr. Aoibheann McMorrow<sup>1,2</sup>, Ms. Helen Cummins<sup>3</sup>, Ms. Maureen McGowan<sup>4</sup>, Ms. Sheena Rafferty<sup>5</sup>, Dr. Brendan Egan<sup>6</sup>, Prof. Giuseppe Vito<sup>7</sup>, Ms. Sinead McCarthy<sup>3</sup>, Ms. Clare Corish<sup>7</sup>, Dr. Caoileann Murphy<sup>1</sup>, Prof. Helen Roche<sup>1</sup>

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Sarcopenia is characterised by the age-related loss of muscle mass and strength, which is associated with frailty and loss of independence. The prevalence and determinants of sarcopenia have yet to be characterised in the Irish population. The objectives of this study were to examine the prevalence of sarcopenia and the determinants of muscle mass and strength in a cohort of community-dwelling older adults living in Ireland.

In a cross-sectional analysis, muscle mass, strength and dietary intake were assessed in 275 free-living adults (78 years  $\pm$  7 y). Muscle mass was measured using bioelectrical impedance analysis and muscle strength using a handgrip dynamometer. Dietary intake was assessed by 24-h recall by a Registered Dietitian. Sarcopenia was defined according to the EWGSOP criteria. Linear regression was used to examine the determinants of muscle mass and handgrip strength.

The prevalence of sarcopenia was 31% (33% in men and 28% in women). Gender, age, weight and skeletal muscle mass index (SMI) were predictors of handgrip strength ( $p < 0.01$ ;  $R^2 = 0.578$ ). Similarly, gender, age, weight and handgrip strength were predictors of SMI ( $p < 0.01$ ;  $R^2 = 0.536$ ). No significant associations were found between diet and SMI or strength.

Our data indicate that sarcopenia is a common condition among the community-dwelling older adults in Ireland. Our findings support previous work demonstrating that age and gender are important predictors of muscle mass and strength in older adults. Further work is required to elucidate the role of nutrient intakes in the development and progression of age-related muscle mass and strength loss.

## Salicornia ramosissima J. Woods - A valuable forgotten resource

**Miss Maria Lopes**<sup>1</sup>, Prof. Maria da Conceição Castilho<sup>1,2</sup>, Prof. Carlos Cavaleiro<sup>1,2</sup>, Prof. Fernando Ramos<sup>1,2</sup>  
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Soil salinisation and the shortage of freshwater are major and growing problems throughout the world. Halophytes, as plants extremely tolerant to salt and drought, can be a key tool to ensure food security. They include a broad spectrum of plant types, including those belonging to the Salicornia genera. Salicornia L. can be found in temperate and tropical areas around the world, and has been exploited in saline and arid areas as feed and oilseed crop. Despite the long history of consumption in Asian countries, Salicornia sp potential as food has not received the attention it deserves. Salicornia ramosissima J. Woods (Chenopodiaceae) is a Portuguese native species that spontaneously grows on the salt pans and salt marshes along the coast. It has been considered a “salt-pan pest”, and only most recently the gourmet cuisine gained interest in its use, given its strong salty taste. Studies on its nutritional and functional value are scarce but promising. Therefore, we decided to assess for the first time the chemical composition of S. ramosissima from Mondego Estuary (Figueira da Foz region), Portugal. On a dry weight basis, S. ramosissima is composed of 5.12% protein, 0.36% lipids, 10.36% dietary fibre, and 46.94% ash. 85.62% is moisture. The most abundant minerals found were chloride 222.80 (mg/g), sodium 156.57 (mg/g), magnesium 7.77 (mg/g), potassium 7.40 (mg/g), and calcium 1.99 (mg/g). Taken together, our findings highlight the potential of S. ramosissima as a valuable ingredient for use in traditional and functional food industry, particularly for its extremely-rich mineral content.

## Bioprospecting of five microalgae strains as a potential source of health protective compound

**Mrs. Monika Jiru**<sup>1</sup>, Prof. Milena Stranska-Zachariasova<sup>1</sup>, Mrs. Kamila Hurkova<sup>1</sup>, Dr. Petr Kastanek<sup>2</sup>, Prof. Jana Hajslova<sup>1</sup>

