AOAC International Midwest Section Annual Meeting & Exposition
June 3-5, 2024
Oak Brook Hills, Illinois

MONDAY || JUNE 03, 2024 || MORNING

MONDAY: 6/3/2024
12.00 - 5.00 PM: REGISTRATION (SOUTH FOYER)
3.00 - 4.30 PM: BOARD OF DIRECTORS MEETING (MALLARD HALL)

1.00 -4.00 PM: Vendor Exhibits setup time
4.00- 5.00 PM: Vendor Visits

5.00 - 6.00 PM: GENERAL MEETING (BALL ROOMS F-J)

- **Welcome**.
- **Jay Alappat**: President, AOAC Midwest
- **Sebastien Moulard**: President, Merieux Nutrisciences, “Collaborative Innovation: Tackling New Food Safety Challenges Together”
- **Mary Kay Krogull**: President, AOAC INTERNATIONAL, *Evolution of AOAC INTERNATIONAL sections*
- **Kate Mastovska**: Deputy Executive Director & Chief Science Office, AOAC INTERNATIONAL, *AOAC INTERNATIONAL Science Program Highlights*
- Sessions on “Speed Networking” and “Cultivating Emotional Agility through Mindfulness”

6.00 - 8.00 PM: SOCIAL HOUR & DINNER (SPONSORED BY MERIEUX NUTRISCIENCES) BALLROOMS F-J

TUESDAY || JUNE 04, 2024 || MORNING

Breakfast: 7.30- 9.00 AM (THE MARQUIS TENT)
Registration: 8.00- 9.00 AM (SOUTH FOYER)

TUESDAY: 6/4/2024

ANALYSIS OF AGRICULTURAL PRODUCTS, FERTILIZER AND BIO-STIMULANTS
9-12.30 PM, Ballroom E-J (8 Presentations)

Session 1: ***Developments in Fertilizer Methods***
Chair: James Bartos, Purdue, jbartos@purdue.edu
Abstract: *This segment contains presentations in the field of fertilizers and related products. In particular, some of the main instrumentation and techniques used and their advancements will be discussed. Techniques that allow for greater productivity, sensitivity, ease of use, and/or expanded capabilities will be shared.*

Presentation 1: *Observation and Determination of Residual Sulfur in Phosphate Fertilizers Using ICP-OES*
James J. Camberato, Peng Li*, Robert L. Nielsen, Office of Indiana State Chemist, pengli@purdue.edu

Abstract: *Sulfur deficiency in soil has been observed globally and throughout the Midwest the last two decades and is likely attributed to a consistent decrease in atmospheric sulfur deposition from coal and power plants. Thus, sulfur fertilization has been significantly increased for many crops to ensure plant productivity and health. A group of researchers at Purdue University agronomy department found a reduced crop response to intentional S fertilization over five years. In this presentation, we will discuss the potential impacts of incidental applications of S from phosphate fertilizers. The concentration and variability of incidental sulfur in hundreds of phosphate fertilizer samples collected at the Office of Indiana State Chemist between 2021 to 2022 were determined using a citrate disodium (CD)-EDTA extraction and ICP-OES method, which is the official AOAC 2015.18 single lab validation (SLV) method for analyzing P and K in fertilizers. The forms of sulfur as total S, sulfate-S and elemental-S were also determined by the official AOAC method 980.02 gravimetric sulfur method using a small portion of the samples from the former sample pool. The results from the two different methods were compared for three different granular P fertilizers: monoammonium phosphate (MAP), diammonium phosphate (DAP) and triple superphosphate (TSP) samples. The SO4-S determined by CD-EDTA extraction and ICP-OES method (AOAC 2015.18) was more than 93% of total SO4-S determined by the gravimetric sulfur method (AOAC 980.02) in all three granular P fertilizers. The amounts of SO4-S in the MAP, DAP and TSP equivalent to ~42–52% of the S removed in the grain of a single crop.*

Presentation 2: *Microwave Digestion Options for Elemental Analysis of Agricultural Products for Every Workflow*
Macy Harris, Sam Heckle, Layla Abu-Al-Halaweh, Ricky Chomicz*,CEM Corporation, macy.harris@cem.com
Abstract*: Accurate trace metals analysis not only starts with sample prep but also choosing the right equipment to provide the analytical results. Using fertilizers as the sample preparation example, this presentation will discuss microwave digestion options and tools, some of the best ways to use them, and how to achieve the best sample prep possible to meet the demands of busy agricultural testing labs.*

Presentation 3: ***Nitrogen and Sulfur Determination in Fertilizers: Optimizing the Analytical Approach using Combustion Analysis***

Jeffrey Gast* and Lloyd Allen, LECO Corporation, jeffery_gast@leco.com

Abstract: *Nitrogen and sulfur represent key components in fertilizers and are important for proper soil management. Accurate determination of nitrogen and sulfur is important for quality control during fertilizer manufacturing and allows the end users to have confidence in the material they are inputting to their crops. There are several different analytical approaches for performing this analysis via combustion, and optimizing the technique used can improve the utility of the final result. Features of the sample such as homogeneity, nitrogen and sulfur concentrations, sample type (solid or liquid fertilizer), and precision requirements all play a role in guiding the analyst toward the best analytical approach. This presentation will outline several instruments used for nitrogen and sulfur determination in fertilizers and will highlight the differences in these analytical approaches. Data and method recommendations will be presented from a variety of different fertilizers covering a range of nitrogen and sulfur content materials*

Presentation 4: ***Strategies for Increasing Productivity and Efficiency by ICP-OES***

Chris Conklin*, Agilent Technologies, christopher.conklin@agilent.com

Abstract: *ICP-OES continues to be a workhorse technique in many labs due to its ease of use and robustness. Advances in the technology have seen gains in not only performance but also in automation. Instruments are able to provide better insight into their operation to help lab personnel keep them running their best. Additionally, systems and accessories are more routinely automating tasks, freeing analysts to focus on more valuable tasks. Larger ICP-OES workflow solutions include automatic preparation of standards and dilution of samples. This talk will examine various hardware and software tools for ICP-OES that now exist to help analysts make the most of their time in the lab. We will look at overcoming challenging matrices, reducing reanalysis, and automating repetitive tasks*

Session 2: ***Advances in Animal Feed Methodologies***

Chair: Darrell Clinton, Office of Indiana State Chemist, dclinto@purdue.edu

Abstract: *The animal feed segment will focus on upcoming major labeling changes for pet foods and on new or revised instrument techniques that can improve productivity, efficiency, and detection related to minerals/metals, vitamins, and related analytes of interest*

Presentation 1: ***Pet Food Label Modernization***

Katie Simpson*, Office of Indiana State Chemist, ksimpson@purdue.edu

Abstract: *In 2015, the Association of American Feed Control Officials (AAFCO) began the Pet Food Label Modernization (PFLM) project to update the Pet Food and Specialty Pet Food Model Regulations that set the model for labeling requirements. The goal was to standardize labeling formats to enhance transparency and improve consumer understanding. Multiple rounds of consumer research were conducted to gain understanding on how pet food labels can better communicate important information. AAFCO sought input from industry, state and federal regulators, and public comments on the draft PFLM Model Regulations and made revisions based on the feedback. The PFLM Model Regulations for Pet Food and Specialty Pet Food were accepted by AAFCO membership in August 2023. The PFLM Model Regulations are effective as of January 1, 2024. AAFCO recommends that state feed regulatory programs use enforcement discretion for a period of up to six (6) years for implementation*

Presentation 2: ***Use of ANKOM FLEX Vitamin Extractor for B-Vitamin Determination***

Darrell Clinton. Ph. D* and H. Dorota Inerowicz, Office of Indiana State Chemist, dclinton@purdue.edu
Abstract: *The determination of water-soluble B vitamins in animal feed can be a challenging analytical procedure with most current methods using high performance liquid chromatography (HPLC) with ultraviolet (UV) or fluorescence (FLR) detection. In this study, the analysis of several B vitamins; thiamine (B1), riboflavin (B2), niacin (B3) and pantothenic acid (B5) were analyzed in feed using Ultra Performance Liquid Chromatography (UPLC) with a Waters Xevo TQ-S micro triple quadrupole mass detector. The application of mass spectroscopy reduces preparation and analysis time and does not involve derivatization agents which may be used in HPLC with other types of detectors. Recently, OISC purchased the FLEX extractor from Ankom to assist OISC in it's quest for a single laboratory validation of water-soluble B-vitamins in feed as well as using the Ankom FLEX extractor to prepare feed samples for D3 determination. The results will show a comparison of extraction methods between the preparation of several B-vitamins using a hot-water bath and shaker to the extraction performance of the Ankom FLEX extractor.*

Presentation 3*: Development/Findings for Analyzing Vitamin A in Animal Feed by UPLC/UV*
Michael Ewbank, Office of Indiana State Chemist, ewbankm@purdue.edu

