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International Symposium

Food Fraud Prevention and Effective Food Allergen Management

Rockville, MD, USA

30 October – 1 November 2019

MoniQA Association 2019, www.moniqa.org

Symposium overview

The MoniQA Association and the United States Pharmacopeia warmly welcome you to the 3rd International MoniQA Symposium on Food Fraud Prevention and Effective Food Allergen Management!

An exciting program with practical litigation cases, with strategies and methods for detecting and combating food fraud, will be presented by renowned speakers from around the world. Join us in Rockville and meet the 'Food Detectives' and get to know modern technologies that are looking for the 'unknown' and learn about legal regulations and get practical advice for protecting your branded products, for correct food labeling, and ultimately for maintaining consumer trust. The symposium program includes speakers from science and innovation, from industry, public authorities, international organizations, law firms and food research institutions.

In addition to the traditional main topics related to food fraud prevention and effective food allergen management, the 3rd edition of this International Symposium will also focus on the Food Law, its implications for industry and food businesses in international trade, export and import liabilities, critical issues of food integrity and food safety related issues governed by national and international regulations and standards, impacts of international trade agreements. This symposium will address food authenticity, food fraud and the need for simple labeling as major drivers for the food industry to rapidly develop new analytical technologies. While the food industry must adapt its programs addressing food safety and quality to meet the ever-changing regulatory and legal landscape, law firms specializing in food issues must also ensure that their knowledge of these changes is up to date when defending the rights of consumers and/or food producers.

This meeting in Rockville brings together international experts in the fields of food authenticity and food allergens, as well as various food industries, SMEs, research institutions, associations and regulatory bodies, all having a different stake in food safety. This special mix of scientific and practical input to the symposium will be a valuable opportunity to grow your knowledge base, learn from practical experiences, and exchange ideas with peers.

We wish you a successful event!

Dr Bert Pöpping, *President, Germany* and Dr Roland Poms, *Secretary General, Austria*

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Fighting food fraud: a consumer perspective

KEYNOTE ADDRESS

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The US Pharmacopia and the National Consumers League share a commitment to fighting food fraud, also referred to as economically motivated adulteration (EMA). USP's Food Fraud Database 2.0 is a great tool and we applaud USP for developing it. With data informed by scientists and food fraud experts from academia, industry and regulatory agencies, the new database offers better coverage of the historical information on instances of food fraud, Food fraud is a global economic and public health problem, costing industry an estimated 10 to 15 billion dollars annually (https://www.gmaonline.org/downloads/research-and-reports/consumerproductfraud.pdf) and affecting as much as 10% of the global food supply.

Identifying food fraud is a challenge for consumers since the grocery aisles are filled with products that make unsubstantiated claims, are adulterated by ingredients that aren't listed on the label and are, in fact, dangerous for human consumption, and 'all natural' when ingredients include artificial flavors, colors or preservatives. There are many good actors in the food industry who want to do the right thing but cannot confirm ingredients in the supply chain – that's where USP and other tools – like Amazon's Project Zero are so important.

The problem for consumers is that too many companies – even reputable ones – deliberately engage in deceptive labelling, marketing and advertising to consumers. I'll be discussing specifics. We also need to increase consumer trust and support new food safety regulations recently finalized by the US Food and Drug Administration (FDA). But mostly we hope to be speaking to the good actors at today's forum; for you, the goal is to provide brand protection and give assurances to consumers that you are doing all that you can to protect your supply chain from adulteration and counterfeits.

Assuming you are actively working to provide consumers with honest labelling, advertising and ingredients, the best way to increase your chances of preventing the next food fraud incident in your supply chain is to make use of the USP database and other tools. But also, to use focus groups and consult with consumer organizations on honest labelling and marketing of products. Thank you for inviting me to join you for this critically important discussion.

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A brief introduction to food fraud information sources

ORAL 1

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Michael will describe the need for information sources on food fraud and food crime, outline what is available globally and suggest recommendations for the future.

Food fraud and food crime are insidious threats to supply chain integrity, consumer confidence and safety, and the reputations of businesses and regulators which the horse meat scandal of 2013 reawakened multiple efforts to combat. By its nature, food fraud is rarely obvious, requiring some form of bioanalytical investigation to uncover. But what to test for and how? Clearly, we can learn much from what has happened in the past – where information sources become essential. Such sources include (but are not limited to):

- The Elliott Review (https://st3.ning.com/topology/rest/1.0/file/get/1030798?profile=original) provides a still relevant primer for all who wish to learn about food fraud and food crime and how to guard against it.
- The scientific literature and media reports
- Food Authenticity Network (http://www.foodauthenticity.uk) – a unique open access website that brings together global information on food authenticity testing, food fraud mitigation and food supply chain integrity in one convenient location. It also ensures that stakeholders have access to a resilient network of laboratories providing fit for purpose testing so consumers can have increased confidence in the food they buy.
- Decernis (https://decernis.com/) originally USP, this platform provides an intelligence-gathering solution and food fraud database using big data analytics providing insights and trend analysis with automatic monitoring and tracking of global events and regulations.
- European Commission – the Commission provides a monthly food fraud summary from the JRC (https://ec.europa.eu/knowledge4policy/food-fraud-quality_en) and other guidance, and the RASFF portal (https://ec.europa.eu/food/safety/rasff_en) that now enables searches of EU alerst for food fraud.
- Horizon scan (https://horizon-scan.fera.co.uk) – a subscription based service that monitors global food integrity issues, allowing you to plan and ensure consumer safety and protect brand identity.

For the future trend analysis in real time is the obvious goal along with intelligence led prediction of previously unknown combinations of fraudulent practices and commodities.

Preventing food fraud: an industry perspective

ORAL 2

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Following the Horsemeat scandal found in some Burgers, Barilla started an ongoing journey with the aim to counteract possible Food Frauds. Barilla needed practical steps to engender trust in the supply chains, maintain transparency, product Integrity and diligent working practises. A Food Fraud Risk Assessment Model was created by a technical team. The model has to take into account the criminal mindset behind a Food Fraud and focus on the following points:

- Scout new technologies to reveal food frauds
- Define unconventional testing to prevent food frauds
- Suggest new parameters to be monitored for food frauds detection
- Review vulnerabilities
- Provide info on risky supply chain/suppliers

The model was the applied as pilot test on a chosen supply chain, tested, revised after results evaluation and finally transformed, from project and in place activities in a whole process.

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International standards development for food authenticity and allergen detection

ORAL 3

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Standards development is carried out by a number of different organizations (Standards Development Organizations, SDOs) according to their own or generally accepted guidelines. The development of international standards, also known as Public Standards or Voluntary Consensus Standards, is based on six principles: transparency, openness, impartiality and consensus, relevance and effectiveness, coherence (no duplication), and development dimension (inclusivity). These criteria are also covered by World Trade Organization in the Agreement on Technical Barriers to Trade which obliges WTO members to ensure that voluntary standards do not create unnecessary obstacles to international trade. Most SDOs operate in the spirit of these principles. Private standards may be developed by consortia of like-minded for-profit organizations or companies, specifically for auditing purposes. However, such standards are less likely to adhere to all or any of the six principles listed above.

The development of a standard by an SDO generally starts with a proposal for adoption of a new method, guidance or specification to address a particular issue. Most proposals come from individual members of groups from an affected industry. If accepted, the text is drafted and critiqued by a group of experts through rounds of consultation until a final version is agreed upon. In some cases, the standard is put into practice or made available for public comment prior to final acceptance and publication. SDOs can be sector specific e.g. AACCI – cereals and grains, AOACI – food and fertilizer, AOCS – fats, oils and oilseeds or broader in scope such as ASTM, CEN and ISO covering a wide range of disciplines through the activities of experts on numerous committees and subcommittees. ISO also develops standards to cover general topics such as quality management, safety, social responsibility, laboratory QC/QA, reference material production, proficiency testing and conformity assessment. All SDOs produce standards to primarily meet the needs of trade and relevant industry partners.

Standards for determining the authenticity of specific foods have been developed in only a few situations. Food Chemical Codex Identity Standards have been written for refined olive oil and pomegranate juice. The International Olive Council and several national and international standards describe the use of methods to authenticate different grades of olive oil. A number of committees at Codex Alimentarius were approached to fulfill this need as was ISO/TC 34 – Food Products and some of its commodity-based subcommittees. A background document was produced for discussion at the Codex Alimentarius Committee for Food Import and Export Inspection and Certification Systems (CCFICS) that referred to early work performed by USP. The current CCFICS project is to identify gaps in existing Codex standards. The European Committee for Standardization (CEN) has produced CWA 17369 – Authenticity and fraud in the feed and food chain – Concepts, terms, and definitions, whereas ISO has a number of general standards aimed at fraud, anti-bribery and corruption. However, none is specific to a particular food, food ingredient or food type. Specifications for food additives and food ingredients as developed by JECFA and USP Food Chemicals Codex are useful in the identification of products though they were developed to provide standards of identity rather than to specifically address authenticity. Food allergen analysis is not well covered by the major SDOs. ISO has not addressed this issue, but CEN 275/WG 12 – Food Allergens, has produced a number of standards and foodstuff-specific reports and technical specifications for different food allergens. AOAC International has published general guidance on food allergen analysis and also a method using LC/MS. Obviously these are rapidly advancing area and more standards are expected.

Food fraud: benefits and challenges of non-targeted methods

ORAL 4

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The food fraud detectives inside and outside the laboratories face quite some challenges. Fraudsters get smarter and the number of adulteration (that we know of) constantly increases. Using single analyte or group analyte methods seems a losing battle. As an answer, scientists came up with what is termed non-targeted or untargeted methods. These methods could be used to detect not only the known-knowns but also the known-unknowns and unknowns-unknowns. And looking at the number of non-targeted methods developed, a PubMed search reveals that the number of publications on this topic constantly increases. In the year 2000, less than a hundred publications can be found on this topic, and since 2017, more than a thousand methods have been published per year. At present, no less than 9000 publications on non-targeted methods can be found. The challenge for the food fraud detectives is: which of the many methods are fit-for-purpose? Most standardization bodies tend to shy away from standardizing methods that target known-unknowns and unknown-unknowns, but some have embraced the challenge. This presentation will discuss the advantages and disadvantages of non-targeted methods and the challenges to standardize them.