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Microalgae belong to the oldest living organisms on the earth and during their long existence under adverse conditions (UV radiation, oxygen deficit, high salinity, high pressure, etc.), they have created new effective defense systems and metabolic pathways. Secondary metabolites of microalgae showing various antioxidant, antimicrobial, antitumoral and anti-inflammatory effects can therefore be of great benefit in the field of nutrition or food / feed technology and biotechnology. Although several strains are nowadays being industrially used (*Chlorella*, *Spirulina*, *Dunaliella* spp.), so far unexplored microalgae exist in nature. The present study aims to focus on the bioprospecting of selected Chromophyta, Chlorophyta, Rhodophyta, Heterokonta and Cyanobacteria microalgae strains (41 samples). For this purpose, simple and high-throughput extraction-fractionation strategy based on consecutive extraction with water – aqueous methanol (80:20, v/v) followed by hexane/isopropanol (50:50, v/v) was developed. Both the biological activity tests (antioxidant activity, enzyme inhibitory assay), as well as the non-target screening utilizing ultra-high performance liquid chromatography coupled with high resolution tandem mass spectrometric detection (U-HPLC–HRMS/MS), were realized. Moreover, by using the specialized software for HRMS/MS data mining, main lipid species (triacylglycerols, (lyso)phospholipids etc.) were characterized. Among these samples, seven showed high antioxidant activity (scavenging activity > 50%), two samples then high elastase inhibitory activity, and one sample high  $\alpha$ -glucosidase inhibitory activity (inhibition > 50%). This work was supported by OPPC (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503), NPU I (LO1601 - No.: MSMT-43760/2015), by TACR project No TE01020080 and by European Union's Horizon 2020 research and innovation programme under grant agreement No 692195 (MultiCoop).



## Immunological characterization of exosomes from blood to develop biomarkers against endocrine disruptors in *Cyprinus carpio*

**Mr Solomon Oguche Alhassan**<sup>1</sup>, Mr. Guillermo Eduardo Valenzuela Nieto<sup>1</sup>, Prof. Dr. Marc Muller<sup>2</sup>, Prof. Dr. Gudrun Kausel<sup>1</sup>

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Environmental contaminants such as estrogen-like compounds, exert endocrine disrupting effects, however, the organismic response is not well understood.

We have shown that gene expression of a panel of genes is modulated in different tissues in response to estrogen treatment in adult male carp. On the other hand, it was shown that most cell types seem to release extracellular vesicles for cell-free intracellular communication and signaling. In blood membrane vesicles of 40 – 100nm diameter called exosomes have been identified as enriched carriers of intracellular molecules with their content varying according to cell type, function, and physiological state and thus might be useful for predicting exposure effects to environmental endocrine disruptors.

Therefore methods for blood exosome isolation were enquired and marker proteins were selected, as positive markers cluster of differentiation molecules (CD9, CD63, CD81), Tumor Susceptibility Gene 101 protein (TSG101), Alg2-interacting protein X (Alix) and as negative markers the integral protein of the endoplasmatic reticulum (Calnexin) and the Golgi matrix protein GM130. From adult male carp blood fractions enriched in exosomes will be further characterized to reveal biomarkers in response to estrogenic exposure.

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## Delivering the nutritional needs by fortification of staple food with under-utilized plant species: A Review

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### Abstract

Under-utilized plant species (UUPS) are rich in nutrients especially micro nutrients that are absent in other staple foods. Its potential for fortifying major staple foods in Africa is a novel approach. This study seeks to review available body of evidence on the fortification of staple foods in Africa using UUPS, and suggest the way forward for effective nutritional and health benefits. The review revealed that; fortification of Sweet potato with cowpea and peanut was found acceptable with dense nutritional properties. Substitution of orange flesh Sweet potato by Bambara groundnut increased the magnesium, phosphorous, potassium and iron in the composite snacks. Composite flour from local Banana, Soybean and Maize had high levels of potassium and sodium and appreciable levels of Iron, Zinc and Manganese. Combination of Oat bran, Soya flour and Maize showed high-fibre and high-protein levels. The fortification of malted Sorghum flour with Soy flour increased the protein and mineral content while the anti-nutritional factors decreased. Tiger-nut based beverage fortified with Vigna racemose legume, enhanced the protein, ash, carbohydrate and mineral contents. Cereal-legume fortified with orange flesh Sweet potato improved the vitamin A content. Sweet potato, Avocado pear and Turkey berry blends improved the nutrient content. The fortification of staple food with UUPS is promising. Fortifications of Rice with UUPS are needed and have good prospects in Africa. More designed feeding trials are required to verify the impact on reducing under-nutrition and hidden hunger in consumers.

Keywords: Under-utilized plant species, Fortification, Nutrition, Malnourishment, Hidden Hunger

## Cost Effective Method for Fortification of Rice with Iron and Zinc to Combat Micronutrient Malnutrition

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Rice is one of the staple foods of India. However, rice is a poor source of essential micronutrients – iron (4-5 ppm) and zinc (8-15 ppm) which are far less than the recommended daily allowance of iron (17-35 mg) and zinc (8-12 mg). As per WHO 2008, micronutrient malnutrition which mainly includes deficiencies of iron and zinc affects two billion people globally and causes almost one million deaths annually.

Among various approaches for combating the micronutrient deficiencies, food fortification is one of the cost effective approaches. Therefore, paddy samples of rice varieties were fortified by parboiling process using edible grade ZnSO<sub>4</sub> and NaFeEDTA as fortificants. Iron and zinc content in the polished rice samples so obtained were in the range of 34-45 ppm and 30-40 ppm, respectively. The present method of fortification is simple and economically cheaper as it can be carried out with existing facilities of parboiling.