Abstract*: Ultra-High Performance Liquid Chromatography (UPLC) is becoming more prevalent in determining Vitamin A concentrations in various matrices in the analytical laboratory industry, replacing High Performance Liquid Chromatography (HPLC). The determination of Vitamin A concentration in animal feed and pet food is one of the most frequently tested assays in the Feed Laboratory of the Office of Indiana State Chemist. Therefore, this laboratory is examining the possibility of switching to an UPLC method for determining Vitamin A using a slightly modified method published by Waters Corporation™. The overnight saponification using potassium hydroxide was used for the extraction procedure (in-house method). A comparison between the two analytical instrument methods (HPLC vs UPLC) was completed using different feed types in a wide range of guaranteed concentration. The presence of different drugs and antibiotics was also considered in the study. The current extraction method provides a large amount of unwanted material placed on the chromatography columns if the samples are not purified properly. Determining the limitations of the UPLC method is needed to provide the necessary scope/criterion and feasibility prior to a single laboratory validation, which would provide the expected benefits using UPLC-UV.*

Presentation 4*: Use of ICP-MS for Simultaneous Determination of Essential Minerals and Heavy Metals*
Yan Cheung, Application Scientist, Agilent, yan.cheung@agilent.com

Abstract*: In this presentation, the key features of inductively coupled plasma-mass spectrometry (ICP-MS), and the fundamentals behind this popular analytical technique will be discussed. The importance of matrix tolerance, interference removal, software capabilities, and linear dynamic range are the main focus of this presentation.*

TUESDAY: 6/4/2024

NOVEL FOODS FROM ALTERNATIVE PROTEIN SOURCES: CURRENT STATUS, ADVANCES AND CHALLENGES

9-12.30 PM, CONFERENCE DINING ROOM (6 Presentations)

Session 1: *Safety, Quality and Acceptability of Novel Foods.*

Co-Chairs: Walt Brandl, Regional Director of Chemistry- North ***America***, walter.brandl@mxns.com
Kate Mastovska, Chief Science Officer, AOAC INTERNATIONAL, kmastovska@aoac.org

Abstract: *The booming population (estimated to reach 10 billion by 2050) has raised concerns on safe and secure food supplies. To address this challenge, new sources of alternative proteins are emerging. These novel protein options, often derived from plants, insects, lab-grown cells, or fermentation, are relatively new to the scientific community and customers. Production methods are under development, and safety protocols are being established. Despite the unknowns, the future of alternative proteins looks promising. Increased investment in sustainability, innovative processing technologies, and a growing trend towards flexitarianism are all fueling this market. The approval of cultivated meat in the US is a prime example, and many companies are trying to join this space. By 2030, the global alternative protein industry is projected to reach a staggering $200 billion.*

Ensuring the safety of these novel foods requires testing methods that go beyond traditional approaches requiring assessments of potential allergens, novel processing techniques, and unique microbial risks. Guaranteeing the quality of novel foods involves not just nutritional content; it involves the ability to monitor and accurately measure various residues and contaminants that might arise from novel production technologies and raw material sourcing.

The acceptability of novel foods from alternative protein sources hinge on consumer perception. Novel foods made with unfamiliar ingredients or production methods pose unique challenges for sensory evaluation. Traditional taste tests might not capture the full picture of consumer acceptance for these innovative products. Mimicking the texture and mouthfeel of familiar foods can be difficult with novel ingredients, making it hard to predict consumer preferences based solely on chemical composition. Off-flavors or unexpected aromas can be deal breakers for consumers, so extensive sensory testing is crucial to identify and address these potential issues early in development. Cultural perceptions and established flavor profiles can also influence how consumers receive novel foods, requiring careful consideration during sensory evaluation. The keys to improve the consumer acceptability and market value for novel foods from alternative protein sources would be to achieve organoleptic and sensory equivalency to traditional protein products. The session will address and discuss the challenges in developing alternative meat and dairy.

This session will delve into the current landscape of novel foods and the unique challenges associated with bringing these new foods safely to the market. The audience will gain a clear understanding of the need for comprehensive testing practices that can ensure the safe and successful integration of novel foods into our food system Presentations will also highlight the chemistry of organoleptic sensory attributes of these products; tests to understand these aspects; and possible solutions.

Presentation 1: *Safeguarding EU Consumer's Health while Supporting Food Innovation: EFSA's Update of the Novel Foods Scientific Guidance*

Ermolaos Ververis[1*], Ermolaos.VERVERIS@efsa.europa.eu
[1]*Nutrition & Food Innovation Unit, European Food Safety Authority (EFSA), Parma, Italy*

Abstract*: In the European Union (EU), the classification of "novel food" encompasses products not significantly consumed by humans before May 15, 1997. Novel foods can be newly developed foods and*

food ingredients, food produced using new technologies and production processes, as well as food which has been traditionally consumed in non-EU countries and are distinct, by law, from GMO products. EU market access for such products requires pre-market authorization, involving, a rigorous safety assessment of novel food application dossiers submitted by food business operators. The safety assessment of these products is performed by the European Food Safety Authority (EFSA), which also provides guidance to applicants on scientific requirements for dossier submission.

The EFSA Novel Food Scientific Guidance is undergoing its first revision of scientific content since 2016, mandated by the European Commission. This update aims to incorporate recent EU regulatory changes in the field, leverage advancements in food research and innovation, and capitalise on EFSA's experience since the centralisation of the EU assessment process in January 2018. Key areas addressed in the update include compositional data, identity, production processes, toxicology, nutrition, exposure, and allergenicity, covering a range of products spanning from cell culture-derived foods and precision fermentation food ingredients to plant extracts, novel protein sources and engineered nanomaterials. Stakeholder engagement plays a pivotal role in shaping the guidance, with EFSA actively soliciting input from academia, researchers, authorities, consultants, and industry stakeholders through various channels such as colloquia, webinars, and public consultations.

This work will elucidate the EU's novel food regulatory framework, EFSA's safety assessment principles, and the main revisions proposed in the updated guidance document, scheduled for finalization in June 2024

Presentation 2: ***Organoleptic and sensory analysis of Novel Foods***

Walt Brandl*, Merieux Nutrisciences, walter.brandl@mxns.com

Abstract: *Consumer acceptance of novel foods has been challenging to say the least. Not the least of the resistance comes from the flavor profile of unknown foods. A trepid consumer can be hyper-sensitive to unexpected flavor notes in a novel food. Combining thorough chemical profiling covering both volatile and non-volatile components along with sensory verification can now be combined with machine learning to interpret the vast amounts of data produced, refine it and make accurate predictions on consumer acceptance. This talk will present some of the most useful analytical techniques that can be used to create an accurate flavor profile of a novel food.*

Presentation 3: ***An Examination of Heavy Metals in Alternative Dairy Milks by Microwave Digestion and ICP-MS***

Macy Harris, Samuel Heckle, Layla Abu-Al-Halaweh, Ricky Chomicz*, CEM Corporation, macy.harris@cem.com

Abstract: *Plant-based milks are becoming increasingly popular alternatives to dairy milk. While some plant-based milks, such as soy and almond, have become mainstream, more and more alternative milks, such as coconut and oat milks are also increasing in popularity. These non-dairy milks are derived from nuts, seeds, and other plant-based sources. These 'milks' are appealing because they follow consumer trends for dairy-free, lactose-free, and vegan products.*
All plants are grown in soil, which has a naturally occurring concentration of metals. Many plants and nut trees are effective bio accumulators of inorganic compounds. Plants uptake metals from soils via the root and vascular system and can concentrate in the leaf, fruit, and flower. As these plants are processed into downstream products (such as non-dairy milks), plants grown in contaminated soil can accumulate heavy metals, increasing a consumer's heavy metal exposure. The heavy metals known as the big four

(As, Pb, Cd, Hg) are of particular concern due to their potential toxicity. In this study, metal concentrations are measured and compared for plant-based milks and cow's milk. The metals are measured after microwave digestion and ICP-MS analysis of the milks.

Session 2: ***Reference methods and materials in Novel Foods.***

Co-Chairs: Daniel Kim, Laboratory Director, Merieux Nutrisciences, Markham, daniel.kim@mxns.com

Kate Mastovska, Chief Science Officer, AOAC INTERNATIONAL, kmastovska@aoac.org

Abstract: *Novel foods can have benefits in improving access to healthy foods and lowering the impact and strain on the environment and resources, but can come with challenges. Not only should the novel food appeal to the consumer, but be safe to consume, offer some health benefit, and not be nutritionally disadvantageous to the consumer.*

Many methods available for the analysis of traditional foods. However, they may require validation to ensure that the methods are suitable for a novel food. Reference materials are important for developing and refining existing analytical methods. They can be used to verify the accuracy and precision in the identification and quantification of specific components within a novel food. This can contribute to better understanding of its nutritional profile and the potential hazards when consuming a novel food. Reference materials can also be used in comparative studies across different laboratories, such as proficiency testing and can enable laboratories to verify the accuracy of their results.

Thus reference materials are necessary in ensuring the accuracy and reliability of food analysis. They play an important role in method development, quality assurance, and contributing to the safety and integrity of the food supply chain.