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Food integrity with new analytical technologies: unlocking the truth

ORAL 5

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Fraud in food and beverage products include misrepresentation or tampering with packaging and labelling; adulteration, normally replacing a higher quality, original material with one of lesser quality one or extending a product by adding an adulterant; and misrepresentation of product origin. Increased complexity in the food and beverage supply chain has provided greater opportunity for economically motivated food and beverage fraud. Consequently, legislation has been enacted globally to protect food and beverage products with respect to production processes and product labelling. The combination of legislation and food fraud practices demand a reliable, high throughput and cost-effective analytical techniques that can identify food and beverage products that are not what they are claimed to be. Detecting food and beverage fraud can be achieved using next generation sequencing and stable isotope fingerprints because these technologies can differentiate between food and beverage samples which otherwise share identical chemical or similar genetic composition. We briefly explore how these technologies really detect food and beverage fraud based on the unique problem you are trying to solve.

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The role of public standards in protecting supply chain integrity

ORAL 6

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Complex food supply chains create multiple opportunities to damage the integrity of food ingredients and the food supply, either intentionally or unintentionally. It is important to recognize that ingredient integrity relies on being able to demonstrate that a substance has the expected composition at any step in the supply chain; that is, that each substance meets a standard for identity, purity, and levels of contaminants. These standards are most effective and easily communicated when they are developed and made available in a transparent manner by independent experts. Useful standards are actionable when they include both the explicit specifications needed to determine whether a particular sample of an ingredient meets the definition for that substance (i.e., acceptance criteria) and the methods needed for making that assessment. This presentation will provide examples of why standards are necessary to prevent fraud and protect quality and how ingredient standards can be used as part of a supply chain control program.

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Capacity building on food fraud and IPR compliance Lebanon & MENA

ORAL 7

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The WTO issues many agreements in protecting World Trade, such as SPS Agreement (Sanitary and Phytosanitary Measures), TBT Agreement (Technical Barriers to Trade) and TRIPS (Trade-Related Aspects of Intellectual Property Rights), among others. The TRIPS Agreement has been issued in 1995 and is the most comprehensive multilateral agreement on intellectual property. TRIPS Agreement introduced global minimum standards for protecting and enforcing nearly all forms of intellectual property rights (IPR), including those for patents. In Lebanon intellectual property is an open registry and does not require a review process of existing patents or registered brands or trademarks. Moreover, in the case of Agrofood sector, consumers are exposed to fraudulent activities and products in the: olive oil, dairy, and herb & spice industry, to list a few. Recent food fraud in Lebanon centered on adulterated pickles that were not legally branded, trademarked, and adulterated with chemical colorants banned in the EU. During the International Association for Food Protection (IAFP) Conference in 2015, many case studies where exposed by the US FDA and fraudulent activities in Lebanese product were documented. This paper aims to address Food Fraud and have developed capacity building courses on local topic for the benefits of MENA food exporters. The fraud vulnerabilities of general food products supply chain were examined. The SSAFE food fraud vulnerability assessment tool, which comprises of 50 indicators categorized in opportunities, motivations, and control measures was used to extract 8 questions from it backed up by literature review with a general background for getting insight into these fraud vulnerabilities. 5 companies participated in the study all are of two sectors: bakeries and oil industries. This was backed up by a meta-analysis that highlights information on trademarks, branding, IPR, by those cases and studies, to urge the Lebanese & MENA governments to move forward in issuing laws addressing fraudulent activities and protecting intellectual food property, by signing and harmonizing TRIPS for the benefit of food industry and to protect IPR and related innovation, brand image and quality of MENA foods, for the benefit of food safety and security.

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US regulatory requirements regarding food fraud prevention

ORAL 8

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This session will provide an overview of US regulatory requirements regarding prevention of food fraud (or economically motivated adulteration) and intentional adulteration. We'll explore requirements grounded in the text of the Food, Drug, and Cosmetic Act and its implementing regulations, and consider FDA's interpretations of those requirements as articulated in FDA guidance and enforcement actions.

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Death by a thousand AMPs: the future of food fraud prosecutions in Canada

ORAL 9

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A critical question in the quest for effective enforcement against food fraud is whether governments should pass more laws or if the food fraud problem simply requires attention and resources from regulators and enforcement authorities. In Canada, enforcement agencies have found that prosecuting food fraud in the traditional, criminal, realm shares much with white collar crime prosecutions: it's expensive, resource-intensive, and often presents uncertainty, even with the most favourable facts. A judicial finding of *Mens rea* – a guilty mind, intent – is a high bar to reach in the prosecution of food fraud charges. For example, the Canadian Food Inspection Agency (CFIA) has instructed the Public Prosecutor (the 'Crown') to charge food fraud defendants with fraud under the Criminal Code approximately 8 times since 2016. However, in each case, the Crown has chosen to abandon fraud charges to seek guilty pleas to regulatory crimes instead, resulting in comparatively minor sentencing given the damage to the integrity in the food value chain. Proving that a defendant has intent beyond a reasonable doubt and not simply 'loose controls' has proven to be a significant barrier to bringing fraud charges.

The Safe Food for Canadians Act (SFCA) has brought, among other things, an expansive potential use for Administrative Monetary Penalties (AMPs). The AMPs are significantly limited – a maximum fine of CAD$ 25,000 – but the burden is only on the balance of probabilities, not beyond a reasonable doubt. Further, the accused does not have a defence of due diligence or several other benefits traditionally bestowed upon an accused. The Canada Agricultural Review Tribunal (CART) oversees these AMPs and is expected to see a massive increase in the use of these penalties. This includes as a supplementary tool in instances of food fraud where traditional approaches are too complicated, time consuming or expensive, relying on the number of instances of fraud to multiply fines making the global amounts meaningful.

If the CFIA uses the CART-governed AMP framework successfully and in good faith, the low cost of enforcement will lead to an effective, death-by-a-thousand-AMPs, approach to enforcing against fraudulent activities in the food sector and creating a public, regulator-driven, model for other countries in the battle against food fraud. If the CFIA oversteps the use of AMPs as a supplementary tool, it's likely that the judicial system will severely limit the reach of this new tool. At the same time, the AMP framework will provide virtually none of the protections that are traditionally afforded to those who are being unfairly prosecuted.

I will discuss the merits related to this made-in-Canada approach to food fraud prosecutions and to present potential problems for consideration, using recent food fraud prosecutions as case studies, and what this means for food value chain stakeholders.

The EU Regulation 2017/625 on official controls and food frauds related provisions

ORAL 10

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The European Regulation 2017/625 on official controls and other official activities performed to ensure the application of food and feed law – published on the EU Official Journal on 7th April 2017 – will be entering into force on 14th December 2019.

The presentation will address which are the new provisions that will impact on food frauds prevention and mitigation and potential implementation issues. The new Regulation avoids providing a long expected and strongly asked definition of 'food fraud', adopting a lateral approach to the topic. First, art. 9.2 about the general rules for official controls, clearly includes food law infringements through fraudulent and deceptive practices within the scope of the Regulation:

> 'Competent authorities shall perform official controls regularly, with appropriate frequencies determined on a risk basis, to identify possible intentional violations of the rules […] perpetrated through fraudulent or deceptive practices.'

On such cornerstone, the Regulation build a soft but comprehensive framework that will oblige the competent authorities of all the Member States to be more proactive on the topic, within their borders as well as at the entry points into the EU. The designation of a European Union reference center for the authenticity and integrity of the agri-food chain is provided and tools to rapidly exchange information between competent authorities (e.g. AAC – Administrative, Assistance and Cooperation) have been developed. Finally, clear principles about the repressive systems and penalties that Member States will have to put in place to fight food fraud's plague has been established.

The presentation will cover such provisions, offering in the meantime a quick overview about recent EU Commission initiatives against food fraud. The aim of the speech will be not only to present such news, but also to figure out future challenges and changes in the EU food system as we know it. The EU market is a composite puzzle of Member States, with different internal structures and authorities: along years, one of the main challenges for the Union proved to be the uniform implementation of theoretically harmonized provision. To build an effective strategy against food frauds a common approach will be absolutely mandatory.

On the industry side how this provision will impact? Even if a specific vulnerability assessment has not been required by the Regulation, how the controlled food business operator will show their compliance and demonstrate that they are trying to mitigate the risk?

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Intentional adulteration – tips for conducting internal investigations

ORAL 11

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A company receives a cluster of consumer complaints alleging foreign contaminants in their retail food products. How should the company investigate the issue? How should the company respond to consumers? This session will take a real-life case study and explore the steps companies should take when faced with intentional adulteration in their manufacturing facilities. This session also will discuss requirements of the Food Safety Modernization Action (FSMA) Intentional Adulteration Rule.

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Authenticity of coconut water – a critical review

ORAL 12

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The fruit of the coconut (*Cocos nucifera* L.) is extensively grown and its parts marketed for many purposes. The inner part of the nut (endosperm) is made up of two edible parts: (a) a white kernel (the 'meat', dried kernel is referred to as copra from which oil is extracted); and (b) 'coconut water' contained within the kernel. Thus, coconut water is the clear colourless liquid extracted, without the coconut meat, directly from the inner part of the coconut fruit. Coconut water is practically sterile in the coconut and is widely consumed as a drink, prized for its delicate, albeit labile, flavour when fresh. Coconut water has attracted religious symbolism in Asia, reputed health benefits and, containing phytohormones (auxin, cytokinins and gibberellins), has been investigated as a growth medium and biocatalyst for microorganisms and plants. Although not ideal, it has been used direct from the coconut *in extremis* as a short-term intravenous hydration fluid.

Consumption of coconut water in Europe is relatively small compared with Asia and South America which account for over 90 % of world consumption. However, in recent years there has been a dramatic increase in the UK and global demand for coconut water due to its reputed potential as a sports drink and a natural isotonic drink. The increase in its consumption, potential variation in composition and potential for adulteration make a review timely of the approaches deployed for its authentication. In a recent UK sampling exercise 60% of imported products described as coconut water were found to contain added sugar albeit the sample size (n=7/12) was small, and some of the implicated brand owners questioned the conclusiveness of the analytical procedure.

Coconut water contains sugars, minerals, vitamins, amino acids enzymes, volatile aromatic compounds and other biochemical compounds. The composition of raw coconut water is determined by a range of factors however, owing to the organoleptic superiority of water from young green coconuts, maturity at harvesting is the most influential factor in yield of water and its composition. Published compositional data have been critically reviewed and this presentation reports consensus sugars data that distinguish coconut water from fruit juices and provide an authenticity guide for water from young (<9 month) coconuts.