Feeding trails showed that the mean haemoglobin level increased from 6.77 g /dl on 0th day to 10.36 g /dl on 60th day and 9.25 g /dl on 0th day to 10.83 g /dl on 60th day, respectively, in cases of severely and moderately anaemic experimental subjects. There was no significant change in the haemoglobin level of the anaemic subjects in control group.

Hence, it can be concluded that the present process of fortification is simple and cost effective and the fortified rice obtained by this process can be an effective solution to prevent the deficiency of iron and zinc.

## Millet Sourdough Products: A healthy, Nutritious and Safe Foods.

**Assoc Prof Adewale Olusegun Obadina<sup>1</sup>**

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Millet has the potential to promote food security, if the right technology is applied for processing it into edible food products. The use of sourdough technology can be a strategic measure to enhance the nutritional quality, palatability, health promoting components, and consumer appraisal of millet products. Hence, different extruded products were developed from millet exploring the spontaneous fermentation method and the effect on the nutritional, sensory and safety properties of the products (bread, extruded snacks and baked snacks) were investigated. It was confirmed that sourdough fermentation could be exploited for processing millet sourdough products with improved nutritional quality and overall consumer preference in terms of appearance, colour, texture, flavor, taste and overall likeness. This will most certainly alleviate malnutrition and other nutritional diseases particularly in those developing countries that are most affected by these diseases.

Keywords: Millet, Sourdough, Nutritional, Safety, Fermentation

## Comparative efficacy of *Jatropha curcas* in the management of wood termites (*Macrotermes bellicosus*)

**Doctor Simon Idoko Okweche**<sup>1</sup>, Ms Comfort Nnah

<sup>1</sup>*University, Calabar, Nigeria*

The efficacy of *Jatropha curcas* in the management of wood termites, (*Macrotermes bellicosus*) was carried out in the Teaching and Research Farm of the Department of Forestry and Wildlife Resources Management, University of Calabar. The experiment consisted of 5 levels of *J. curcas* oil (0, 0.5, 1.0, 1.5 and 2.0 mls) and a corresponding quantity in powder of 0, 0.5, 1.0, 1.5 and 2.0 g, replicated 4 times and arranged in Randomized Completely Block Design (RCBD). Each concentration was tested on 20 unsexed adult wood termite placed in grave yard of 8cm x 8cm. Data on mortality rate was taken at 12 hourly up to 72 hours. The result from the experiment showed that *J. curcas* oil was significantly efficacious compared with *J. curcas* powder. At 12, 24, 48, 60 and 72 hours after application, percent mortality of oil to powder were 20:12, 22:14, 40:25, 60:40, 68:45 and 70:50 % respectively. It was observed that there was progressive increase in mortality rate due to increased concentration and time duration. The management of termite using *J. curcas* should be encouraged due to its environmental friendliness and should also be incorporated into integrated pest management (IPM).

## CONTRIBUTION OF NON-TIMBER FOREST PRODUCTS TO WELFARE OF RURAL COMMUNITIES AROUND FOREST RESERVES IN NIGERIA

**Prof. Rabiu Sani<sup>1</sup>**

<sup>1</sup>*Atb University, Bauchi- Nigeria, Bauchi, Nigeria*

The study was conducted in eight communities which are contiguous to the Afaka Forest Reserve in Kaduna State, Nigeria. The respondents were 204 household heads of which 181 were involved in Non-timber forest products (NTFPs) collection in the year 2015. The Gini coefficient for total income in the area was 0.3427 which was decomposed to reveal an NTFP income Gini index of 0.4426 and a relative contribution to total income inequality of 0.0178, meaning that a 10 percent rise in NTFP incomes will result to a 1.78 percent increase in total income inequality in the area. The Foster-Greer-Thorbecke (FGT) analysis revealed a poverty headcount, poverty gap and poverty severity were 44, 21 and 12 percent respectively. The semi-log regression model showed that the variability (adjusted R<sup>2</sup>=0.4803) of NTFP income (as an index of welfare) was partly due to the incorporated model variables. In particular, tertiary education (with negative relationship), types of NTFPs collected and core poverty status were all highly significant (P<0.01). Gender, household size, primary occupations (like farming and hunting), and incomes from other sources were also significant (P<0.05). The reduction in income inequality and poverty indices from NTFP incomes show that NTFPs contribute to welfare in the study area. Improvement in the genetic quality of some NTFPs, provision of affordable modern storage facilities and establishment of cottage industries are suggested as panacea to some of the problems encountered in collecting and using NTFPs in the study area.