Presentation 1*: The Vital Role of Reference Materials in the Analysis of Novel Foods*

Daniel Kim*, Laboratory Director, Merieux Nutrisciences, Markham, daniel.kim@mxns.com

Abstract*: Novel foods can have benefits in improving access to healthy foods and lowering the impact and strain on the environment and resources, but can come with challenges. Not only should the novel food appeal to the consumer, but be safe to consume, offer some health benefit, and not be nutritionally disadvantageous to the consumer.*

Many methods available for the analysis of traditional foods. However, they may require validation to ensure that the methods are suitable for a novel food. Reference materials are important for developing and refining existing analytical methods. They can be used to verify the accuracy and precision in the identification and quantification of specific components within a novel food. This can contribute to better understanding of its nutritional profile and the potential hazards when consuming a novel food. Reference materials can also be used in comparative studies across different laboratories, such as proficiency testing and can enable laboratories to verify the accuracy of their results.

Thus reference materials are necessary in ensuring the accuracy and reliability of food analysis. They play an important role in method development, quality assurance, and contributing to the safety and integrity of the food supply chain

Presentation 2*: Rapid and Efficient Hydrolysis of Plant-based Foods for Amino Acid Content*

Bobbie Mcmanus*, Alicia Stell and Benedict Liu, CEM, alicia.stell@cem.com

Abstract: *There is an increased public interest in plant-based ingredients, particularly for use as alternative proteins. Determination of the amino acid content is an important part of the overall evaluation of protein quality and digestibility score, in general, but specifically for plant-based*

ingredients. Rapid feedback on amino acid content enables adjustment to optimize production, control costs and produce high-quality products while meeting these important criteria. Herein, a rapid, efficient, and safe hydrolysis, followed by precolumn derivatization and LC-UV analysis is used to determine amino acid content of plant-based foods, including alternative tuna and chicken. Samples were hydrolyzed via a 30 minute microwave-assisted hydrolysis method and amino acid content was determined within an hour. This method was compared to amino acid content determined via traditional manual hydrolysis methods and the data reported herein to determine the most accurate, safe, and rapid process for amino acid determination in these alternate protein sources

Presentation 3: *Elute-and-Shoot: Improving sample extraction by harmonizing elution solvent and liquid chromatography*

Eoin P. Quinlivan*, Merieux Nutrisciences, eoin.quinlivan@mxns.com

Abstract*: Phase extraction (e.g., solid or liquid-liquid) is commonly used to purify samples prior to chromatography. However, if the elution solvent and chromatographic phase are incompatible, additional sample processing (e.g., solvent exchange, sample dilution) may be required. This additional workup can be costly in terms of effort, materials and sensitivity. Here we provide 3 examples where existing methods are modified to optimize extraction performance while harmonizing the sample solvent and chromatographic technique:*

Example 1: Sample dilution is the easiest way to harmonize sample solvent with the chromatographic conditions. A typical approach is to dilute the sample to closely match the starting chromatographic conditions. However, this approach can cause loss of sensitivity due to sample over-dilution: By modifying FDA LIB4421 (by optimizing the dilution-factor) we were able to increase Melamine and Cyanuric Acid sensitivity (LOQ in infant formula < 35 ppb; FDA Action Level = 125 ppb) without affecting chromatographic performance.

Example 2: However, it may be advantageous to use dilute the sample (especially for sensitive assays), if it negates the need for solvent exchange. By modifying AOAC Official Method 2000.08 for Aflatoxin M1 we were able avoid having to exchange the buffer. By optimizing the elution volume and sample dilution sample volume only increased two-fold, while chromatographic performance remained unchanged. Thus, assay sensitivity (LOQ in milk < 0.002 ppb) ; FDA Action Level = 0.5 ppb), without having to dry down and reconstitute the sample.

Example 3: If the elution solvent isn't compatible with the chosen chromatography: Change the chromatographic phase. Acrylamide is commonly assayed by Reverse Phase HPLC. But, as Acrylamide is poorly retained on reverse phase chromatography, small (<5%) amounts of organic solvent in the sample can cause retention shifts. However, the high (100%) acetonitrile content of the eluate makes it ideal for HILIC chromatography.

TUESDAY 6/4/2024

CURRENT STATUS AND PROGRESS IN THE ANALYTICAL LANDSCAPE OF DAIRY AND DAIRY PRODUCTS

9-12.30 PM: Mallard (2 Presentation)

Session 1: *Harmonization of Methods, Activities/Participation across Organizations AOAC, ISO/IDF, CODEX*

Chair: David Ellingson, Eurofins, David.Ellingson@ft.eurofinsus.com
Abstract: *Understanding what methods can be used for regulatory purposes for commercial sale of milk, dairy products, and infant formula within the United States and across the globe in different countries can be a complex task. Creating a system where standardized and fully vetted methods are available that are acceptable for regulatory in all countries is the easiest way to combat this issue. This session will go into detail on how organizations such as IDF, ISO, AOAC, and CODEX are working together to build this system of methods that can be used. This session will also go into how manufacturers deal with this system and their experience with meeting regulatory requirements.*

Presentation 1: *International Standard Harmonization for Milk and Dairy Products*
Wendy Warren*, Scientist and Branch Chief of Standards Division, AMS Dairy Program – USDA, wendy.warren@dallasma.com
Abstract: *As the U.S. dairy industry expands export markets, engagement with international standards activities is essential to ensure domestically produced products meet global expectations, and that the analytical standards used for assessment are technically aligned. This session will provide an overview of international standards development processes within the International Organization for Standardization (ISO) and International Dairy Federation (IDF) for analytical evaluation of milk and dairy products, along with opportunities for alignment with AOAC Official Methods as it relates to Codex. Furthermore, the importance of analytical method harmonization and stakeholder participation will be discussed.*

Presentation 2: *Navigating Global Dairy Standards Through Commercial Chaos*
Laura Loysen*, QA and Technical Services Manager, Hoogwegt, l.loysen@hoogwegtus.com
Abstract: Pending

TUESDAY 6/4/2024

ANALYSIS OF AGRICULTURAL PRODUCTS, FERTILIZER AND BIO-STIMULANTS

9-12.30 PM Mallard (3 Presentations)

Session 3: Beneficial Substances and Biostimulants

Chair: James Bartos, Office of Indiana State Chemist, jbartos@purdue.edu
Abstract: *Plant Biostimulants is a rapidly growing area of interest and development. Clarifying what qualifies as a Plant Biostimulant and how to get safe and effective products to market has received international attention. This session will focus on the process developed for getting these products to market in North America and some of the challenges with identifying and quantifying the active ingredient(s) and/or organisms. Continued rapid growth in this area is anticipated with the need to develop and validate many test methods. Some of the challenges, options, and objectives regulators and manufacturers face will be discussed in this session.*

Presentation 1: *Developments in Biostimulant Path to Market and Method Needs*
James Bartos*, Office of Indiana State Chemist, jbartos@purdue.edu
Abstract: *Plant Biostimulants are a new area of rapidly growing interest and development. A Plant Biostimulant is a substance(s), microorganism(s), or mixtures thereof, that when applied to seeds, plants, the rhizosphere, soil or other growth media, act to support a plant's natural nutrition process independently of the biostimulant's nutrient content. The plant biostimulant thereby improves nutrient availability, uptake, or use efficiency, tolerance to abiotic stress, and consequent growth, development, quality, or yield. While some of these products are in use in*

other countries and/or may have existed prior to the creation of this category, a clear path to market in North America has been limited. The regulation of these products has fallen to the Association of American Plant Food Control Officials (AAPFCO). This presentation will provide a brief history of AAPFCO's involvement in this process and its efforts to help bring safe, effective, and properly labeled products to market. A critical part of the pathway to market includes the need for test methods to confirm the active ingredient(s) and its concentration. As a result, the need for many new test methods over the next several years exists. Some of the challenges will be the wide range of different product types, formulations, expertise, instrumentation, and sheer number of new products that fit into this category. This presentation will primarily focus on the efforts, progress, and outlook for bringing these products to market.

Presentation 2: *Biostimulant Methods Development: Shifting from EPA to AAPFCO mindset using Silicon as a case study*

Wendy L. Zellner, Ph. D*., Zellner's Research Solutions, LLC, ZellnersRS@outlook.com

Abstract: *We can all agree that pesticides and growth regulators are more stringently tested and regulated when compared to fertilizers or animal feed. EPA regulated chemicals and pesticides typically contain one or two active ingredients responsible for their effects. However, efficacy of biostimulants rely on multiple components working in concert. Switching from a mindset of 'active ingredient' to 'beneficial substance' has made it challenging to develop test methods that address industry's wants, while fitting within the capabilities of state-funded laboratories. While these laboratories may have less resources than their federally-funded counterparts, there are opportunities to develop test methods that are stringent, reproducible, and cost-effective to ensure efficacy and industry standards are maintained. Lessons can be gleaned from the development of the 5-Day Sodium Carbonate-Ammonium Nitrate Extraction Method for Soluble Silicon (ISO 19747:2020), which involved compromises between industry stakeholders as well as amongst state regulators in order to have an acceptable test method for these products. Additionally, methods and labeling already in place through AAFCO could be adjusted to fit the needs and criteria for biostimulants regulated under AAPFCO, decreasing the time and cost for lengthy methods development. Switching the focus from a single or handful of active ingredients to a more general nutritional assessment of biostimulants will provide industry with methods that are accepted by state officials, benefiting the end user with meaningful label information. All-in-all switching to an AAPFCO mindset benefits everyone involved.*

Presentation 3: Plant Biostimulants– *Determination of Microbial Beneficial Substances/Plant Biostimulants in Fertilizer Products, Availability of Methods and Challenges.*

Dancia Wu*, Office of Indiana State Chemist, scharfd@purdue.edu

Abstract: *Plant beneficial microorganisms are increasingly being recognized as an important category of many plant biostimulants to improve plant growth and stress tolerance. However, enumeration and identifying the specific microbes can be challenging. This presentation explored methods for determining the beneficial substance(s) of microorganisms in fertilizer products.*

Some of the key points that covered in the presentation:

- *Importance of biostimulants and the role of microorganisms.*
- *Challenges of enumeration and identifying plant beneficial microbial in fertilizer products.*

• Available methods for determining beneficial substances of microorganisms and limitation of those methods.
• Our effect and testing results for mycorrhizal fungi and microorganisms in fertilizer products.