While international standards are as yet lacking there is a global good practice guide to coconut water production and several local standards. The presentation collates and presents these. The standards described above rely for the most part on classical analytical techniques. More advanced approaches have been proposed including Raman spectroscopy, FTIR, NMR in combination with chemometrics, GC-MS and IRMS. These have been applied to investigate composition and processing but also, in some cases, with the direct intention to detect adulteration. In particular Raman spectroscopy appears to have significant potential as a rapid accurate analytical method for the detection of abnormalities in sugar ratios within coconut water. Equally, ^{1}H NMR and chemometrics can be utilised as a potential diagnostic marker for partial substitution of fresh coconut water with extrinsic components such as sugar mixtures. Stable carbon isotope ratio mass spectrometry (SIRMS) analysis is a powerful authentication approach widely applied for exogenous sugar and geographic origin. IRMS investigations have been successfully applied by several research groups for the detection of added C_4-plant sugars in coconut waters.

SYMPOSIUM: LABELLING, ANALYSIS AND EFFECTIVE FOOD ALLERGEN MANAGEMENT

Food industry perspective on effective food allergen management to address food safety challenges

OPENING SPEECH

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Undeclared food allergen is a well-recognized public health concern due to the potential for severe and life-threatening reactions in allergen sensitive consumers. The food industry has an obligation to protect consumer health and produce safe foods for all consumers. This food safety priority is non-negotiable. Food manufacturing is a complex process, and low level of allergen can find its way to manufacturing facilities by comingling from farm to table. This presentation will review the industry challenges, labeling requirements and threshold needs. In addition, a risk-based allergen management strategy is highlighted.

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The face of food allergy – consumer attitudes; industry insights and recommendations to build trust

ORAL 13

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Food allergens are on the rise. Recent data reported by Food Allergy Research and Education FARE indicate an increase in food allergy diagnosis, reaching as high as 32 million American adults and 5.6 million children living with at least one food allergy. Current data suggests that 10% (25 million) of all adults in the U.S. have at least one food allergy. Food allergies can also affect an individual later in life, resulting in an increase in total diagnoses over time. The 2019 IFIC Foundation Food & Health Survey, suggests slightly higher results. In the survey of 1000 American households, 17% say they, or someone in their household, is living with a food allergy. Additional F&H data suggests that over one-third of consumers know someone with a food allergy outside the home. Combined, these data suggest that more than half (54%) of Americans, 'know' someone with a food allergy; at home, work or play. With rates suggesting an increase in diagnosis as well as clinical data to suggest late onset of food allergies, it's important to understand perceptions and behaviors regarding food allergen management for meals at home and away from home. Cristikas *et al.* estimates '150 people (children and adults) die each year from all food allergies combined.' The food industry is dedicated to allergen management and proper labelling to indicate the presence of allergens in packaged foods. However, the recent CDC's report 'How to address food allergies' states that 'most food allergic reactions occur in restaurants.' Anecdotally, food companies who are dedicated to managing allergens in packaged foods, are typically on the lower end of consumer trust. With only a small fraction of restaurant staff being trained in food allergens, what does this mean for the food allergic family yearning to eat out for a special occasion. What stress does 'may contain' have on the primary shopper and how should I call attention to the restaurant that even the slightest contamination can cause a reaction. These are just some of the quality of life challenges affecting today's food allergic consumer. These challenges affect the family, the school and the community at large. These challenges can also occur later in life presenting an unexpected lifestyle change. This presentation will increase attendee awareness of individuals living with food allergies; family, friends and colleagues. Attendees will understand how to address challenges facing families and provide tangible recommendations to build trust for the food industry. In addition, this presentation will provide a deep dive into consumer responses to consumer confusion regarding 'may contain' and recommendations to improve understanding. This session will provide insights into perceptions about effective and not so effective labelling and suggestions aimed at streamlining the variety of statements in use today.

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Gluten-free: food safety vs voluntary consumer choice?

ORAL 14

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About 2%-5% of the adult population suffers from one or more food allergies. Amongst children these numbers seem to be twice as high. Although not a food allergy, celiac disease is often mentioned in the same context. It is estimated around 1% of the population suffers from this gluten triggered auto-immune disease. Extrapolating this number to the number of households, this would mean that the number of people affected (directly or indirectly) is probably 3-4 times higher.

Food allergies and intolerances have a significant socio-economic impact. Individuals suffering from a celiac disease and their family or household members are facing several additional costs. In the case of medical care and hospitalization additional healthcare expenditures are made. But beyond those direct costs, individuals with celiac disease face various indirect costs like loss in productivity and quality of life. For many years the availability of gluten-free products, the price as well as the sensory (and nutritional quality) was a major issue.

The choice of gluten-free products, as well as the quality with respect to taste has improved a lot though the past 5-10 years for a simple reason: the demand for gluten-free products has increased in a spectacular way. In the UK 22% of the consumers consume gluten-free products; up to one third of the consumers in the US avoid gluten in their diet. Gluten-free has become a major trend in food production and number of consumers interested to buy gluten-free products is still rising. In fact 'free from' is one the growth drivers in the food industry and offers new possibilities for the food industry.

To protect consumers with celiac disease and to assure consumers are informed in a correct way about the presence of gluten in their food, labeling legislation is in place in a growing number of countries all around the world. In order to meet this legislation and customer requirements, gluten-free food producers should have a solid gluten management in place. This means of course the food industry has to invest in quality assurance with respect to gluten, e.g. testing, but also invest in production capacities (dedicated production line or at least an effective cleaning regime). Also sourcing of raw materials and monitoring of suppliers may lead to higher production costs.

This presentation will give an overview of the socio-economical aspects of 'gluten-free' from the consumers and the industry's point of view.

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Identifying, curating and harmonising clinical data to identify minimum eliciting doses for food allergens in the ThRAll project

ORAL 15

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Mandatory food allergen labelling is helping help food-allergic consumers practice food avoidance but the presence of unintended food allergens and precautionary allergen labelling (PAL) is problematic. Surveys of foods with and without PAL indicate confusion and a lack of a coherent approach, with some foods having been found to contain significant levels of allergens and yet not carrying a PAL. The use of risk-based approaches to managing allergens in foods is addressing this confusion but requires access to good quality data from clinical studies to allow levels of allergens in foods that are considered to be generally safe for most food-allergic consumers. Following on from other EU initiatives, such as EuroPrevall, MoniQA and iFAAM, the European Food Safety Authority with the UK Food Standards Agency (FSA) and the Federal Agency for the Safety of the Food Chain (FASFC) are cofunding the ThRAll project which seeks to support the application of risk-based approaches to food-allergen management. One objective is to develop a harmonized quantitative MS-based prototype reference method for the detection of multiple food allergens. A second objective is to develop tools to support the collection, curation and harmonisation of data on oral food challenges, which are used to define thresholds and minimum eliciting doses. This is being achieved through the development of common clinical protocols, which take account of aspects such as the dose progression required to identify both no observed and lowest observed adverse effect levels, consistent methods for recording the symptoms during a food challenge and provide ways of supporting implementation of severity scoring. The selection of appropriate allergenic food ingredients and the types of food matrix used in food challenges is also taken into consideration, along with the needs blind the taste, texture and appearance of allergenic ingredients whilst retaining palatability and representing foods as they are commonly consumed. Challenge data for allergenic foods on Annex 2 of the EU Food Information for Consumers regulation are being collected using a REDCap database, which are then being curated to provide a publicly accessible curated, data-analysis data set. The activity is focused on foods such as walnut, cashew, pistachio, fish, crustacean and molluscan shellfish. For those foods for which threshold data are collected which exceed 30 subjects responding with objective symptoms, dose distributions will be modelled using interval censoring survival analysis and compared with published dose distributions, where available.

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Application of mass spectrometry methods for food allergen analysis and management: opportunities, hurdles, and needs

ORAL 16

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A number of research groups throughout the globe have developed innovative and effective mass spectrometry (MS) methods for food allergen detection, but we have yet to see widespread adoption of these methods across laboratories or by the food industry for food allergen management purposes. In addition, both the specificity criteria for detection and the strategies for quantification using MS vary considerably among method developers. This talk will leverage both research and industry experiences to discuss the areas in which MS methods may have the most benefits, the hurdles that are encountered with the use of MS methods, and the needs that should be addressed to make the methods applicable to allergen management.

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The xMAP Food Allergen Detection Assay: a solution for the simultaneous detection and identification of multiple food allergens to address current and future needs

ORAL 17

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Safeguarding of food allergic consumers is dependent on food labels declaring the presence of allergens. The undeclared presence of food allergens may be due to a labelling error, inadvertent cross-contact, use of ingredients containing or derived from food allergens, use of shared equipment, and manufacturing practices. To ensure the accuracy of labelling disclosure statements, analytical methods are required. Validated ELISAs are the most commonly employed methods because they are easy to use and do not require expensive equipment or specially trained technicians. ELISAs are ideal for testing for a single, known food allergen. However, this is often not the case when dealing with regulatory samples, especially when responding to a consumer complaint. All too often, consumers suffer from multiple food allergies, a prevalence estimated at >30% of the allergic population. Further, details of the manufacturing process which may alter the allergenic proteins are often not known. The Food Allergen Labelling and Consumer Protection Act of 2004 (FALCPA) requires the identification of tree nuts, crustacean, and fish species, with the only legumes that must be declared being peanut and soy. Since, the concentration of food allergens may vary considerably along with that of any potentially cross-reactive food, cross-reactivities as low as 0.0005% have been a problem when testing a commodity for inadvertent cross-contact. Previously, the FDA addressed some of these ambiguities by requiring concurrence between two ELISA test kits that relied on different extraction and analytical protocols. However, with an increased need to test for multiple allergens in a sample and the globalization of the marketplace increasing the possibility of novel, cross-reactive foods being present, the use of ELISA test kits became untenable.

The xMAP Food Allergen Detection Assay (xMAP FADA) was developed with Radix BioSolutions to address the problems associated with the use of single-analyte ELISAs. The xMAP FADA can simultaneously detect 15 food allergens plus gluten and by employing two capture (complementary) assays per analyte and two extraction protocols, has built-in confirmation. The use of color coded magnetic beads gives the xMAP FADA a plug-n-play design that makes it adaptable to meet future needs or be tailored to the specific needs of the end-user. Further, the requirement that the antibodies display high affinity for the target analyte and a very high degree of specificity is not necessary and sometimes undesirable. By exploiting the potential of antibodies to cross-react with homologous antigenic proteins, the xMAP FADA can distinguish cross-reactive proteins from the target analyte. Indeed, the unique specificities that govern the antibody binding profiles make it possible to reliably detect and identify antigens not specifically targeted by an antibody. As such, many of the control material samples necessary when employing an ELISA are no longer necessary; an advantage when analyzing unique food products for which allergen-free control samples are not available.