## GAPS AND NEW CHALLENGES ON ANALYSING RISK MANAGEMENT AMONG SMALL RUMINANTS KEEPERS IN NIGERIA.

**Prof. Rabiu Sani<sup>1</sup>**

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The study examined the gaps and challenges on risk management strategies among goat keepers in Kano State, Nigeria. Data were analysed using descriptive statistics and multinomial logit regression. The result revealed that the average age of respondents was 36 years, 95.1% were female, with average household size of 7 persons and majority (71.6%) had no formal education with more than 12 years of production experience. Also, 75% of the respondents had no extension contact and all the respondents relied mainly on family labour. The respondents identified diseases and parasites, improper health practices, inappropriate breeding practice, poor feeding and poor housing as production risk; low price of output, inadequate market, and high cost of input as marketing risk. Similarly, lack of credit facilities and government policy were the financial and institutional risk, respectively. Most of the perceived risks to goat keepers were rated high. The risk attitude showed that majority (60%) of the respondents were risk averse while 23.3% and 16.7% were risk takers and risk neutral, respectively. The multinomial logit regression result showed that age, production experience and household size were significant at 10%, 5% and 1%, respectively. The study recommended that stakeholders should focus on creating a cost effective livestock insurance coverage against risks associated with goat production to improve integrated food and nutrition security in Nigeria and Africa in general.

## Closing Millet Yield Gap through Site-Specific Fertilizer and Plant Population Recommendations Model in Senegal

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Farmer's yield of pearl millet (<0.8 t ha<sup>-1</sup>) are still far below the potential for new varieties (over 3 t ha<sup>-1</sup>). One of the primary driver of gap seem being the flat fertilizer recommendation of 68.5 kg of N, 22.5 kg of P and 22.5 kg of K, developed in the 1970s and applied across the country despite the diversity of soil and precipitation gradient. Additionally, millet continues to be sown at only 12,300 seed holes ha<sup>-1</sup> compared to over 55,000 for maize. Twenty mineral fertilizer treatments using simple fertilizers (Urea, DSP KCl) were used to establish N, P, and K response functions under low (12,300) and high (24,600 seed holes ha<sup>-1</sup>) two improved pearl millet varieties (Suna 3 and Thialack 2) yield response. First results show that for both varieties higher sowing density significantly increased grain yield. However, Thialack 2 was more responsive to higher sowing density. Increase in N application also significantly enhanced grain yield of both varieties with optimum yield obtained with 100 kg N ha<sup>-1</sup> but, induced significant soil NO<sub>3</sub> contamination. Across all treatments maximum grain yield of 3545 kg ha<sup>-1</sup> was obtained for variety Thialack-2 at high planting density and application of about 100 kg of N ha<sup>-1</sup>. This design has now been replicated across five of Senegal's agro ecological zones (300-450 mm yr<sup>-1</sup>; 450-500 mm yr<sup>-1</sup>; 600-700 mm yr<sup>-1</sup>; 700-800 mm yr<sup>-1</sup>; and 1000-1100 mm yr<sup>-1</sup>) in order to develop fertilizer response functions and soil maps to provide site-specific fertilizer and plant population recommendations.



## Causes of Household Food and Nutrition Insecurity: Implications for Food Sustainability in Kano State, Nigeria

**Mrs. Ifeoma Anugwa**<sup>1</sup> Prof. Agwu Ekwe Agwu<sup>1</sup>

<sup>1</sup>*University Of Nigeria, Nsukka, Nsukka, Nigeria*

As an agricultural extension educator, I provide nutrition education to farm families because I discovered that most of them are food insecure. Thus, I encourage them to adopt sustainable agricultural technologies that will both increase food production as well as the nutritional value of food consumed. I chose to participate in the theme 'Delivering the nutritional needs for the 21st century global population' because in the course of my research on the causes of food and nutrition insecurity, I discovered that some of the major nutritional challenges to support improved health and well-being of rural farmers were inadequate nutrition education arising from poor agricultural extension services, large family size and poverty. Thus, farmers mainly consumed carbohydrate containing food and less of diversified diet due to their lack of adequate information on nutritious diet. As a result of my vision of eradicating hunger and malnutrition globally and in Nigeria, I have participated in a number of food security projects such as the West African Agricultural Productivity Programme (WAAPP) during which I was actively involved in disseminating information on high yielding maize and cassava varieties with improved nutritional contents to rural farmers. I trained them on how to apply sustainable agricultural practices in order to achieve the desired result of improving their nutritional well-being. In order to improve the protein intake of farmers, I also participated actively in training farmers on engaging in catfish production. To ensure sustainable production and consumption of catfish, we established fish ponds in four different farming communities.