Session 5A: ***Agricultural Soil Analysis***
Chair: Dylan Tanner, FOSS, dtanner@fossna.com
9-12.30 PM: Mallard (2 Presentations)
Abstract: *This segment contains presentations in the soil testing area within the scope of agriculture. The topics include testing methodologies and mentality for addressing soil testing. What should be tested and how. What are some of the new methods on the forefront of soil and agriculture testing and how should we go forward in addressing the needs of agriculture.*

Presentation 1: *A different kind of soil testing*
Dustin Sawyer*, MSc, Rock River Laboratory, Inc., dustin_sawyer@rockriverlab.com
Abstract: Soil testing is a generic term that takes on different meanings depending upon the audience. At AOAC meetings, it usually centers on EPA or other environmental regulations. This presentation we'll explore the different and interesting world of agricultural soil testing; a type of soil testing that may be new to some. While there are some similarities between environmental and agricultural soil testing, the differences may be surprising. Participants will learn about the chemistry, equipment, regulations, challenges, and the very purpose of agricultural soil testing and may even find a new subject of interest.

Presentation 2: *Evaluating Laser-Induced Breakdown Spectroscopy (LIBS) for rapid estimates of soil carbon content*
Ryan Stewart* and Spencer Toru Patrick, School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA ryan.stewart@vt.edu
Abstract: *Uncertainties abound related to carbon storage and cycling in soils, making it difficult to assess whether efforts to sequester atmospheric carbon are working. Current methods to estimate soil carbon concentrations typically require lengthy preparation times and require the samples to be combusted or chemically reacted. Laser-induced breakdown spectroscopy (LIBS) is a tool for measuring the elemental composition of soil samples, and holds promise for rapid and accurate quantification of soil carbon content. We are currently assessing the ability of a handheld LIBS scanner to analyze carbon concentrations for different agricultural soils. We performed one study to determine the best sample preparation method, and found that hydraulically pressing samples to 400 MPa was superior to using glass slides or entraining the samples in curable fluids. Our second study has focused on assessing whether calibration curves generated from a site at one time will suffice for repeated samples, an important feature of soil carbon sequestration work. The results suggest that calibrations may hold for multiple years under certain conditions, but are confounded by heterogeneities in soil properties such as texture and by management practices such as tillage and use of over-winter cover cropping. This presentation is ultimately designed to share progress and lessons learned thus far about the potential for LIBS to become more widely used for soil carbon assessment.*

LUNCH (SPONSORED BY SCIEX AND OROCHEM): 12.30-2 PM (THE MARQUIS TENT)

TUESDAY

2-4 PM: CURRENT STATUS AND PROGRESS IN THE ANALYTICAL LANDSCAPE OF DAIRY AND DAIRY PRODUCTS

2-4 PM, Ballrooms F-J (5 Presentations)

Session 2: Advancement in Technology, Rapid Methods/Automation/AI

Chair: Dino Holmquist, Eurofins, dino.holmquist@ft.eurofinsus.com

Abstract: *The use of rapid methodology, data analytics, and automation to provide us with results faster and allow us to make decisions much sooner is key to greater throughput and profitability. Additionally, how we also create "green solutions" is as of equal importance to the technology evolution. The dairy industry continues to evolve and this session will highlight some of the advancements that are currently taking place. This session will provide a variety of areas where the analytical community has demonstrated advancements towards these goals*

Presentation 1: *Leveraging Artificial Intelligence and Data Analytics to Improve Dairy Product Quality and Safety*

Nicole Helen Martin, PhD*, University of Cornell, nhw6@cornell.edu

Abstract*: In recent years there has been rapid innovation in the use of large data sets to advance the food industry. This emergence of Industry 4.0, where the development and implementation of artificial intelligence tools have already begun to play a role in optimizing, predicting, and improving food quality and safety. Here we will discuss examples of these tools that have been developed and their practical application in the food sector.*

Presentation 2: *Changing the Rumen Microbiome in Dairy Cattle for Improved Animal Health and Reduced Methane Emissions*

Matthias Hess, PhD*, University of California-Davis, mhess@ucdavis.edu

Abstract: Pending

Presentation 3: *Advancements in FTIR: Enhancing Performance and Application Expansion*

Alli Wilson* and Roman Kwasiborski, FOSS, aeramo@fossna.com

Abstract: Pending

Presentation 4: *CPMG-NMR, a practical and safer replacement of extraction methods for total fat determination in dairy samples*

Colin Simpson , Bobbie McManus*, Actalia Cecalait, Poligny Cedex, France, Matthews, NC, CEM Corporation, bobbie.mcmanus@cem.com

Abstract: *For more than 30 years, NMR technologies have been used in the analysis of dairy products for fat content. A recent improvement in the application of NMR technology, adapting the CPMG pulse sequence, has removed the need for calibrations, creating a reliable, greener alternative to reference methods like Mojonnier, Weibull-Berntrop, Rose Gottlieb, and others. Utilizing CPMG-NMR, it is possible to determine accurate fat content of any dairy sample without calibration development or manipulation, nor prior analysis by reference methods. Additionally, this technique requires no solvents, a critical safety*

improvement over the hazardous and environmentally unsound chemical extraction reference methods. With an estimated 40 million fat analyses done on processed dairy products globally, there is a large potential to greatly reduce solvent needs and waste. Data comparing CPMG-NMR to traditional extraction methods for thirty dairy samples ranging from 0.42-44.33% fat, resulted in sd (standard deviation) of 0.12% across the whole range, and a statistically insignificant bias of 0.9999, for a near perfect correlation coefficient between the CPMG-NMR and reference methods. Comparing robustness of the methods, the reproducibility of CPMG-NMR method (R = 0.26g/100g) is lower than the expected reproducibility of the reference methods (R = 0.40g/100g). This presents an opportunity for the dairy industry to shift from environmentally hazardous chemical extraction methods to a greener and more simple method. All without sacrificing the precision of these reference methods and improving reproducibility of fat content determination beyond what is currently expected within the dairy industry.

Presentation 5: *The Potential use for Raman Spectroscopy in Dairy*

Dean Roberts and Sudagar Dhaliwal*, Bruker, Sudagar.Dhaliwal@bruker.com

Abstract: Vibrational Spectroscopy has been established as a critical tool for ensuring the safety, quality, and nutritional integrity of dairy products. Spectroscopic techniques such as mid-infrared (MIR) and near-infrared (NIR) are powerful tools for the rapid and non-destructive analysis of raw ingredients, intermediates, and finished dairy products. Yet Raman as a spectroscopic technique has not been extensively explored in the dairy industry. The pharmaceutical industry on the other hand has established Raman as an essential tool for monitoring both upstream and downstream manufacturing stages.

MIR electromagnetic radiation has wavelengths ranging approximately from 2.5 to 25 micrometers (µm) or equivalently, from 4000cm-1 to 400cm-1 in wavenumbers. NIR spans wavelengths from around 780 to 2500 nanometers (nm). Both MIR and NIR radiation interacts with molecules in a material at energy levels corresponding to the vibrational and rotational modes of the molecules. This interaction leads to absorption of specific wavelengths of light, which can be used to identify and characterize chemical compounds. Information on the chemical composition of dairy samples can be obtained using both MIR and NIR, enabling the quantification of key components such as fat, protein, lactose, and total solids content. Furthermore, as automation scales in the dairy industry, NIRs ease of deployment for use in real-time process monitoring of liquid, semi-solid, and solid dairy samples allows industry to take well established benchtop techniques transfer methods online so that variation can be scrutinized and controlled in real-time to increase overall manufacturing yield.

Raman spectroscopy relies on inelastic scattering of photons, providing information about molecular structure and chemical bonds in dairy samples. When laser light interacts with the molecules, most of it is scattered elastically (Rayleigh scattering), meaning its energy and frequency remain unchanged. However, a small fraction of the scattered light undergoes inelastic scattering, known as Raman scattering. The scattered light then carries the fingerprint of energy transitions, reflecting the vibrational and rotational modes of the molecules in the sample. This allows for the identification of specific molecules and complex structures. Raman's high specificity and sensitivity make it valuable for detecting minor constituents and monitoring subtle changes in dairy products during processing and storage. Raman's has weak scattering effects on water, limiting its detection capabilities, this presents new spectroscopic avenues to be explored that were previously masked by waters signal strength in the MIR/NIR.