The xMAP FADA has been extensively validated with each of the 16 targeted analytes incurred in various food matrices, including baked muffins, dark chocolate, orange juice, and meat. It has also been successfully applied to regulatory case samples where the definitive identification of an allergen was critical, or an acceptable ELISA method was unavailable. The ability of the xMAP FADA to detect and distinguish between related botanical products was also examined, along with the effects of roasting on legumes and tree nuts. When the potential number of undeclared allergens present made identification using the xMAP FADA problematic or processing modified the allergenic proteins making antibody-based detection and identification unreliable, orthogonal methods (e.g., mass spectrometry and DNA-based) were employed. Together, the use of such multiplex methods resolved the analytical questions and limitations associated with the use of single analyte-based methods.

Simultaneous quantification of major food allergens using fluorescent multiplex array

ORAL 18

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Quantification of food allergens is important for dose assessments as part of food allergy prevention, for risk assessment, safety monitoring and for effective food allergen management. Generic immunoassays for 'total protein' (e.g. peanut, milk, egg) do not measure specific allergens and their specificity is poorly defined. Our goal was to use a molecular approach to food allergy to develop a multiplex array capable of simultaneously measuring allergens of known clinical importance (e.g. Ara h 6, Ara h 3, Bos d 5, Gal d 1). These are the 'active ingredients' in foods to which allergic patients react and their molecular structures are known. The aim was to develop a multiplex array capable of measuring all food allergens that are regulated in the US, Europe and Japan in a single test.

The multiplex array was developed on the Luminex xMAP system. Microspheres coupled to specific monoclonal antibodies were used for allergen capture. Biotinylated specific monoclonal or polyclonal antibodies were used for detection. Reference standards were purified allergens with protein content determined by amino acid analysis and purity established by mass spectrometry. Full method validations were performed to determine parameters of linearity, range, limits of quantification and detection, accuracy and precision. Inter-laboratory performance was compared. Food products were analysed using a multiplex array and the results were compared with ELISA and with mass spectrometry. Environmental samples from schools in the Boston area were also compared to assess food allergen exposure in classrooms.

Method validations were completed for 12 major food allergens. Standard curves for all analytes allow for quantification over a broad (4-log) dynamic range. Limits of detection were as low as 0.01ng/ml (1.0E-5 ppm). Intra- and inter- assay accuracy and precision of three samples assayed in triplicate on four occasions passed acceptance criteria within the range of 70-130% recovery and a CV of <15%. Inter-laboratory variability was 14-18%. The specific allergen content of food products (e.g. Nutella, milk, egg, cashew, hazelnut, Bamba) and the NIST SRM 2387 Peanut Butter Standard correlated with the food ingredients. The results for individual allergens in the array correlated with ELISA and showed broad agreement with protein abundance by mass spectrometry. Analysis of the school samples showed that milk allergen Bos d 5 was found at high concentration in dust extracts and table wipes, along with peanut and egg allergens.

A quantitative, accurate and precise multiplex immunoassay (MARIA for Foods) was validated for the simultaneous detection of major food allergens. Measurement of specific food allergens ('active ingredients') by MARIA:

- Improves consistency, reproducibility and standardization of food allergen detection
- Enables direct comparison with other test methods – ELISA and mass spectrometry
- Enables raw materials to be screened for allergen contaminants
- Enables cleaning processes to be validated for allergen control
- Improves risk assessment of allergic reactions due to allergen contaminants in the food industry and other environmental exposures (homes, schools)
- Enables risk thresholds to be based actual allergen doses and exposures.

Completion of a 17-plex array will allow all allergens that are regulated by food laws in the US, Europe and Japan to be measured simultaneously in a single test. MARIA is an important forensic tool for 'food detectives', regulators, and food processors, to reduce food fraud and improve food allergen management. Most importantly, application of this technology in the food industry should reduce the risks of accidental exposure for food allergic patients.

Multiplex competitive ELISA and western blot analysis for the detection and characterization of gluten in fermented-hydrolyzed foods

ORAL 19

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Celiac disease affects approximately 1 in 141 individuals in the United States, requiring adherence to a strict gluten-free diet. The prevalence is approximately 1% in regions populated by individuals of European origin. The Codex Standard, the European Commission, and the FDA use a cut-off level of 20 ppm to define gluten-free foods. Accurate quantitation of gluten in fermented-hydrolyzed food is a challenge due to the lack of appropriate reference materials and variable proteolysis. The commercially available methods routinely used to detect and quantitate gluten are not able to distinguish between different hydrolytic patterns arising from differences in fermentation processes, a severe limitation that makes accurate quantitation and, ultimately, a detailed evaluation of any potential health risk associated with consuming the food difficult.

To ensure accurate quantitation of gluten in fermented-hydrolyzed foods, a novel multiplex-competitive ELISA was developed utilizing gluten specific antibodies (G12, R5, 2D4, MIoBS and Skerritt) from nine commercial gluten ELISA kits. The assay was used to evaluate 87 different fermented-hydrolyzed foods belonging to six different categories (20 wheat beer, 20 barley beers, 6 gluten-reduced barley beers, 15 soy sauces, 6 teriyaki sauces, 6 Worcestershire sauces, 6 vinegars and 8 sourdough breads). Western blot analyses, utilizing the same nine gluten specific antibodies and a subset (65 samples) of the same fermented-hydrolyzed foods, were performed as an orthogonal approach that can be used to both confirm the multiplex-competitive ELISA while also providing additional insight into the protein/peptide profile of fermented-hydrolyzed foods. Hierarchical cluster analysis of the apparent gluten concentration profiles, obtained by the multiplex-competitive ELISA and the western blot analyses, was performed using the Ward's Minimum clustering method.

The multiplex-competitive ELISA classified foods based on the degree of gluten proteolysis, indicative of the fermentation process. All of the 20 wheat beers, 32 of the 33 soy-based sauces and vinegars, and all of the 8 sourdough breads generated significant clusters separately from each other (Approximately Unbiased (AU) P-value>0.95). Although the soy-based sauces showed non-specific inhibition with multiple antibodies, their overall profile was distinguishable from the other categories of fermented foods. The barley beers generated complex clustering patterns. Of the 26 barley beers, 25 clustered separately from wheat beers and 24 clustered separately from sourdough breads. By the western blot analyses, the protein/peptide profiles generated by the nine gluten specific antibodies varied in size distribution and intensity dependent on the type of food, with minor differences between related products. Cluster analysis of the estimated gluten concentration values (based on western blot band intensities) distinguished among the different categories of fermented-hydrolyzed foods; comparable to what was observed in the multiplex-competitive ELISA. The specificity of the different antibodies used in the multiplex-competitive ELISA and the western blot analyses towards gliadins, glutenins, and deamidated-gliadin means that the clusters reflect differences in antigenic protein/peptide profiles. Further, unlike the multiplex-competitive ELISA, the western blot analyses distinguished between the presence of antigenic proteinaceous materials and false positives due to the presence of binding inhibitors, as observed with four soy-based sauces and one vinegar.

The developed multiplex-competitive ELISA, along with the western blot analyses, provide insight into the extent of proteolysis associated with various fermentation processes. The use of two orthogonal, complementary approaches should assist in selecting appropriate calibration standards that may be useful in the qualitative and quantitative characterization of gluten in fermented-hydrolyzed food products. Specifically, the western blot analyses make it possible to distinguished between false positive responses and the presence of gluten derived proteinaceous materials while, with the appropriate calibration standards, the multiplex-competitive ELISA could be used to quantitate the gluten content.

Optimization of a targeted, multi-allergen LC-MS/MS method for the quantification of egg, milk, and peanut in food

ORAL 20

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Liquid chromatography (LC)-tandem mass spectrometry (MS/MS) can be used as a complimentary analytical technique to immunochemical-based assays for allergen detection. A targeted, multi-allergen LC-MS/MS method has been previously developed for the simultaneous detection and quantification of egg, milk, and peanut. In bakery products, the method provided reliable detection of each allergen at concentrations as low as 5 mg/kg (ppm) incurred allergen ingredient using fourteen peptide markers from egg (lysozyme C and ovalbumin), milk (beta-lactoglobulin and alpha-S1 casein), and peanut (Ara h 1, Ara h 2, and Ara h 3). In the absence of established guidelines for MS-based quantification of allergens in food, such as acceptable quantification strategies and reporting units, the goal of this work was to evaluate different modes of quantification, establish acceptance criteria, and demonstrate transparency in the utilization of conversion factors. Commercial cookie samples were fortified with 10, 25, and 100 ppm light roast peanut flour, spray-dried whole egg, and nonfat dry milk and then homogenized, defatted, and extracted for total protein content. Sample concentration and trypsin digestion was performed using a modified filter-aided sample preparation (FASP) protocol. Method performance including accuracy, precision, limits of detection, and quantitation were evaluated for different standards (isotopically-labeled $^{13}C^{15}N$ peptide surrogates and chemically homologous protein/peptides), calibrants (synthetic native peptides and allergen reference materials), and instrument platforms. The samples were analyzed on multiple LC-MS/MS platforms under both nano- and standard-flow LC conditions using a multiple reaction monitoring (MRM) method on a 6500 QTRAP (Sciex) or nano-LC conditions using a parallel reaction monitoring (PRM) method on a Q Exactive (Thermo). All MS data were analyzed using Skyline software. The suitability of each method for routine analysis was evaluated to provide a robust workflow that can be used in support of allergen management within the food industry and the protection of consumers with food allergies.

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Real-time PCR based allergen detection at FDA-CFSAN

ORAL 21

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The United States' Food Allergen Labeling and Consumer Protection Act (FALCPA) of 2004 mandated that the presence of allergenic proteins derived from the eight major food allergens must be declared on food labels. In order to determine the accuracy of labels, FDA relies on highly sensitive and highly specific analytical methods. Work at FDA-CFSAN is currently focused on the use of multiple orthogonal methods, including antibody-based protein detection and DNA-based real time PCR detection, to provide the most thorough and definitive assessments of complex samples. FALCPA names eight major allergenic foods and food groups which account for over 90% of allergic reactions, and DNA is a suitable indicator for the presence of allergens in four of these. This talk will provide an overview of work at CFSAN on the development and validation of real time PCR methods for detection of crustacean shellfish, finfish, peanut, and tree nuts. All of the real time PCR methods developed at CFSAN have demonstrated linearity over 6-8 orders of magnitude and limits of detection of approximately 0.1-1 mg allergenic food per kg food matrix. This body of work has demonstrated the importance of targeting the DNA of high copy number organelles to yield low limits of detection in real time PCR assays for food allergens. Sample preparation and DNA extraction techniques developed as part of this work have been effective across allergens and across food matrices. These methods have been robust in a wide variety of finished food products and thermal food processing conditions. Studies beyond initial development and validation have shown that some of these PCR methods are orders of magnitude more sensitive than commercially available ELISA assays, and they have also been used to help clarify ambiguous ELISA results in certain regulatory samples. Overall, this work has demonstrated that well-developed PCR assays are highly valuable as a sensitive and robust method for detection of allergenic foods at trace levels in complex products.