## Nutritional Challenges of Agricultural Students in Selected Tertiary Institutions in Enugu State, Nigeria

**Mrs. Ifeoma Anugwa**<sup>1</sup>, Prof. Elizabeth Onwubuya<sup>1</sup>, Ms Christaina Ali<sup>1</sup>

<sup>1</sup>*University Of Nigeria, Nsukka, Nsukka, Nigeria*

I am a lecturer and PhD student with specialty in Agricultural Extension Communication. Furthermore, I have conducted a number of research on food and nutrition security issues. I have also received training and attended conferences on food security issues wherein I gained knowledge on different perspectives of food security. The knowledge I gained has enabled me to teach my students nutritional education effectively. I chose to participate in the theme 'Delivering the nutritional needs for the 21st century global population' because in the course of investigating the nutritional challenges of agriculture students in selected tertiary institutions in Enugu State, Nigeria, I observed that the majority of the students were food insecure, even though they had a high dietary diversity. Also, the most frequently purchased and consumed food by the students were foods containing poor nutritional contents such as sweetened drinks and fast foods, while they purchased less of more nutritious foods such as fruits and vegetables. The students noted that although they received nutrition education on balanced meals, but they were faced with challenge of inadequate funds to purchase and consume more nutritious food. Also, the prevailing high food price makes them consume cheaper and malnourished foods that will basically supply the energy they need to pursue their academic activities. Thus, I had advocated that the government, through the school authorities, should establish an educational policy aimed at providing balanced meals with adequate nutritional contents that are affordable to students so as to improve their food and nutrition security situation.

## International Regulatory Framework for Fish Safety and Quality: Africa navigating under two regulations

**Professor Yemi Akegbejo-Samsons<sup>1</sup>**

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Developing countries contribute over 50% of the world fish trade and this has continued to rise. About 58% of fish consumed in the EU come from non-EU waters, mainly developing countries. Fish trade between Africa and the rest of the world is regulated via a complex overlap of multilateral and bilateral trade agreements. The US Food and Drug Administration announced its adoption of final regulations to ensure the safe and sanitary processing of fish and fishery products (Seafood HACCP Regulation). Currently many countries apply HACCP to control seafood safety, however its implementation induces trade barrier and harms international trade in seafood markets. In a similar vein, the New European Union (EU) regulations ban all 'commercial' consignments of smoked fish from Africa (and other non-EU countries) from entering the EU region. Application of these two regulations pose a big challenge for effective fish trade for Africa. This paper examines the implications and effects of these two regulations on fish trade on the developing countries especially Nigeria. It highlights various WTO Agreements and the major challenges faced by the region in the development of Fish Inspection and Quality Control Systems such as poor infrastructure, inadequate technical/scientific expertise and poor understanding of regulations. The paper provides measures to overcome the unfair trade barrier. This includes building capacity (human resources and equipment) for an effective quality and safety assurance systems, improvement of infrastructure, establishment of testing and referral laboratories, building of good and credible scientific database, through regular sampling and analysis of samples.

## Building capacities for Fish production in Africa: The role of Aquaculture

**Professor Yemi Akegbejo-Samsons<sup>1</sup>**

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Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world. In 2010, Africa contributed 7 597 427 million tonnes, or 9% of global caught supply, representing a regional increase of 6.8 times from 1 109 387 tonnes in 1950. In that year, fish catches and aquaculture totalled some 158 million tonnes valued at US\$ 217.5 billion. In 2011, the African total capture fisheries and aquaculture production dropped slightly to about 8 995 518 tonnes (6% of world total), of which 1 398 091 tonnes came from aquaculture and 7 597 427 tonnes from capture catches. Overall though, Africa's contribution to world fishery production has grown from 5.9% in 1950 to 8.1% in 2011. The global population is increasing and, in order to maintain at least the current level of per-capita consumption of aquatic foods, the world will require an additional 23 million tons thereof by 2020. This additional supply will have to come from aquaculture This paper looks at the current fish production level from aquaculture in Africa. The paper highlights the contributions from the different African countries while the major constraints to increased production such as climate change, high feed and seed cost, degraded ecosystems etc. are discussed. The paper identified Egypt, Nigeria and Ghana as some of the leading producers. The paper recommends a national and regional aquaculture plan and strategy that will mainstream aquaculture into key planning and policy instruments in Africa.

## Effect of women's off-farm economic activities on rural households' per capita food consumption

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This paper attempts to investigate the direction of influence if any, of non-farm income generating activities of women on the per capita food consumption of rural households in Nigeria. Primary data was obtained through a multistage sampling technique from 200 rural farming households. Data obtained was analysed using descriptive statistics such as frequency, percentages etc. Econometric tools such as ordinary least squares regression and Logit regression models were used. The Coping Strategy Index was used to determine the degree of severity of hunger among the rural households. Findings showed 69% were hungry. Male headed households, experience of climatic shocks and the use of coping strategies which revolved around asset sales, significantly reduced households per capita food consumption, while an increase in the number of adult female household member as well as of number of working household members significantly increased households' per capita food consumption. There was a stronger correlation for working female members of the rural farming households between the number of off farm income generating activities and per capita food consumption of such households compared to that of their male counterparts. Comparing between the male headed households and the female headed households, the mean per capita food consumption was significantly higher for the female headed households.

## Global challenges and issues call for global Food Security and Nutrition Governance

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It is recognized that the transformation of agriculture in all developed, emerging and developing countries is an effective condition for reducing inequalities not only because agriculture is the source of food and nutrition security for a considerable part of the world's population, but also because it produces positive structural impacts on human capital, health, education, etc.