In this presentation we will discuss commonalities and differences between NIR and MIR measurements as applied to benchtop analyzers and discuss early work examining the feasibility of Raman spectroscopy for both in-line and Benchtop analysis of Dairy samples

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TUESDAY

2-4 PM: ADVANCES IN DIETARY SUPPLEMENTS
Conference Dining Room (6 Presentations)

Session 3. Advancement in Quality Testing of Dietary Supplements
Chair: Jonathan DeCenzi, NOW Health Group, jonathan.decenzi@nowfoods.com

Abstract: *Dietary supplements (DS) are generally natural products intended to complement one's diet and encompass a wide variety of commodities such as vitamins, minerals, botanicals, amino acids, enzymes or probiotics. Like many commodities, DS materials can be subject to accidental or intentional adulteration and contamination that could potentially compromise the effectiveness and safety of the products. As such, the FDA requires that DS manufacturers are responsible for ensuring their products are not adulterated or misbranded and that each ingredient must meet specifications for identity, purity, strength, composition and for limits on contamination. Given the complexity of DS and the evolving scope of contaminants they are subject to, it is essential that the analytical techniques employed for testing keep pace with the regulatory requirements. This session will cover a variety of specialized analytical techniques developed to keep DS testing regimes current with the available technology, with particular emphasis on mass spectrometry*

Presentation 1: *Quantitation of Organic and Inorganic Arsenic Species in Kelp, Marine Oils, and Emerging Nutraceutical Ingredients by ICP-MS*
Dr. Jeffrey Buth*, Anna Plocicka-Okladlo, Katarzyna Banaszewski, Aaron Secrist, NOW Foods, jeffrey.buth@nowfoods.com

Abstract: *The determination of total arsenic in vitamin and nutritional supplement raw materials and products is routinely performed to ensure product safety. The ability to additionally separate and quantify individual arsenic species provides vital information for safety assessment, as the toxicity of inorganic species, As (III) and As (V), far outweighs that of organic ones. NOW foods has developed and validated a HPLC-ICP-MS method that speciates and quantifies As (III) and As (V), as well as common organic forms, arsenobetaine (AsB), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA), in materials such as kelp and marine oils. Solid samples are block digested in nitric acid while oil samples are microwave digested in nitric acid with hydrogen peroxide. HPLC separation of arsenic species is performed using an anion exchange column with a highly basic ammonium carbonate mobile phase gradient. Time-resolved ICP-MS analysis is performed at m/z 75 using helium collision gas. Signal drift is corrected by the post-column introduction of As (V) internal standard. Calibration is performed using a series of standards of each individual arsenic species. The method yields mass balances of 88-108% of speciated arsenic relative to the independently determined total arsenic. Spike recoveries of 100 ± 10% were obtained for most species in fortified samples, while all fall within 100 ± 20%. The %RSD of the sum of arsenic species in replicate samples is routinely less than 5%. The speciation method provides a valuable tool in assessing the safety of emerging nutraceutical ingredients such as fish oil gummies. It*

also enables the determination of total arsenic from the speciated forms for materials that suffer from interference in conventional total arsenic ICP-MS analysis such as vitamin B-12.

Presentation 2: *Development of Quality Control Methods for Juniper Berries by HPTLC and HRAM LC-MS*

Sara Plowman* and Mark Krzeszowiec, NOW Foods, sara.plowman@nowfoods.com

Abstract: *In dietary supplements, manufacturers need to verify raw materials used in formulation not only for the safety of customers but also to fulfill regulatory requirements. Use of an adulterated or misidentified material can have numerous consequences including reduced efficacy or even adverse events. Two quality control methods have been developed to identify juniper (Juniperus communis) berries for use in dietary supplements. Presently there is no official method for identification of the fruit of Juniper communis; the current methods focus on the identification of essential oil. The first method HPTLC and can differentiate between seven common juniper species: J. communis, J. virginiana, J. horizontalis, J. scopulorum, J. osteosperma, J. chinensis, and J. deppeana. Samples were a combination of commercially available botanical reference material as well as commercial samples and field collected specimens. In addition to HPTLC, high resolution accurate-mass LC-Orbitrap mass spectrometry with statistical analysis was employed to help identify marker compounds which will be further used to aid in identification of common juniper and discriminate from other species. Several potential marker compounds were identified to aid in differentiation of the species.*

Presentation 3: *Streamlined Sample Preparation for LC-MS/MS and GC-MS/MS Multi-residue Pesticides Analysis in Botanicals and Teas Using Novel Mixed Mode Cartridge Pass-through Cleanup*.

Jerry Mueller, QC MS Specialist II NOW Foods, jerry.mueller@nowfoods.com

Abstract: *One of the biggest challenges in routine pesticide residue analysis in botanicals is addressing the complex and varying nature of those matrices. Historically, separate preparations for LC-MS and GC-MS were necessary to achieve acceptable and consistent recoveries in botanical matrices. The LC-MS method utilized an AOAC QuEChERS extraction followed by a MgSO4, primary secondary amine (PSA), and C18E d-SPE cleanup step. The GC-MS method utilized an original QuEChERS extraction followed by an SPE cleanup containing PSA and graphitized carbon black (GCB). The GCB necessitated an elution solvent containing toluene to free the planar analytes from the GCB. These separate methods come with complications of allocating additional time to sample preparation, high cost per sample due to different sorbents used, and introducing points of contamination by additional interactions with the sample. These points make it difficult to implement on a larger or routine scale. There was a need for sample preparation that can cleanup a diverse amount of matrices while not trapping any pesticides. A method was developed with Agilent's Captiva EMR that answered many of the above problems. This was accomplished largely in thanks to the unique composition of these cartridges that can trap pigments without the drawback of also trapping planar analytes. The new method utilizing the Captiva EMR cartridges allowed a simultaneous preparation for analysis by both LC-MS and GC-MS, saving on analyst time and cost per sample. This method has shown an increased number of analytes able to be recovered on difficult matrices such as Ginger Root Powder, Peppermint Essential Oil, Turmeric Extract Powder, and various other botanical extracts. All monitored analytes met the SANTE guidelines for analyte recovery and linearity. This streamlined procedure has allowed an uninterrupted workflow that is flexible enough to handle a wide range of botanicals and robust enough to produce consistent data.*

Presentation 4: ***Targeted and Non-Targeted LC-MS Approaches to Analysis of Pharmaceutical Adulterants in Dietary Supplements and Ingredients***

Lukas Vaclavik*, Eurofins, Lukas.Vaclavik@ft.eurofinsus.com

Abstract: *The surge in the use of dietary supplements has coincided with a rise in adulteration incidents, wherein synthetic pharmaceuticals were illicitly added to these products by unscrupulous producers. The adulterants used span across various classes, including but not limited to phosphodiesterase type 5 (PDE-5) inhibitors, weight loss drugs, anti-inflammatory and hypoglycemic agents, or anabolic steroids. The aim of this fraudulent practice is to develop or enhance the biological effects of the supplement product and represents a worldwide issue associated with significant health hazard to consumers. To combat this fraudulent practice, there's a pressing need for analytical methods capable of reliably identifying and quantifying synthetic drugs in dietary supplements. Mass spectrometry (MS), along with hyphenated techniques such as liquid chromatography-mass spectrometry (LC-MS), represents crucial tools in this pursuit. In this presentation, we will discuss the development, optimization, and validation of targeted methods for high-throughput screening and reliable quantification of wide range of pharmaceutical adulterants in high-risk supplement classes. Additionally, LC-MS-based workflows allowing screening for and elucidating the structures of unexpected adulterants and emerging designer drugs in dietary supplements will be discussed*

Presentation 5: ***USP <561> Pesticides and the Three Bears***

Walt Brandl*, Merieux Nutrisciences, walter.brandl@mxns.com

Abstract: *Table 5 in USP General Chapter <561> prescribes pesticide compounds and residue limits that are to be applied to the assessment of Articles of Botanical Origin. We examine the USP pesticide list in terms of the "Goldilocks Principle", that is, is it too lenient, too stringent or just right. We will also discuss the relevance of the residue list in terms of current pesticide usage and the applicability of this assessment to various product types which may be covered by other regulatory regimes. The presentation is intended to provoke discussion and critical thinking when selecting a pesticide residue method for dietary supplements.*

Presentation 6: ***Quality Testing RoadMap for In-Process and Product Release Criteria: From Components to Final Soft Gel Manufacturing: Use of FTIR to GC/MS and LC/MS***

Asha Oroskar* , Xuejun Zang and James Lowe, Orochem Technologies, Inc, asha@orochem.com

Abstract: *Satisfactorily completing Identity testing, Microbial Load, and Potency of the Active component(s) for Incoming Components, Active ingredient potency with the custom formulated blend, Final product QC testing for potency, and microbial load, are three critical QC steps expected of contract soft gel manufacturers. Based upon the FDA requirements and the manufacturers' promise of quality followed, the manufacturer offers, in-house, in-process testing, in addition to third-party testing as release criteria for the final soft gels. An extensive workflow review demonstrating in-house, in-process tests, and QC test methods established as Release Criteria for a wide variety of softgels, such as Omega-3s from Fish and algal oil, CBD from Plant origin, Vitamin D3 and K2, Astaxanthin, Krill Oil, and other common supplements will be presented.*