The undeclared presence of allergens in foods: a need for specific tree nut peptide markers

ORAL 22

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Undeclared food allergens are the leading cause of FDA food recalls, accounting for 30-40% of all food recalls. Compliance with food labeling regulations and the implementation of effective allergen control plans require the use of reliable analytical methods for detection of allergens in complex food products. Mass spectrometry-based approaches have recently emerged as an orthogonal confirmatory technique for allergen detection, due to their ability to provide specific identification among closely related allergens. For tree nuts, the selection of species-specific peptide markers can be challenging because of limited protein sequence information and the absence of well-characterized reference materials. The goal of this work was to establish a workflow for the selection of walnut peptide markers, which includes the implementation of a comprehensive protein database and the empirical evaluation of closely-related food ingredients and commercial food commodities. Raw and roasted varieties of common tree nuts (English walnut, black walnut [raw only], pecan and hazelnut) and commercial walnut-containing samples were ground, defatted, and extracted for total protein content. Sample extracts were concentrated and digested using a modified filter-aided sample preparation (FASP) protocol and LC-MS/MS data were evaluated applying a parsimony-driven global proteomics workflow. A two-tier strategy was applied to select candidate peptide markers whereby traditional selection criteria including empirical MS identification, physiochemical properties, and specificity were complimented by differential peptide-based profiling of peptide markers in processed (e.g. roasted) ingredients and peptide presence in commercial food commodities. Candidate peptides (5 peptides per protein family) were selected from priority (high abundance) allergen protein families as the foundation for targeted MS method development. Using walnut as a case study, this work establishes criteria for the selection of candidate peptide markers in tree nut proteomes, promoting transparency in the development of LC-MS/MS-based methods for food allergens. Moving forward, the availability of improved protein databases and reference material will provide a more comprehensive characterization of allergen ingredients and help establish highly-specific confirmatory workflows for the detection and quantification of allergenic foods.

Global overview on food allergen labeling regulations: harmonization vs. consumer protection?

ORAL 23

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The lack of legislative harmonization is one of the main obstacles to international trade of food. Different countries have different labeling rules in place, different nutrition declarations and – when it comes to allergens – different lists of substances regulated, different exemptions and different positions about the so called *'precautionary allergen labeling'* statements (PAL, e.g. 'may contain …').

These factors create a highly complex legal environment for companies operating in several countries, hinder the creation of unique labels to comply with different market requirements and oblige companies to keep into consideration in their allergen management procedures a wider range of substances than the ones listed in their own country. For instance, if you are operating in Europe and marketing your products also in Japan or US, you might have to consider as an additional allergen the buckwheat (for Japan) or an extended list of tree nuts, including pine nuts or coconut (for USA). It has to be remembered that a breach in allergens' legislation will cause in most countries food recalls and – eventually – criminal prosecution, especially when consumers get injured.

The presentation will offer a brief overview of such complexity, of the impact on day-to-day business operations and of the potential consequences for brand owners. New emerging challenges about allergen labeling will also be addressed, in particular the one brought by e-commerce, food delivery services and catering activities.

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Consumer analytical devices: the good, the bad and the ugly

ORAL 24

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The number of portable/mobile food safety testing devices is continuously increasing. While in 1984, the number of publications about mobile food safety testing devices amounted to 15 in that year, it increased crossed the 100 publication/year mark in 2003 and in 2018 the number had risen to over 500 manuscripts on this subject. While this per se is a very encouraging development and has the potential to shift the frontiers of food safety testing, there are a number of risks associated with it, which require mitigation.

The presentation will discuss the pros and cons of portable devices in laymen's hands, the different types of portable devices, the needs for standardization and validation as well as providing examples of devices already on sale for consumers.

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Food allergen analysis, reporting and interpretation – how to make them fit for purpose

ORAL 25

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Food allergen analysis is one of the cornerstones of risk assessment and risk management of this epidemic disease. However, the three main approaches to routine food allergen analysis, ELISA, PCR and LC-MS/MS have come under scrutiny and need to be improved. Walker *et al.* have described the framework to address problems detecting and quantifying allergenic proteins in foods. Gowland and Walker have described food allergen cases in the civil and criminal courts of the UK, an adversarial common law jurisdiction. This presentation will update both aspects.

Part of the answer lies in accessing and using clinically and industrially relevant reference materials (RM). We will describe what are currently the optimum such RMs available from LGC and MoniQA. For example LGC provides two peanut allergen QC materials (a low-allergen matrix material and peanut flour) and a RM kit containing (a) a representative food matrix gravimetrically incurred with 5 allergens at clinically and industrially relevant concentrations; (b) the food matrix devoid of the target allergens; and (c) the allergenic raw materials themselves. The allergen materials, hens' egg white powder, skimmed cows' milk powder, hazelnut powder, walnut powder (both partially defatted) and almond powder (full fat), have been characterised by proteomics (UoM, led by Professor Clare Mills). The matrix has been gravimetrically incurred at 10 mg allergen protein for each allergen. This presentation will provide a summary of the work, and will describe the steps taken to ensure the relevance and characterisation, including homogeneity and stability of RMs.

Equally, MoniQA have made available RMs which will be described.

But there are key questions that must be addressed:

- How should RMs be used?
- Will they address all the current issues besetting allergen analysis?
- How should fit for purpose data be reported to ensure customers derive maximum benefit from their laboratories?
- How should results be interpreted?
- What do the courts demand, and have recent UK court of appeal decisions changed the landscape?

These questions too will be addressed in this presentation with practical answers signposted focused on improving our analytical performance. A model report will be discussed. Our aims are to benefit people with allergies, businesses supplying food, bioanalytical companies, and regulators seeking to protect consumers on a level business playing field.

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MoniQA's food allergen reference material program

ORAL 26

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In 2013 MoniQA initiated a Task Force on the development of food allergen and gluten-free reference materials. The Task Force is an international group comprised of several SDOs (Standardisation Organisations), industry representatives, policy makers, test kit providers and method developers, analytical companies, as well as representatives from various universities. This international group works towards consensus on the specific requirements and the design of global food allergen reference/testing materials and gluten-free standard materials. For this purpose, MoniQA has liaised with the EU funded project iFAAM, the Prolamin Working Group and Australia's Vital concept group.

The aim of MoniQA's initiative is the publication of a Guidance Document on the special requirements and production of food allergen reference materials. Accompanying research and the optimization of the production scheme to provide basic and incurred reference materials, spiked samples and extracts have been initiated. This paper will give insight in the challenges, controversial views, and the design of the reference materials and the current state of affairs. First details will be given on the characterization of the milk material and the basic matrix material based on a gluten-free rice cookie. For the gluten-free analysis the selection criteria and characterization of the candidate wheat varieties have recently been completed.

The quality of reference materials is critical for accuracy and comparability of analysis results. Reference materials must be sufficiently homogenous, stable and traceable. Usually extensive material characterisation and testing for homogeneity and stability of the material precede the availability of reference materials. Ideally a certified reference material (CRM) shall be used, which has been validated by accredited institutions and is subject to strict quality testing. Certified reference materials usually come with a certificate with information on the methods used for validation/assigning a value, the measurement uncertainty and traceability of the numerical value of the analyte's concentration in the material or the analyte's purity. According to ISO/IEC 17025, accredited laboratories are required to use certified reference material. At this point the currently available knowledge base and methodological abilities do not allow to certify food allergen reference materials according to international standards requirements, however, for the currently available internationally validated materials the international task force led by MoniQA Association is discussing appropriate procedures for the certification of the offered food allergen reference materials according to ISO Standards.

The first validated Reference Materials for Food Allergen Analysis are now available and can be ordered from MoniQA Association. The first set of materials includes testing materials for milk allergen analysis comprising a Positive Control (SMP-MQA 092014, characterized dried skim milk powder, validated protein content), Negative Control (BLANK-MQA 082015, based on a gluten free cookie), and 2 Incurred Materials: LOW-MQA 102016 (SMP incurred in gluten free cookies, milled, concentration approx. 3.54 mg/kg milk protein, validated) and HIGH-MQA 082016 (SMP incurred in gluten free cookies, milled, concentration approx. 17.7 mg/kg milk protein, validated).

The materials were produced by Trilogy Laboratories USA (MoniQA Member since 2013) and have been commercially available starting 01 January 2017 through MoniQA Association. All materials come with a data sheet and a reference certificate to the analytical results, a measurement uncertainty and validation information. Distribution and shipment of the materials is subcontracted to Authorized Distributors among the MoniQA Member Institutions.

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International standards for food authenticity and allergen detection from ISO TC 34/SC 16 horizontal methods for molecular biomarker analysis

POSTER 1

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ISO Technical Committee 34 'Food Products'/Subcommittee 16 'Horizontal methods for molecular biomarker analysis' works to ensure that standardized biomolecular testing and laboratory criteria are reproducible and technically sound reducing potential disputes between exporting and importing nations and increasing predictability in world trade. Harmonized, easy to handle methods of analysis with defined patterns and known nomenclatures bring more customers to the market. SC 16/TC 34 has increased international stakeholders' participation in standardizing biomarker testing, improved the quality and relevance of these standards and continues to increase transparency in international markets, particularly for food authenticity, varietal identification and genetically engineered products. ISO standards have been adopted by Codex Alimentarius and many governments throughout the world. The International Organization for Standardization (ISO.org) was formed in 1946. It is an independent, non-governmental voluntary consensus standard body based in Geneva, Switzerland with a membership of 163 national standards bodies. The US ISO member is the American National Standards Institute (ANSI.org) a consortium of US standardization organizations. ISO TC 34/SC 16 was created in 2008. There are 42 participating countries. Its scope is, Standardization of biomolecular testing methods applied to foods, feeds, seeds and other propagules of food and feed crops. The US delegation responsible for developing the US position for standards development in food authenticity and allergen detection is called the US Technical Advisory Group (TAG). It was delegated to the American Oil Chemist's Society (AOCS.org) by ANSI. AOCS also hosts the TC 34/SC 16 international secretariat. TC 34/SC 16 has published 20 standards with another 16 under development. Recently published standards include: Technical Specification ISO/TS 16393 Molecular biomarker analysis – Determination of the performance characteristics of qualitative measurement methods and validation of methods, International Standard ISO 16578 Molecular biomarker analysis – General definitions and requirements for microarray detection of specific nucleic acid sequences and International Standard ISO 20813 Molecular biomarker analysis – Methods of analysis for the detection and identification of animal species in foods and food products (nucleic acid-based methods) – General requirements and definitions. More on food authenticity and allergen detection work in ISO TC 34/SC 16 is provided.