There is an urgent need to rethink our agricultural model for a better effect and impact on the food and nutrition security of populations: 1 billion people still suffer from starvation nowadays.

Indeed, facing the global challenges of achieving zero hunger and the structural nature of undernourishment, it might be accepted that governance is the missing ingredient in the classical modalities of interventions to cope with poverty, food and nutrition insecurity.

Policies, strategies and programs for food security, nutrition, social protection and sustainable agriculture must reflect a deep analysis of the situation and promote the best technical solution to an issue through multisector approaches based on a constant and permanent dialogue between all stakeholders. The objective is to achieve coordinated political action in different areas at different levels for better synergy between sectors to have a better impact on populations well-being.

Since 2016, with funding from EU, the Food and Agriculture Organization of United Nations (FAO) is experimenting with the Government of Senegal, a project to strengthen the governance of food security and nutrition. Indeed, this project aims to strengthen the formulation, monitoring and evaluation of agricultural policies and programs for a greater impact on FSN

## The impact of Drought tolerant maize varieties on household food security and Nutritional in Benin

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In the context of climate change, some climate-smart innovation like Drought Tolerant Maize varieties packages had been disseminated on all the heard of Benin's territory, to increase productivity, yield, income, food security, nutritional status, and poverty. This paper examines the impact of Drought tolerant maize varieties adoption on household food security and nutritional status, using country-wide cross-sectional data of about 518 maize farming households in Benin. We used food consumption scores and anthropometric scores as outcome indicators of food security and nutritional status, respectively. The instrumental variable approach was used to identify causal effects of Drought tolerant maize varieties adoption on food security and health. We found significant differences in some key socio-economic and demographic characteristics between adopters and non-adopters of Drought tolerant maize varieties. To control for such differences and allow a causal interpretation of the impact of Drought tolerant maize varieties adoption, we estimated the Average Treatment Effect. Our analyses indicated that adoption of Drought tolerant maize varieties adoption significantly increased household food security by 15 percentage points. This helps severely food insecure households to achieve acceptable food security status by enabling them to acquire cereals and tubers, pulses, vegetables, and fruits on a daily basis. There was no significant impact of Drought tolerant maize varieties adoption on nutritional status. Our findings point out that Drought tolerant maize varieties can play an important role in fighting against food insecurity in Benin.

## Challenges of Operating Environment for Nutrition Research Improvement in Sub-Saharan Africa

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Good nutrition is central to the sustainable development agenda that is taking shape in the form of the Sustainable Development Goals (SDGs) now in sub-Saharan Africa. Inherently sustaining, good nutrition flows throughout the life cycle and across the generations. It promotes individual resilience in the face of shocks and uncertainties generated by climate change and extreme price fluctuations. It supports the generation of innovations needed to meet the joint challenge of improving the lives of current and future generations in ways that are environmentally sustainable. Good Nutrition is the bedrock of human well-being. For young children, good nutrition status averts death and equips the body to grow and develop to its full potential. Over the course of the human lifespan, it leads to more effective learning at school, better-nourished mothers who give birth to better-nourished children, and adults who are likelier to be productive and earn higher wages. In middle age, it gives people metabolisms that are better prepared to ward off the diseases associated with changes in diet and physical activity. Without good nutrition, people's lives and livelihoods are built on quicksand.

The paper finds that evidence to show that improvements in nutrition status will make large contributions to SDGs on poverty, food, health, education, gender, and employment.

Almost all countries in sub-Saharan Africa suffer from high levels of malnutrition. It is clear that the low-income countries do not have a monopoly on malnutrition problems and that the high-income countries do not have a monopoly on nutrition solutions.

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## GENOTYPE BY ENVIRONMENT INTERACTION ANALYSIS OF PROVITAMIN-A CASSAVA IN SIERRA LEONE

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Assessment of GEI effects of a given trait is useful in understanding its varietal stability. Provitamin A cassava genotypes have featured so distinctly in biofortification because they have an increasing level of micronutrients, such as carotenoids. The study aimed to determine GEI among 30 provitamin-A accessions and select stable genotypes with high carotenoid levels and dry matter across three contrasting environments. Data on pre-harvest morphological parameters were measured at 3, 6 and 9 MAP while yield, dry matter and total carotenoid content were measured at harvest (12 MAP). Data were subjected to GGE Biplot (v4.1) to determine GGE interaction, stability and graphical identification of genotypes with specific adaptation. Highly significant differences were observed among genotypes (total carotenoid content) and sites (dry matter and total carotenoid content). Dry matter varied between 35% and 54%. Total carotenoid content varied between 5 and 13  $\mu\text{g/g}$ . Genotypes TR 1182 and TR 1313 were the most ideal for both traits. Njala and Pendembu were ideal for selecting superior genotypes for total carotenoid content and dry matter. These appeared to be the best genotypes (TR 1182 and TR 1313) for food technologists and nutritionists to use for feeding programmes to combat vitamin A deficiencies in Sierra Leone.