TUESDAY

2-4 PM: CURRENT STATUS AND PROGRESS IN THE ANALYTICAL LANDSCAPE OF DAIRY AND DAIRY PRODUCTS

2-4 PM, Mallard (4 Presentations)

Session 3: ***Hot Topics in Microbiology: Sanitation/EMPs/Advanced Diagnostics***

Chair: Tim Stubbs, Innovation Center for US Dairy, Tim.Stubbs@dairy.org

Abstract: *Sanitation and hygienic conditions in manufacturing are of extreme importance in the dairy industry. This session will provide discussion on certain dry cleaning and sanitization methods along with the challenges encountered. The use of environmental monitoring programs (EMPs) will also be a topic that will be discussed in depth and how this has evolved. Additionally, the availability of new advanced diagnostics and sequencing that can be used in the dairy industry and the situations where these can be of optimal use will be covered.*

Presentation 1: ***Sanitation Methods for the Low-Moisture Food Processing Environment***

Hilary Green, PhD*, ORISE Postdoctoral Fellow, FDA, Hilary.Green@fda.hhs.gov

Abstract: *Dry cleaning and sanitization methods remove debris and microbial pathogens from food-contact surfaces without the use of water. These sanitation methods are used in facilities that produce low-moisture foods since the presence of water can lead to microbial growth. However, ensuring the removal and/or control of potential hazards such as allergens and pathogens without the use of water can be challenging, particularly when the food is also high-fat or sticky in nature. This presentation will cover research on dry sanitation approaches including their implementation feasibility and effectiveness.*

Presentation 2: ***EMPs for the 21st Century: Enhancing EMPs to Meet Ever Changing Needs for the Dairy Industry***

Patrick Bird*, Sr. Manager Scientific Affairs, bioMérieux, patrick.bird@biomerieux.com

Abstract: *Environmental monitoring programs (EMPs) serve as crucial tools in evaluating the hygienic conditions present during food processing. For manufacturers of low moisture ingredients, EMPs may include sampling for pathogens (Salmonella), index organisms (Listeria spp.) and/or indicator organisms (Enterobacteriaceae (EB), coliforms), however, often little is understood on how to interpret the results for the latter two types of analysis. Is testing for index or indicator organisms sufficient to keep a manufacturing facility safe? Is there a single type of test that can be used to provide all necessary information to a manufacturer? What is the best process to ensure efficiency in an EMP? This presentation will provide an overview on the evolution of EMPs, understanding the difference between index and indicator organisms analysis, and how digital readiness tools provide a future roadmap for EMPs.*

Presentation 3: ***Beyond the ACTGs - Practical Applications of Advanced Diagnostics and Genome Sequencing for the Dairy Industry***

Darryll Barkhouse, PhD*, Director Genomics Discovery Center, bioMérieux, Darryll.BARKHOUSE@biomerieux.com

Abstract: *Many advanced diagnostic tools are now available to the dairy industry. These technologies have progressed past the academic stage and are mature enough to be deployed for routine use and troubleshooting. The list of technologies covers a wide range of resolution, ensuring an effective and affordable technology exists to match the diverse issues relevant to the industry. Examples in this toolbox include pathogen mapping, whole genome sequencing, metagenomic sequencing, amplicon sequencing and allele specific qPCR. This presentation will discuss how to determine the right tool for the situation, how WGS can provide the resolution need to maintain productivity, and how to translate results into actionable items.*

Presentation 4*: Isolating the Bacillus cereus group from dairy products using a new chromogenic agar*

L. Restaino, R&F Products, lrestaino@rf-products.net

Abstract: *This presentation will review the performance of a new selective and differential chromogenic medium, R&F Bacillus cereus Group (BCG) agar. This agar selects for strains belonging to the Bacillus cereus group based on phosphatidylcholine phospholipase C activity, a virulence factor. Colonies are more easily differentiated from non-Bacillus cereus group species compared to tradition media using egg yolk and lecithinase.*

VENDOR VISITS: 4-5.30 PM, TUESDAY

SOCIAL HOUR & DINNER: 5.30 TO 7.30 PM (SPONSORED BY MERIEUX NUTRISCIENCES: THE MARQUIS TENT)

WEDNESDAY || JUNE 05, 2024 || MORNING

Breakfast: 7.30- 9.00 AM (THE MARQUIS TENT)
Registration: 8.00- 9.00 AM (SOUTH FOYER)

WEDNESDAY: 6/5/2024

ANALYSIS OF AGRICULTURAL PRODUCTS, FERTILIZER AND BIO-STIMULANTS
9-12.30 PM, Ballroom F-J (9 Presentation)

Session 4: ***Chemical Residue Analysis***

Chair: Ken Kise, Iowa Dept. of Agriculture and Land Stewardship, Ken.Kise@iowaagriculture.gov
The chemical residue session will have presentations on potential contaminants of pesticides in soil, produce, and food. These analyses assure the safety of the food we consume.

Presentation 1: *Multi-residue Analytical Method for the Confirmation and Quantification of 500+ Pesticides in Fruit and Vegetables*

Maria Laura Pati, Autumn Payne*, Nicola Barbieri, Alfredo Fantastico, Piero Pontrelli, Perkin Elmer, autumn.payne@perkinelmer.com

Abstract: *Pesticides are primarily utilized in the agricultural sector and contain one or more active substances. From the point of application, pesticides can be transported through various media, and ultimately be deposited on plants and animals humans consume. While some of these compounds have not been found to be harmful, others may have toxic properties to humans and animals, as well as pose a danger to our environment and ecosystems.*

A total of 503 pesticides were investigated and analyzed in this study and measured in three matrices - apple, orange, and lettuce, to cover a range of both fruit and vegetable-based samples of varying composition and acidity. Sample extraction with acetonitrile, followed by a dispersive solid phase clean-up step was utilized. Development of a multi-residue pesticide analysis using PerkinElmer QSight 220 triple quadrupole mass detector coupled to the LX50 ultra-high performance liquid chromatography (UHPLC) system allows for rapid and reliable results.

The LOD values for 97% of the 503 measured pesticides are equal or below 1 ng/ml. The LOQ values for 90% of the 503 compounds are equal or below 2 ng/ml, and thus easily meet the required MRL values as set by the EC 396/2005. All pesticide calibration linearities are better than 0.99 in the working range of LOQ-100 g/ml.

The QSight 220 LC/MS/MS system provides a robust and reliable platform for reproducible results in compliance with regulatory limits. Utilizing the PerkinElmer system, confirmation, and quantification of hundreds of pesticide analytes in fruit and vegetable matrixes was measured in positive and negative polarity switching mode, allowing for greater efficiency and higher laboratory throughput. Further, the automated time-managed MRM algorithms optimize dwell times for fast acquisition rates without compromising data quality.

Presentation 2: *Analysis of Fentanyl and Derivatives by SPME and Twister technology*.

Jean-Paul Schirlé-Keller*, Yoko Johnson, Minnesota Department of Agriculture, jean-paul.schirle@state.mn.us

Abstract: *Drugs such as fentanyl are at the forefront of the opioid epidemy due to their potency. Their lethal effect at low dose is not only a threat to recreational users, but it can also be a threat to unsuspecting population if laced into common items such as drinking water. We investigated the use of SPME fiber and Twister® technology to determine levels of 4 fentanyl derivatives. No solvent extraction was needed, speeding up analysis time: a 5-point standard curve and 1 sample sequence can be run in under 6 hours, extraction included. We were able to achieve quantitative results with LODs of 25ppt and 0.25ppb with SPME and Twisters, respectively, with reasonable reproductivity. Extractions in 3 types of commercial drinks showed effects of flavor and carbonation on extraction efficiency.*

Presentation 3: *Challenges of Matrix Effect in Mycotoxin Screen Analysis by HPLC-Mass Spectrometry*

Atiq Rahman, Ph. D*, Senior Scientist- Mass Spectrometry, Merieux Nutrisciences, atiq.rehman@mxns.com

Abstract: *Mycotoxins are natural products produced by fungi and are possible sources of contamination in grain and milk products. The Matrix effect poses a big challenge in analyzing mycotoxin contamination in food by HPLC-Mass spectrometry. The present talk will focus on different strategies used to develop an efficient dilute-and-shoot method for mycotoxin analysis by mass spectrometry*

Presentation 4: *Perfecting Pesticides: Leveraging Technology to Decode Multi-Analyte Analysis*

Emily Burke-Kaniewski, MS*, Chemist III, Merieux Nutrisciences, emily.kaniewski@mxns.com

Abstract: *Pesticide testing is an important aspect of food safety and quality testing. With over 17,000 pesticide products currently on the market, screenings can quickly become a long and complicated process. This presentation will discuss common issues faced in pesticide screenings and techniques used to overcome these challenges as well as new developments that are being made to improve the screening process*