Setting up the foundations for the reliable confirmation of food authenticity in official food control

POSTER 2

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Food authenticity is currently one of the major topics in the field of food analytics (e.g. according to article 25 and 26 of Regulation (EU) 2017/625). With the development of new analytical technologies such as liquid or gas chromatography coupled to mass spectrometry (LC/GC-MS), isotopic ratio mass spectrometry (IRMS), nuclear magnetic resonance spectroscopy (NMR), or next-generation sequencing (NGS), powerful tools are ready to join the food forensic toolbox. However, even though such techniques typically show high potential in scientific applications, their fitness for purpose for routine analyses has yet to be proven in most cases. So far, most of the newly developed methods are either in-house validated or not validated at all. Determination of the reproducibility is especially important for routine application over a wide range of laboratories, therefore making inter-laboratory validation studies for these methods mandatory. Moreover, since the new analytical technologies have been transferred just recently to the food analytics, there is still a lack of standardization of the methods themselves but also for the validation procedure of such methods.

In order to meet the requirements of the official authorities responsible for food surveillance in Germany and Europe, it is requested to validate and implement these methods into the German 'Official Collection of Methods of Analysis and Sampling' (ASU) and to subsequently transfer the validated methods to the German (Deutsches Institut für Normung, DIN) and European (European Committee for Standardization, CEN) standardization bodies. Following a suggestion of the Federal Office of Consumer Protection and Food Safety (BVL) which has been submitted by DIN, CEN has taken the initiative to institute a new technical activity (CEN/TC 460 'Food Authenticity'; scope: Standardization of analytical methods for verification of food authenticity and data evaluation aspects including validation concepts and terms and definitions. The methods shall be validated if possible).

The first meeting of the new CEN/TC 460 took place in Berlin on 14 June 2019. There, the technical committee decided to establish the working groups (WGs) 'Concepts, terms and definitions'; 'Species analyses using DNA-based methods'; 'Coffee and coffee products' and 'NMR analysis'. Further actions included the proposal from UNI to create a WG on 'Stable Isotope Analysis' and the proposal from NEN to create a WG on 'Validation concepts of non-targeted methods.' Apart from the endeavors on the European level regarding the analytics of food authenticity, there have already been several German activities regarding this topic. On 28 February 2019, DIN founded a new working committee 'Food Authenticity' in Berlin including the WGs 'Molecular Biological Species Analysis', 'Coffee' and 'NMR'. Furthermore, in 2018 and 2019, the office of the ASU at BVL constituted several new § 64 LFGB (German Food and Feed Act) WGs. These WGs include the WGs 'Mass Spectrometric Protein Analysis' (founded in March 2018), 'MALDI-TOF', 'NMR' (both February 2019), 'NGS – Species Identification' and 'NGS – Bacteria Characterization' (both March 2019). The foundation of a new WG regarding IRMS is planned for the end of 2019. Within the new WGs, the applicability of new methods is discussed by the expert members with the aim to validate those methods through inter-laboratory validation studies and to develop general guidelines for such studies.

The poster presents the experimental design of pilot studies. The aim of such studies is to unify the sample preparation and the data evaluation considering different analytical equipment in a single procedure.

The use of specific swine detection methods to ensure Halal authenticity

POSTER 3

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In the past few decades, Halal meat has had growing sales with Muslim communities totaling nearly 25% of the world population. The qualification of Halal, *permitted* as per Islamic Shari'ah, addresses attributes that refer to the method of production and establishes that products must be free of any prohibited ingredients, such as pork, animals slaughtered improperly and other intoxicants. Despite preventive measures, food industries might fail to produce food which is not correctly described and may be contaminated with pork derivatives. Analytical tests in meat have increased in recent years due to the discovery of species adulteration in processed products.

To ensure Halal authenticity, food safety enforcement authorities perform controls at each stage of the agrifood chain, and Halal entities are responsible of certifying goods apt for consumption by Muslims through coherent measures and adequate analytical monitoring. Our laboratory analyzed a total of 507 samples supposed to be Halal using a highly sensitive analytical method (sensitivity > 0.0005%) to discover that a significant proportion of the samples analyzed presented traces of pork DNA. Such small amounts of pork DNA might end up adulterating the final products due to accidental contamination during processing, thus rendering it Haram, or *non-permitted*.

The present study highlights the importance of implementing specific and sensitive analytical surveillance methods to ensure the authenticity of Halal products.

The use of specific animal detection methods to minimise food adulteration

POSTER 4

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Over the years, the food industry and authorities have developed food safety management systems to improve the resilience of supply chains to food fraud, mostly directed to prevent the fraud opportunity. While it is not the intention of food fraud to harm consumers, such act might cause distrust and even illness. This was the case in 2013 when EU authorities revealed the presence of uncontrolled horse meat burgers that were supposed to contain 100% beef. Generally, food fraud does not impose a health hazard, but in some ways they are more dangerous because the raw materials and quality control actions are unknown and untraceable.

Thus, addressing fraud should focus on being proactive in prevention and detection. Raw material monitoring should be performed using appropriate analytical methods for the verification of authenticity. Our laboratory analyzed a total of 173 beef products to discover that a significant proportion of them had been adulterated with water buffalo meat, *Bubalus bubalis*. Once the adulteration event had been characterized, prevention measures were taken, and a surveillance plan was effectively set up. Following the fraud detection event, beef products are routinely analyzed for buffalo and results show absence of unexpected ingredients.

The present study highlights the effectiveness of implementing analytical surveillance to ensure the authenticity of food by minimizing vulnerability to fraud and mitigating the consequences of food fraud.

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Next Generation Sequencing (NGS) workflow applied to the analysis of commercial spices and herbs products

POSTER 5

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The use of DNA-based testing methods is increasing in the food sector. DNA analyses can be a helpful tool for analysis of many food products and can address some of the present concerns about adulteration and authenticity. Several analytical methods have been proposed to answer the specific topic of species composition in foods. Next Generation Sequencing (NGS) has been found to be a suitable tool for food analysis including spices, herbs, seasonings, etc. In the present study, we show how an internal NGS workflow was setup and tested for species composition of real food seasoning samples. NGS was used for the testing of several commercial samples of different spice and herb mixtures. The results obtained will be discussed based on the labeling of the products relative to the type of sample and species mixtures.

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DNA extraction protocols for Next Generation Sequencing food authenticity application

POSTER 6

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The Thermo Scientific™ NGS Food Authenticity Workflow is based on next-generation sequencing technology to identify meat, fish and plant species. With semi-automated workflow and an extensive database thousands of species can be identified and more than a hundred food and feed products can be simultaneously analyzed. This proposes a challenge for sample preparation due to the high workload and time required to manually extract DNA from multiple samples. To overcome this issue an automated extraction method was developed using the Thermo Scientific™ KingFisher™ Flex Purification system.

The automated KingFisher Flex Purification System was compared to the manual method using the Imegen™ GMO Extraction Kit (Thermo Fisher Scientific) spin column protocol for DNA extraction in the NGS Food Authenticity Workflow. DNA from 48 samples of various food categories including dried, frozen, liquid and canned foods was extracted using both methods and then sequenced with Ion™ GeneStudio™ S5 System according to the NGS Food Authenticity Workflow. The sequencing data from both methods was compared to evaluate equivalency. The sequencing results obtained using the KingFisher Flex protocol showed excellent equivalency to meat and fish products when compared to sequencing results obtained with the manual GMO Extraction Kit . The study demonstrated that the automated KingFisher Flex workflow reduces hands-on-time by around a half compared to manual extraction with the GMO Kit.

Comparison of DNA extraction protocols for down-stream food authenticity Next Generation Sequencing application

POSTER 7

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DNA extraction is a crucial part of successful sequence analysis when studying the species authenticity of food products. The Thermo Scientific™ NGS Food Authenticity Workflow relies on next-generation sequencing technology to identify meat, fish and plant species using DNA extracted from foods, feeds and ingredients. With semi-automated workflow and extensive database thousands of species can be identified and more than a hundred samples can be simultaneously analyzed.

The advantage of the NGS method is the unmatched capacity to identify species without the need to specifically target only a limited set of species. As multiple species are analyzed from a variety of sample types the DNA extraction method needs to perform robustly regardless of the variables.

This study was conducted to compare the performance of two DNA extraction kits designed for food samples. Foods from different categories were tested to challenge the method including heavily processed foods, fresh and frozen foods, ready-to-eat meals, liquid foods and dried food products. After DNA extraction, the sample libraries for sequencing were prepared with the SGS™ All Species ID DNA Analyser Kits for meat, fish and plant species. Following library preparation samples were prepared for sequencing on Ion™ Chef™ Instrument and sequenced on Ion™ GeneStudio™ S5 Sequencer. Results analysis and reporting was automated through the SGS™ All Species ID Software.

Next Generation Sequencing for detection of meat, fish and plant species in pure and mixed species samples

POSTER 8

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Food authenticity and fraud are topics of high interest in the food industry and highly controlled by authorities. The complexity of the food supply chain is challenging the abilities of analytical tools used for traceability of ingredients for food production. The most common method to verify species substitution and species identification is Real-Time PCR. However, PCR testing is limited by the number of targets that can be simultaneously identified and differentiated. This can be critical, especially when testing highly processed and complex food that often contain multiple different species.

The introduction of Next Generation Sequencing (NGS) into the food sector revolutionize food authenticity testing with its untageted approach which enables accurate detection and differentiation of thousands of different species in each sample. In this study the technical experts from Thermo Fisher Scientific and SGS Molecular supported scientists at Nestlé Research in the use of the Thermo Scientific™ NGS Food Authenticity Workflow to test for meat, fish and spices/herbs species detection and identification at a variety of different spike levels (1 to 100%) and combinations of species (up to 5 different species combined into a sample).