Index terms: Provitamin-A, Total carotenoid content, Dry matter, Yield, sites

Micronutrient malnutrition is a burgeoning health problem in Sierra Leone. The adoption and consumption of these newly developed bio-fortified genotypes selected from this study would enhance nutritional efficiencies in Sierra Leone.

## The catabolite control protein CcpA affects phytate solubilisation in *Paenibacillus polymyxa*

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Using soil-dwelling microorganisms to improve phosphorus accessibility for plants is a promising strategy for more sustainable and ecological crop management; it is therefore critical to understand the basis of microbial phosphorus solubilisation. The ability of the rhizobacterium *Paenibacillus polymyxa* to solubilise organic and inorganic phosphorus has been demonstrated, but the mechanisms involved are not clear.

We employed a combination of mutant library screens, promoter assays, RNAseq and plant growth experiments for a systematic genetic analysis of phytate solubilisation by *Paenibacillus polymyxa* and its effects on the plant.

Compared to the wildtype strain, deletion of catabolite control protein A ( $\Delta$ ccpA) resulted in a 9-fold increase in expression of phytase, an enzyme known to solubilise phytate; however,  $\Delta$ ccpA showed a 50% decrease in phytate solubilisation and a significant reduction in leaf weight of *Arabidopsis thaliana* grown on phytate, suggesting additional gene products other than phytase are at play. Indeed, RNA sequencing revealed 70 *Paenibacillus polymyxa* genes that are differentially expressed in  $\Delta$ ccpA in presence of phytate. Among those are an uncharacterised phosphatase and genes of mixed acid fermentation, potentially contributing to the observed phenotype.

In conclusion, we present the first data on the genetic programme underpinning phytate solubilisation by *Paenibacillus polymyxa*.

## The contribution of food composition data to attaining food security – South African case study

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Most governments have committed to the set of Sustainable Development Goals established by the United Nations (UN) to be achieved by 2030. Subsequently the governments have drafted, or are in process of drafting, policies and programmes which aim to answer to these global requests. South Africa provides a unique case study: in that despite economic growth, undernutrition has not improved when compared to other industrialised nations, while at the same time, diet-related non-communicable diseases and obesity have exponentially increased. Access to healthy food is a constitutional right of all South Africans, and towards increasing food security and improving population health, various policies, programmes and regulations have been developed and implemented by the government to rectify the situation. The paper presents an overview of food composition within these public health policies, programmes and regulations and unpacks the important role of accurate food composition data.

## BREEDING ADAPTED ORANGE-FLESHED SWEETPOTATO FOR SUDANO-SAHELIAN ZONE OF BURKINA-FASO TO ADDRESS FOOD SECURITY AND MALNUTRITION

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Orange-fleshed sweetpotato production and consumption is settling down in West Africa with its specificity in the Sudano-Sahelian zone. In this area, the rainy season is shorter with period of drought of varying lengths. To better fit in this agro-ecosystem suitable sweetpotato varieties with yield performance close or higher than this of the farmers' varieties are expected. A total of 118 newly developed sweetpotato varieties including some orange-fleshed varieties have been planted at INERA station of Farakoba one month before the end of rainy season for end-season drought screening. Growth parameters, agronomic characteristics at harvest after three months (90 days), and parameters as perceived by farmers: tolerance to drought and weevil, performance compared to the local varieties, disease symptoms, planting material potential, root appearance and attractiveness, root quality were used for variety selection. Rainfall data has also been recorded for the growing period.

Eight varieties were obtained with yield performance of 6 to 12 T/ha with good weevil resistant; among them two varieties were orange-fleshed with dry matter content of 26 and 28%. The photosynthesis activity of these eight varieties suggested good material that can fit to the local condition where end season drought and weevil constitute the major constraint to sweetpotato production in Sudan-Sahelian zone of Burkina Faso.

Key words: sweetpotato, end-season drought, yield, quality, preferences

## Improving the food knowledge and dietary intake of primary schoolchildren: the DAIRE project

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Project Daire will work with primary schools in the Derry region, with the aim of improving knowledge of the food supply chain and dietary choices in primary schoolchildren. Two interventions will be developed, working with primary school principals, staff and children, to ensure relevance and usability:

1) The “engage” intervention will develop and deliver educational activities linked to food and aligned with the primary curriculum. This programme will focus on the development of knowledge, skills and understanding underpinned by subject areas such as Geography, History and Science and Technology. Activities could include: videos, lesson plans, worksheets, games, talks, visits from experts, and practical activities such as experiments that can be conducted in the school environment. The content and delivery will be supported by Food Ambassadors: people working locally in the food industry who wish to take an active role in raising knowledge and understanding of the sector.

2) The “nourish” intervention will develop and deliver interventions to alter the food offered in the school environment, including in the food canteen, working with school catering/procurement systems, breakfast clubs, healthy breaks, and will be supported by the local food industry partners.