Presentation 5: *FGIS and AOAC: Comparing Method Validation Options for Rapid Quantitative Mycotoxin Test Kits*

R. Lucas Gray [*], *Neogen, lgray@neogen.com*

Abstract: In the U.S. Rapid Mycotoxin testing landscape, two major method validation bodies to which test kit manufacturers can apply for approval are the AOAC Research Institute (RI), for Performance Tested Method[SM] *(PTM) certification; or the USDA, for Federal Grain Inspection Service (FGIS) method approval. Although both groups offer method validation of rapid quantitative mycotoxin test kits, they vary widely in their sampling and testing rules, performance requirements, submission and approval process, and significance to potential end users. This presentation will illustrate the complexity of seeking third-party approvals by comparing these two method validation bodies and illustrating the varied goals and incentives facing test kit manufacturer as well as the complexity of the question, "is this a good test kit?"*

Presentation 6: *Determination of PFAS Substances in food and packaging using LC-MS/MS*

Ming Gao[*] and Walt Brandl, Merieux Nutrisciences, ming.gao@mxns.com

Abstract*: Perfluoroalkyl compounds (PFAS) are a subset of fluorinated substances widely utilized since the 1950s due to their unique properties, such as high thermal and chemical stability. PFAS are commonly found in various products like fabrics, paper coatings, non-stick cookware, and firefighting foams. Due to their stability, PFAS persist in the environment, contaminating soil, air, and water sources, potentially exposing populations through food ingestion, inhalation, or direct contact. Human studies on PFAS exposure have shown mixed health effects, including potential associations with elevated cholesterol, uric acid levels, liver issues, hormone disruption and even potential links to cancers. Regulatory agencies, like the FDA, are taking steps to limit human exposure. While there's no federal ban on PFAS in food packaging, some states have enacted regulations. Recently, the FDA announced the discontinuation of PFAS-containing grease-proofing substances in food contact materials. This method outlines the analysis of PFAS in food items and packaging materials for human consumption using LC-MS/MS technology. The procedure involves homogenizing the sample, fortifying it with isotopically labeled surrogates, and extracting PFAS using acetonitrile and formic acid. Clean-up techniques like QuEChERS and solid phase extraction may be necessary for complex samples. LC-MS/MS is then used for analysis, identifying PFAS compounds through multiple reaction mode transitions and retention time matching. Quantitation is achieved by comparing response ratios to calibration standards, with adjustments made for dilution and starting sample mass. In sum, this method provides a comprehensive approach to accurately assess PFAS levels in food and packaging samples, aiding in ensuring consumer safety and regulatory compliance*

Presentation 7: *Novel QuEChERS for testing pesticides in industrial hemp leaves and cannabis oil*

Xuejune (June) Zang[*], Gautham Oroskar and Asha Oroskar, Orochem, june@orochem.com

Abstract: *The QuEChERS method has been utilized to detect final product pesticides and screen incoming fruits, vegetables, feedstocks, and nutraceutical ingredients to quality (overseas) suppliers or qualify ingredients and final products. Based on CA's regulation, several challenging pesticides were analyzed.*

These pesticides are known to have low signals in mass spectrometry or interferences with the components (such as color or cannabinoids) in hemp leaves and cannabis oil. We tested several different QuEChERS formulations and extraction methods (including the standard AOAC method) with pesticides in hemp leaves and cannabis oil to achieve high and consistent recovery of analytes and high color-pigment removal. Finally, QroQ QuEChERS for hemp is used to extract these pesticides in cannabis oil and to analyze pesticides in the purification process. The pesticide extracts are further separated by Gazelle C18 UHPLC column and detected by LC-MS/MS. The pesticide detection ranges are between 0.25-100 ppb, and recoveries are between 85-102%

Presentation 8: *Evaluation of PFAS Levels in Infant Formula based on the AOAC Draft SMPR*

Karl Oetjen, Simon Roberts, Christopher Via*, Sciex, christopher.via@sciex.com

Abstract: *Despite approximately 22 years of research, quantifying the major PFAS exposure routes to humans remains unresolved. Interest in PFAS food analysis as increased in recent years. In the European Union, maximum levels of specific PFAS (PFHxS, PFOS, PFOA, PFNA and sum of the 4 PFAS), in some foods (e.g., meat, eggs, fish, seafood and shellfish) were implemented in 2023. Further, the AOAC has established as working to develop criteria for PFAS analysis in food. A method was developed to analyze 34 PFAS in baby food using a high-end mass spectrometer. In summary, 5 g of baby food was extracted with a Quechers method and dispersive SPE. Chromatographic separation was performed using a PS C18 column (150 mm length, 3 µm particle size). Mobile phases were comprised of "A": water with 10mM ammonium acetate and "B": acetonitrile. Gradient conditions were used with a flow rate of 0.8 mL/min and run time of 13 min. The injection volume was 20 µL. Two transitions per analyte were monitored except for those analytes which did not have stable secondary transitions. Analyte responses were normalized to their appropriate mass-labelled internal standard response. Method detection limits were determined following the protocol described in 40 CFR Part 136. MDLs were generally within the range of 1-10 pg/g, except for 6:2 FTS, PFBA, PFODA and PFPeS which were in the 10s of pg/g. The results demonstrate that low part-per-trillion detection limits in baby food were achieved with the Quechers method and high-end triple quadrupole mass spectrometer*

Presentation 9: ***Extraction and Analysis of glyphosate from soil***

Ken Kise*, Iowa Dept. Agriculture and Land Stewardship, Ken.Kise@iowaagriculture.gov

Abstract: *Initially, the Iowa Department of Agriculture and Land Stewardship lab did not have a method for analysis of glyphosate from soil. Due to the amount of glyphosate sprayed in the State of Iowa to control weeds, a method was needed. The first method we used was developed from Ibanez, et al., which used a strong KOH solution to extract the glyphosate from soil. It required neutralizing the solution after extraction and derivatizing the glyphosate with FMOC-Cl. Our lab currently uses a method developed by the Oklahoma Department of Agriculture, which uses a borate buffer instead of a KOH solution. This eliminates neutralization; the extract is ready for derivatization with FMOC-Cl. As well as glyphosate, this method also works well for glufosinate and AMPA. The instrumental analysis is done on LC/MS/MS with a C18 column.*

WEDNESDAY: 6/5/2024

ANALYSIS OF AGRICULTURAL PRODUCTS, FERTILIZER AND BIO-STIMULANTS

9-12.30 PM, Mallard (5 Presentation)

Session 5B: ***Agricultural Soil Analysis***

Chair: Dylan Tanner, FOSS, dtanner@fossna.com
Abstract: Pending

Presentation 1: ***Mineralization of nutrients from soil organic matter: how, how much, and can we test for it?***

Andrew Margenot, University of Illinois, margenot@illinois.edu

Abstract: Soils contain large reserves of potentially crop available nutrients in organic form that must first be mineralized into inorganic forms, notably for nitrogen (N), phosphorus (P) and sulfur (S). Tests that can predict mineralizable nutrients are a "holy grail" of soil testing and fertility management, because accurate prediction of N, P and/or S mineralized during the growing season enables precise application of nutrients, increasing agronomic and economic fertilizer use efficiency. However, the biological nature of mineralization challenges such precision, in particular the sensitivity of biological processes underlying mineralization to soil moisture and temperature conditions (i.e., weather). Here, we review historical and best-bet attempts today to predict N, P and S mineralization through soil testing, and future needs and directions.

Presentation 2*: Elemental Analysis for Soil Using Laser Induced Breakdown Spectroscopy:*

Michelle Walter, Foss representative, mwalter@fossna.com

Abstract: Pending

Presentation 3: ***Streamline your TDF and Protein Analysis Workflow***

Jerry Richardson, Jared Mayo*, BUCHI, mayo.j@buchi.com

Abstract: *Total dietary fiber and protein are crucial parameters for any facility or laboratory testing food products. By transferring your sample bags from your TDF Analyzer, directly to a Kjeldahl digester, you will eliminate messy and time consuming sample prep. In this presentation we will illustrate how to simplify and streamline the testing process by optimizing workflow.*

Presentation 4*: Automated targeted and non-targeted Orbitrap MS workflow for analysis of more than 40,000 PFAS compounds*

Richard Cochran, Ph.D., Senior Application Scientist, LSMS, richard.cochran@thermofisher.com

Abstract: *The increasing application of PFAS testing in the food, beverage, and food packaging industries has prompted the need for efficient and robust workflows in laboratories. To address this, a novel automated dispersive liquid-liquid microextraction (DLLME) method has been developed specifically for food and beverage laboratories. This method allows for the quantification of targeted PFAS compounds in samples, as well as the identification of additional PFAS compounds through Non-Targeted Analysis (NTA).*

Samples are analyzed using a combination of targeted quantitative and non-targeted screening Orbitrap MS methods. The non-targeted results are then compared against a comprehensive PFAS compound library, which includes over 40,000 PFAS compounds. Additionally, a new in silico predicted transformation library is utilized to enhance the identification process. This new library allows for the identification of more tentative candidate structures, where previously only possible empirical formulas could be determined. To identify potential PFAS compounds, the putative library identifications are

reviewed alongside other factors such as retention time correlated with mass, molecular mass divided by the number of carbon atoms, and mass defect divided by the number of carbon atoms. This multifaceted approach provides a comprehensive and confident means of identifying both known and potential unknown PFAS compounds. Overall, this workflow offers an automated solution for laboratories to quantify known PFAS compounds and explore potential unknown PFAS compounds with confidence.