This untargeted, NGS-based approach for meat, fish and spices/herbs identification was tested. 573 samples were tested including artificial mixtures of species spiked at 1, 5 and 10%, single species samples, reference material and real food samples. The untargeted sequencing workflow includes DNA extraction, library construction, template preparation, sequencing and data analysis. The SGS ALLspeciesID products were used for library construction and data analysis. Template preparation and sequencing were performed using the Thermo Fisher Scientific Ion Chef™ and GeneStudio S5™ Food Protection system. The Thermo Scientific NGS Food Authenticity Workflow was shown to detect and correctly identify 100% of meat (n=49), fish (n=26) or plant (n=39) species at a spike level of 5% or higher). At spike levels 1-5% all species were detected making the workflow appropriate for food species ID analysis.

Application of Next Generation Sequencing to food authenticity testing – study of adulterated beef samples using Ion GeneStudio S5 Food Protection System

POSTER 9

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Following the UK/EU Horse-meat issues of 2013, where a significant amount of horse DNA was found in a number of processed beef products there has been an increased need for routine non-targeted species detection methods. In recent years Next Generation Sequencing (NGS) has been promoted as a useful technique to identify species present in samples containing a mixture of species. Very few studies have looked into application of processed meat products where DNA can be highly degraded. This study applies a commercial NGS system to a range of spiked meat product samples processed to industry standard conditions. The samples consisted of lean beef spiked with varying levels of pork and horse muscle was used to prepare raw, burger, canned meat and cottage pie sample types. Multiple DNA extracts were prepared from each sample type and NGS was performed using SGS™ All Species Meat Analysis kit in conjunction with Ion Chef™ Food Protection Instrument and Ion GeneStudio™ S5 Food Protection System. Results will be presented and relevance to food screening will be discussed.

Tracking sugar addition in food and beverage using isotope fingerprints

POSTER 10

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Complexities in the food and beverage supply chain from the production site through to the consumer have presented significant, and at times relatively easy, opportunity for economically motivated fraudulent activities to occur and be undetected. Consequently, there is an increase in retailer and consumer demand to proof that food and beverage products are what the label claims them to be, including origin, authenticity and ingredient verification.

One of the most known adulteration processes involves the addition of sugar to food and beverages. Detecting the added sugar can be achieved using stable isotope measurements because stable isotopes can differentiate between the sugar already present in the sample from the sugar which is added artificially. Carbohydrates carry an isotope fingerprint, a unique chemical signature which identifies their origin. To visualize this fingerprint, Isotope Ratio Mass Spectrometry (IRMS) can be used, identifying the isotope fingerprint of the product.

In this poster the application of stable isotope fingerprints in detecting sugar addition to food and beverage samples is explored. Data show how stable isotopes offer conclusive answers on questions associated with origin, adulteration and correct labeling of food and beverage products. An overview of the interpretation of isotope fingerprints and the official methods using isotope fingerprints for food and beverage analysis are also provided.

Elemental analysis in food for risk assessment and provenance studies

POSTER 11

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To ascertain the quality of food and food products, the analysis of toxic, essential and nutritional elements has become a routine task for food quality monitoring. Elements such as arsenic, cadmium, mercury or lead can enter the food chain via a series of pathways including, but not limited to, industrial pollution or environmental contamination. Recent public alerts on arsenic or lead contaminations in our daily food or water supply have contributed to increased attention on this particular issue, but there is also a high demand for clear information on nutrients and contaminants from health-conscious consumers.

Elements such as the rare earth's (e.g. Nd, Gd) can cause significant false positive signals on critical analytes such as arsenic or selenium, as a result of the formation of doubly charged ions in the plasma. Elimination of these interferences is crucial to obtain correct results. In addition, the appearance and distribution of these elements may give additional insight into the provenance of foodstuffs.

To keep up with the demands of the market, analytical laboratories need to be capable of analysing a high number of samples, containing both major and trace levels of a variety of elements, in the shortest possible time. This can usually be accomplished by using single quadrupole ICP-MS instruments, with a single measurement mode applied for analysis of all the target elements in a suite. This single mode approach dramatically reduces the measurement time required per sample and reduces analysis cost.

However, some interferences, such as the doubly charged ions of rare earth elements mentioned above, require triple quadrupole ICP-MS instruments to consistently remove them. At the same time as quantifying the target set of analytes, screening a sample set for other analytes that don't require full quantification can be accomplished using a full mass scan, allowing unexpected elements in the sample to be identified, even months after the original analysis.

This poster reviews various strategies, including the use of collision/reaction cell (CRC) technology with both single and triple quadrupole ICP-MS instrumentation, for the accurate analysis of trace elements in different food samples.

Peanut flour protein with defined allergen content for use as reference standard

POSTER 12

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Allergen measurements are widely used for determination of the potency of therapeutic allergenic products, environmental exposure assessments, and for validation of IgE molecular diagnostics. However, few standardized allergen reference materials have been developed. The aim was to produce a standardized peanut flour protein with defined allergen content that could serve as a reference standard for peanut diagnostics or therapeutics.

Peanut flour protein was prepared from roasted and defatted peanut flour using standardized aseptic extraction conditions at pH 7.4. Peanut allergens were quantified in quadruplicate using validated allergen-specific ELISA's (Ara h 1, Ara h 2, Ara h 3, Ara h 6, and Ara h 8) and analyzed by SDS-PAGE, endotoxin assay, and mass spectrometry (LC-MS/MS). Real time stability data were collected from frozen liquid allergens over a period of 24 months.

Peanut flour protein showed excellent reactivity in peanut allergen-specific ELISA assays. Ara h 3 (878 µg/ml) concentrations were the highest, followed by Ara h 2 (371 µg/ml), Ara h 1 (220 µg/ml) and Ara h 6 (217 µg/ml). This pattern was similar to the results obtained by LC-MS/MS. Ara h 3 was the most abundant allergen (61%), followed by Ara h 2 & Ara h 6 (15% each), Ara h 1 (7%) and Ara h 7 (1.5%). Abundance of other peanut allergens and non-allergenic peanut proteins was very low (<0.5%). Endotoxin levels were < 0.03 EU/µg. Real time stability tests of frozen liquid allergens (up to 24 months) showed consistent potency in allergen-specific ELISA and no signs of degradation on SDS-PAGE.

Ara h 1, Ara h 2, Ara h 3, and Ara h 6 are the predominant allergens in roasted peanut flour extracted at neutral pH. Optimized, ISO-9001 compliant, bioprocessing pathways have been established to yield standardized peanut flour allergen with defined allergen profiles which can serve as a reference standard. The low-endotoxin peanut flour protein has applications as a standard for monitoring the composition of peanut diagnostics and therapeutics.

Measurements of specific milk allergens in baked food challenge materials

POSTER 13

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Oral food challenges (OFC) are considered the 'gold standard' to diagnose a true food allergy. Allergists use baked milk food preparations for OFC under the assumption that they contain decreased allergen levels due to baking. However, the effects of baking on specific allergens has not been thoroughly investigated. The aim was to compare levels of major milk allergens and IgE reactivity in uncooked and baked milk challenge materials currently used in clinical practice.

Uncooked and baked muffins were prepared using recipes from Mount Sinai (Jaffe Food Allergy Institute) and the UK National Health Service (NHS). Allergen levels were compared using a two-site monoclonal antibody ELISA for beta-lactoglobulin (Bosd5) and for beta-casein (Bosd11). IgE reactivity was assessed using sera from milk-allergic patients in direct binding and inhibition ELISA.

Bosd5 (β-lactoglobulin) concentration decreased from 680 µg/g in uncooked muffin mix to 0.17 µg/g in baked muffin, representing >99% reduction in Bosd5 allergen. The level of Bosd11 (β-casein) decreased by 30% from 4,249 µg/g in uncooked muffin mix to 2,961 µg/g in baked muffin. Bosd11 levels in the Mount Sinai muffins (n=30) were higher compared to the NHS muffins (n=15) and varied depending on whether the baked muffin was sampled from the top, middle or bottom. Baked muffins retained ~70% of the IgE reactivity in uncooked muffin mix while baked muffin extracts inhibited IgE antibody binding to uncooked muffin by up to 64-96%.

The level of major milk allergen Bosd11 remained high in baked muffins used in oral food challenges. These findings emphasize the potential risk for adverse reactions to baked milk challenges, especially in patients who have high anti-casein IgE antibodies. Measurements of specific milk allergens, together with IgE molecular diagnostics, should improve the safety of food products used for OFC and reduce the risks associated with milk challenges in clinical practice.

Simultaneous quantification of major food allergens using a multiplex immunoassay POSTER 14

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Quantification of food allergens is increasingly important for dose assessments of food preparations used in oral food challenges (OFC), food allergy prevention, and monitoring safety in the food industry. Generic immunoassays for 'total protein' do not measure specific allergens. Our aim was to use a molecular approach to food allergy to develop a multiplex immunoassay capable of simultaneously measuring specific allergens, the 'active ingredients', from peanut, cow's milk, shellfish, egg, cashew, soy and hazelnut.

The multiplex array was developed on the Luminex xMAP system. Microspheres coupled to specific monoclonal antibodies were used for allergen capture. Biotinylated specific monoclonal or polyclonal antibodies were used for detection. Reference standards were formulated from natural or recombinant allergens, with purity established by mass spectrometry. Full method validations were performed to determine parameters of linearity, range, limits of quantification and detection, accuracy and precision of the multiplex food immunoassay.

Method validations were completed for the major food allergens. Standard curves for all analytes allow for quantification over a broad dynamic range. Limits of detection were as low as 0.01ng/ml. Intra- and inter- assay accuracy and precision of three samples assayed in triplicate on four occasions passed acceptance criteria within the range of 70-130% recovery and a coefficient of variation of <15%. Food products and the NIST SRM 2387 Reference Standard were analyzed using the multiplex immunoassay.

A quantitative, accurate and precise multiplex immunoassay was validated for the simultaneous detection of major food allergens. The multiplex array provides a sensitive and efficient tool for measuring specific food allergens, as opposed to generic food source proteins, with potential applications for risk assessment in the food industry and standardization of clinical OFC.