The effect of these interventions will be tested over a 12 month period, with measurement of effects on outcomes including the school food environment, attitudes to food, food knowledge and choices, trust, food behaviour and education, and wellbeing.

## Dietary Interventions: Nanny state or optimising the environment for healthy outcomes? A Global Perspective

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Public Health England (PHE) uses the Eatwell guide as a policy tool to define recommendations for a healthy, balanced diet. The National Diet and Nutrition Survey (NDNS) figures for 2014-16 show that consumption of saturated fat was too high and levels of fruit, vegetables and fibre, too low. PHE has now issued a target to food manufacturers to cut 20% of the calories from a list of foods by 2024.

International dietary guidelines are consistent with the message that a minimum of 400g of fruit and vegetables should be eaten daily. The Nuffield Farming study “Vegetable production for specific nutritional need” looked at dietary guidelines in three countries and reviewed the activities promoting increased vegetable intake.

China takes a prescriptive approach recommending 300-500g vegetables a day, 50% of which should be dark; however, vegetable consumption remains low. South Korea stipulates 2 portions of vegetables with every meal. Population surveys show sufficient micronutrients in the diet but high salt levels are a concern. New Zealand's 5+ a day programme is recognised by 80% of the population, however producer-driven interventions such as the Vital Vegetables range and Vitamin D-enhanced mushrooms have communicated the benefits of high-nutrient vegetables to consumers by adopting the latest health claim regulations. The Chip Group programme has proved successful in reducing fat levels in take-away fried chips without changing consumer behaviour.

These findings suggest that new approaches to optimise the environment for healthy outcomes through informed consumer choice or improving production processes should be considered alongside policy guidelines.

## Investigation of the herd and animal variation attributing to the nutritional properties of dairy colostrum

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Bovine colostrum is becoming widely recognised as a nutraceutical for its bioactive ingredients contributing to immune development and modulation of the intestinal bacterial flora. As the first milk produced after calving, colostrum contains essential growth and immune factors needed for to establish an active immune system and initiate development of the calf rumen. Much research has been dedicated to assessment of the level of antibodies contained within colostrum which can sustain effective immune transfer to the newborn calf. However, growing research indicates potential effectiveness against gastrointestinal disorders and respiratory conditions in humans. Bovine colostrum has been shown to have direct antibacterial and anti-inflammatory effects on human intestinal epithelial cells, whilst others have shown colostrum can help increase endurance, accelerate recovery and promote lean muscle mass during exercise. There is a need to identify the animal and farm management factors which influence the level of beneficial colostrum components. As such, an evaluation of the nutritional elements contained within colostrum produced by 30 Northern Irish dairy farms was undertaken. Samples of bovine colostrum (n=15) were collected from each farm at the first milking, within 12 hours after calving and stored for subsequent biochemical analysis. Information regarding farm level decisions on herd management (n=30) was collected and individual animal records (n=450) were gathered to evaluate the effect of the dry cow diet and dam health status on the level of fat, protein, lactose, immunoglobulin, vitamin and mineral content. It is expected that further analysis of extractable components could supplement health promoting milk products.

## Grain Patterns are Associated with Dietary Fibre Intakes and Diet Quality in Children and Adults

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The current analysis identified the most commonly consumed grain patterns in US children (2-18 years-old) and adults (≥19 years-old) and compared dietary fibre intakes, diet quality, and health parameters in the various grain patterns in comparison to individuals who consumed no main grain foods. The USDA food coding system was used to define main categories of grain foods. Cluster analyses using data from the National Health and Nutrition Examination Survey 2005-2010 identified patterns of grain consumption. In children, consuming bread/rolls, pasta/cereals/rice, and crackers/salty snacks patterns was associated with a higher diet quality (Healthy Eating Index-2010 score: 46.1±0.5, 50.6±1.0 and 46.0±0.4 vs. 42.7±0.9, respectively; p<0.001). In children, dietary fibre intake was higher in five grain patterns as compared to those consuming no grains. Body mass index Z-score was lower in children consuming bread/rolls, quick breads, pasta/cereals/rice, crackers/salty snacks, and mixed grains patterns. Adults consuming cereals, pasta/cooked cereals/rice and mixed grains patterns had a higher diet quality than no grains (54.7±1.0, 54.4±0.6 and 49.5±0.03 vs. 46.8±0.9, respectively; p<0.002). Dietary fibre intake in adults was greater in three of the eight grain patterns identified compared to no grains. Pasta/cereals/rice consumption in adults was linked to lower body weight (79.1±0.7 vs. 82.5±1.2 kg; p=0.009) and waist circumference (95.2±0.6 vs. 98.2±1.0 cm; p=0.004) as compared to no grains. Consuming a variety of grain food patterns in US children and adults was associated with improved dietary fibre intake and diet quality. Additionally, certain grain patterns were associated with lower added sugar intake and improved weight-related measures.



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