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Development of an *in vitro* bio-assay using human intestinal and immune cell-lines to measure the immuno-pathogenicity of food allergens

POSTER 15

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Food allergies are a rapidly growing public health problem that affect >15 million Americans. The FDA's Food Allergen Labeling and Consumer Protection Act (FALCPA) requires that foods containing allergenic proteins derived from the eight major food allergens be declared on packaging. As strict avoidance is the only option for allergic consumers, accurate methods are needed to ensure correct labeling. The currently used Immunochemical methods (e.g. ELISA) detect IgG antigenic epitopes, not allergenic elements. Hence, immunochemical methods may not detect antigenic epitopes that are transformed during food processing, even though immuno-pathogenicity could continue to persist. To address this gap in analytics, an *in-vitro* bio-assay that employs human intestinal epithelial and immune cell-lines to measure the biological effects caused by food allergens was developed. This novel biological activity-based assay compares the allergen-induced immuno-biological responses in Caco-2, HT-29 & T84 intestinal epithelial cells individually, as well as each co-cultured together with THP-1 cells. The goals of this project are to compare the cell signaling and immune modulation induced by different food allergens in a dose-dependent manner, and to further develop an *in-vitro* bio-assay using these cell lines.

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Detection of peanut in legume containing food products

POSTER 16

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Food allergies affect 4% of adults and 8% of children, and each year, about 29,000 cases of anaphylaxis occur in allergic individuals, resulting in roughly 150 deaths. Peanut allergies affect about 0.6% of adults and 0.8% of children in the US are one of the most severe allergies, causing potentially life-threatening reactions. Patients do not generally outgrow peanut allergies, and since there is currently no cure, avoidance of peanut is the only option for the allergic population. The Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) mandates that manufacturers label major allergens on food labels. However, inadvertent cross-contact of allergens is still possible, threatening the health of allergic individuals. Peanuts belongs to the legume family and contain homologous proteins to those present in other legume species. In recent years, foods have been recalled due the presence of peanut in legume containing products. Current commercial ELISA methods face challenges in detecting peanut in legume containing products due to cross-reactivity issues. In this study, the limitations of the ELISA methods have been addressed by using orthogonal methods, including the multi-analyte profiling food allergen detection assay (xMAP FADA) and a DNA-based PCR method targeting regions of the peanut chloroplast genome, to detect the presence of peanut in legume containing food products.

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Cross-reactivity of chili peppers using the xMAP Food Allergen Detection Assay (xMAP FADA)

POSTER 17

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The xMAP Food Allergen Detection Assay (xMAP FADA) is a unique and powerful analytical method for the simultaneous detection of crustacean shellfish, egg, gluten (wheat), milk, peanut, sesame, soy, and 9 tree nuts. Except for crustacean, the use of multiple antibodies for each allergen provides built-in conformational analysis and permits the calculation of complimentary antibody ratios, allowing positive results to be distinguished from cross-reactivity with homologous proteins.

The xMAP FADA was used to analyze common botanicals, including spices. These commodities often contain homologous proteins that cross-react with the antibodies and potentially generate false positives in ELISAs. Cross-reactivity due to homologous proteins can occur as a result of closeness of species or due to coincidence. Compared to traditional ELISA, the xMAP FADA is well-equipped to assess samples containing homologous proteins and distinguish cross-reactivity from detection of target analytes due to the built-in redundancy of the assay. The xMAP FADA's combination of high sensitivity, ability to distinguish between cross-reactive homologues, and ability to quantify the presence of any of the 16 targeted analytes provides a detailed picture regarding the possible presence of food allergens. Included in the evaluation of botanicals used in dietary supplements and spices was 27 chilis, represented by both pre-ground and whole peppers. The 27 chilis displayed qualitatively similar multi-antibody profiles with different quantitative features. All chilis displayed two dominant cross-reactivities: Brazil nut-14 and Hazelnut-29. Though the associated hazelnut-30 responses were comparable to that expected for hazelnut, a similar observation was not observed for Brazil nut-15. Additionally, almost all the chilis displayed moderately strong responses with Walnut-48 and both cashew antibodies, though neither displayed complementary antibody ratios characteristic of these nuts. Overall, the cross-reactivities of the chili peppers were small and easily distinguished from the presence of target analytes using the requirement that both complementary antibodies generated positive responses and that the various secondary endpoints were characteristic of the target analytes. Interestingly, unlike what was observed previously with spices from the Orders Apiales (anise, caraway, cumin, and fennel) and Lamiales (marjoram, oregano, sage, and thyme), no indication of the presence of gluten was detected in commercially pre-ground chili products.

The xMAP FADA proved to be a high-throughput, cost-effective alternative for the detection of multiple food allergens and gluten, while the use of secondary endpoints (e.g., ratio analysis and antigenic profiling) enabled identification, classification, and characterization not possible using single-analyte ELISA technology.

Detection and quantification of allergens in foods and minimum eliciting doses in food-allergic individuals (ThRAll)

POSTER 18

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People suffering from food allergy must strictly avoid their offending food since currently there is no clinical treatment or cure. Accurate analytical methods are essential to support the application of precautionary 'may contain' allergen labelling. An objective of the ThRAll project, funded by the European Food Safety Authority, is the development of a harmonized targeted mass spectrometry-based prototype reference method for the quantification of multiple food allergens in standardized incurred food matrices. The second objective of the project is to establish Minimum Eliciting Doses (MEDs) for the tree-nuts for which data gaps were identified. To this extent, data from the literature, EU-funded projects such as iFAAM and EuroPrevall, and nationally-funded projects in France (such as MANOE) and the UK will be collated and reviewed using these criteria to provide 'cleaned' analysis-ready data sets.

Six priority allergenic foods which are responsible for the majority of food product recalls (namely cow's milk, hen's egg, hazelnut, peanut, almond and soybean), were incorporated in two model foods selected as hard to analyze matrices. One was a chocolate bar with high fat and polyphenol content; and the second a broth powder, which is extensively processed and has a complex protein background from which the allergenic proteins will need to be discriminated.

Signature peptides for the detection of the six allergenic foods were selected using a dual approach. Initially the signature peptides reported in previous studies were reviewed and candidates selected according to specific criteria as sequence length and occurrence of amino acids prone to natural and chemical modifications. This list of makers was then evaluated experimentally. Conditions for protein extraction and purification from the food matrix were optimized using technical aids such as sonication, size exclusion chromatography and C18 solid phase extraction. Untargeted HR-MS/MS analysis was then used to evaluate the effect of the extraction conditions on the detection of candidate peptides. This allowed conditions to be identified, which maximized the number of identified marker peptides and provided maximal coverage of allergenic proteins in each of the food ingredients. The list of candidate peptides identified from the literature was cross-checked with the experimentally identified peptides to produce and experimentally verified list of candidate peptides was collated for further validation by targeted mass spectrometry.

We describe the preliminary results obtained in the development of a targeted mass spectrometry-based method for the multiple quantification of six allergenic foods. The integrated two-front approach, which combines the literature review of marker peptides to the experimental identification of food-derived peptides by discovery mass spectrometry analysis, provided a robust list of signature peptides.

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Reference materials for food allergen analysis

POSTER 19

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Effective food allergen risk assessment and food allergen management are important to protect allergic consumers and to comply with allergen labelling regulations. Such approaches require reliable analytical tools for the detection of allergens in food. Both, reference methods and reference materials are urgently needed to assure the quality, reliability and comparability of analytical results obtained with different methods. Ensuring the correctness of analytical results is crucial to laboratories, since incorrect results may trigger decisions that can cause economic damage or pose a risk to public health. The quality of reference materials is critical for accuracy and comparability of analysis results. Reference materials must be sufficiently homogenous, stable and traceable. Usually extensive material characterization and testing for homogeneity and stability of the material precede the availability of reference materials. Ideally a certified reference material shall be used, which has been validated by accredited institutions and is subject to strict quality testing. The first validated reference materials for food allergen analysis are now available from MoniQA Association at https://www.moniqa.org/. The first set of materials includes testing materials for milk allergen analysis comprising a Positive Control (SMP-MQA 092014, characterized dried skim milk powder, validated protein content), Negative Control (BLANK-MQA 082015, based on a gluten free cookie), and 2 Incurred Materials: LOW-MQA 102016 (SMP incurred in gluten free cookies, milled, 10 ppm skim milk powder, validated concentration 3.5 ppm milk protein) and HIGH-MQA 082016 (SMP incurred in gluten free cookies, milled, 50 ppm skim milk powder, validated concentration 17.5 ppm milk protein). These materials are the outcome of an international initiative (since 2013) led by MoniQA Association that has liaised with the EU funded project iFAAM, the Prolamin Working Group, Health Canada, FARRP, Australia's Allergen Bureau (Vital), and others. Additional food allergen reference materials are in preparation. Next to be distributed are validated reference materials for wheat/gluten and soya.

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Development of a reference material for gluten analysis

POSTER 20

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Celiac disease is a gluten-induced disorder that requires a life-long gluten-free diet. Gluten analysis in food is a great challenge since it is influenced by a number of factors. A certified gluten reference material (RM) to identify these factors, to determine the degree of uncertainty and to validate analytical methods, is currently missing. In the framework of international cooperation, our research group has embarked on a comprehensive research aimed to investigate questions related to RM and selecting a suitable candidate. 23 different wheat cultivars were collected from around the world to map the genetic variability between wheat cultivars and to reduce the number of candidates based on certain selection criteria. We examined mainly the protein and gluten content of the cultivars, the protein composition and the immunoanalytical response by different ELISA methods. Five wheat cultivars (Akteur, Carberry, Mv Magvas, Yitpi and Yumai-34) were selected that may be suitable for gluten RM individually or as a mixture. The selected cultivars were collected from a new harvest year. White flours were prepared on laboratory scale to examine the stability of the cultivars and to study the analytical error resulting from the use of individual wheat cultivars or their blend. Based on our results, the effect of the harvest year is comparable to genetic variability, so it is not possible to select a single cultivar with similar average parameters every year. The use of blend flour will prove to be better, which can reduce not only the degree of genetic variability but also the uncertainty caused by different harvest years. A widely used reference material requires the upscaling of its production, so the flours from the selected cultivars and their blend were produced on pilot scale. Comparative studies with laboratory samples have shown that flours with similar parameters have been successfully produced in high amount and the blend (similar to the lab scale blend) was sufficiently homogeneous. The conclusions drawn for the samples produced under laboratory conditions are also valid for pilot-scale samples. Accordingly, we have a well-characterized homogeneous blend flour from five wheat cultivars which is suitable for gluten RM and ready for use. The protein profile of the reference material seems to be stable and is confirmed by regular stability control by ELISA.

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