Presentation 5: Breaking Ground: Advancements in Soil Biology Methods

Rebecca Harvey, PhD - Woods End Laboratories,

Abstract: *Historically, soil testing primarily involved simple analyses of nutrient content and pH levels. However, a shift in our understanding of the role of soil biological fertility along with advancements in technology have brought about a paradigm shift in soil testing methodologies. This presentation will focus on soil biology testing, exploring its three main types: population analysis, biological activity, and indirect indicators, the challenges associated with this relatively new field and the value it provides in building a more sustainable agricultural system.*

WEDNESDAY: 6/5/2024

NOVEL FOODS FROM ALTERNATIVE PROTEIN SOURCES: CURRENT STATUS, ADVANCES AND CHALLENGES

9-12.30 PM, CONFERENCE DINING ROOM (6 Presentation)

Session 3: ***Allergen testing in Traditional/Novel Foods and potential challenges.***

Co-chairs: *Rakhi Panda, PHD, FDA and Tiffany Miller, Merieux Nutrisciences*

Abstract: How food is grown and/or processed could have a major impact on the sustainability of life. Individuals with food allergies are at risk of having serious health issues, up to and including death, if consuming products that have unidentified allergen residues in them, either due to mispackaging, labeling error or cross-contamination during production. Due to these risks, allergen awareness and testing have become increasingly important in the manufacturing industry due to public safety and regulatory requirements. To date, the capability for testing allergen residues in various ways is abundant. The various methodologies for testing allergen residues in foods, however, also creates the potential for a variety of different cross-reactivity or matrix interference issues that can result in false negatives or positives, particularly in relation to specific food types. Additionally, cross-reactivity or matrix interference issues and false negative/positive results affect the reliability of labeling of food products, which have the potential to cause major problems in production, compliance, distribution, etc., for the manufacturing company. This session will provide an overview of the various allergen testing methodologies and their detection capabilities as they relate to food products, with a specific focus on pea protein and hydrolyzed products. The session will also cover quantitative risk assessment approaches by using allergen product data for allergen labeling decisions

Presentation 1: ***Overview of Allergen testing methodologies and the different tests available for food products****.*

Tiffany Miller*, Mérieux NutriSciences, tiffany.miller@mxns.com

Abstract: *In the US, the number of people with food allergies has been on the rise, doubling each of the last decades. Year over year, more and more people are expected to have at least one food allergy to the following common allergens- Milk, Eggs, Peanuts, Tree nuts, Fish, Shellfish, Soy, Wheat or Sesame. Product testing for Food allergens, as required by FDA regulation, allows manufacturers to inform their consumers of the potential for food allergens present in their consumable products. Currently, there are several methodologies on the market for testing food allergen residues- Lateral Flow testing, ELISA testing, PCR testing and LC-MS/MS testing. These different methodologies also have many different kits available for use with various intrinsic properties for antibody/protein binding of a product. This presentation will cover the characteristics of various allergen testing methodologies as well as their suitability levels for various product testing.*

Presentation 2: *Food Allergens and Gluten Detection Methods and Recent Progress in Solving Unique Analytical Challenges of Fermented and Hydrolyzed Gluten*.

Rakhi Panda, PHD, FDA, Rakhi.Panda@fda.hhs.gov

Abstract: *There is no cure for food allergy or celiac disease (CD), and patients require strict avoidance of allergen and gluten containing products to avoid the ill effects of these conditions. The Food Allergen Labeling, and Consumer Protection Act (FALCPA) and FASTER Act, define nine major food allergens, and their labeling requirements. Regulations issued in 2013 and 2020 define the term ''gluten-free'' for food labeling and establishes the "gluten-free" compliance requirements for fermented or hydrolyzed foods and ingredients. Compliance with food allergen and gluten labeling regulations is challenging due to the presence of unintended allergens and gluten in finished products that may be introduced during the manufacturing process due to insufficient control, introduction of alternative protein sources with unknown allergen or cross-reactivity profiles, and introduction of novel manufacturing processes with unknown effects on food allergens and gluten. Accurate quantitation and accurate allergen/gluten labeling practices are essential for the safety of food-allergic consumers and consumers with CD and requires valid analytical methods. Foods such as those containing hydrolyzed gluten/allergens pose unique analytical challenges that require special attention while developing and validating analytical methods. This presentation will provide an overview of detection methods for food allergen and gluten analysis while specifically focusing on unique analytical challenges associated with fermented and hydrolyzed gluten detection and quantitation. Recent studies to quantify gluten in fermented dairy products and sourdough will also be discussed.*

Presentation 3: *Cross reactivity/False positive issues observed with Soy allergen testing in various foods.*

Eboni Perkins , Mérieux NutriSciences, eboni.perkins@mxns.com

Abstract: *Soy protein is a common allergen with associated allergenic health risks. Soy proteins are composed of a mixture of albumin and globulin proteins. Hydrolyzed soy protein is derived from acid hydrolysis that breaks down soy protein to a varied degree of its amino acids. Contrastly, Pea protein is a plant-based complete protein source, composed of globulin, albumin, prolamin and glutelin protein. It is also vegan-friendly and low in calories making it a great alternative for those looking for soy-free options. Pea protein, however, can have analytical cross reactivity issues with some ELISA Soy kits while some kits may have issues detecting Hydrolyzed Soy protein products. This presentation will provide an in-depth overview of the aforementioned issues in several product types.*

Presentation 4: ***The integration of risk-based approaches for allergen management and labeling decisions***

Joseph Baumert, PHD, Univ. of Nebraska, FARRP, jbaumert2@unl.edu

Abstract: *Food allergic consumers rely upon the accuracy of prepackaged foods labels to ensure the products they purchase are safe to consume. Unfortunately, the ubiquitous use of advisory/precautionary allergen label (PAL) statements such as 'May Contain', 'Processed in a Facility' or similar have caused confusion and frustration amongst consumers world-wide, leading some allergic consumers to ignore these risk communication statements. A harmonized, risk-based approach for decisions around the use of PAL statements would provide more transparency, and therefore, more informed decisions for allergic consumers as they manage their avoidance diets. An ad hoc Food and Agriculture Organization (FAO) and the World Health Organization (WHO) expert consultation on food allergen risk assessment was convened between 2020 and 2023 to evaluate the feasibility of using data from clinical threshold studies to derive reference doses for the priority allergenic food sources. These reference doses are proposed to be utilized in a risk assessment framework which can be uses by the food industry to make decisions around the use of PAL statements. The framework includes elements of quantitative risk assessment where the consideration of finished product consumption quantities and the accuracy of methods for quantification of allergen residues are used to calculate potential allergen residue exposure doses. The potential exposure doses are then compared to the established reference doses to support the risk-based management decisions. The sensitivity and accuracy of the analytical methods used to support this quantitative risk assessment approach is critical. This presentation will cover the framework and recommendations of the FAO/WHO expert committee and the international initiatives utilizing this risk-based approach.*

Presentation 5: ***Allergenicity assessment of insect proteins***

Kitty Verhoeckx, Ph. D, University Medical Center Utrecht, The Netherlands, K.C.M.Verhoeckx-2@umcutrecht.nl

Abstract: *To solve the future food insecurity problem, alternative and sustainable protein sources (e.g. insects, rapeseed, fava bean and algae) are now being explored for the production of food and feed. To approve these novel protein sources for future food, a comprehensive risk assessment is needed according to the European food legislation. Allergenicity risk assessment might pose some major difficulties, when no history of safe use is available, since detailed guidance on how to assess the allergenic potential of novel whole animal or plant foods is not available. At present, the approach relies mostly on the guidance of allergenicity assessment for genetically modified (GM) plant foods (weight-of-evidence approach). However, this guidance focuses on specific proteins and is difficult to interpret for whole organisms. In this presentation we demonstrate a conceptual strategy which address concerns for a "new-use" food; an insect.*

Allergies to new food proteins can result from cross-reactivity in existing sensitized or allergic individuals and thereby thus immediately manifest themselves in elicitation of allergic reactions upon consumption (elicitation phase). Bioinformatic tools such as Allermatch and AllergenOnline can be used to screen for potential cross reactive reactions with known allergens in new organisms. However, in case of whole novel foods thousands of proteins have to be tested and in case of mealworm, protein sequences are not always available. Using IgE binding tests and LC-MS analysis (based on sequence homology) potential allergenic proteins in mealworm were identified (e.g. tropomyosin, arginine kinase) and allergy was confirmed in a DBPCFC with mealworm snacks (containing whole mealworm) in patients with shrimp

allergy. Cross reactive reactions (IgE binding to mealworm proteins) were also found in a House dust mite allergic subpopulation, (not allergic to shrimp or Der p 10.

Allergies to new food proteins may also result from de novo sensitization and development of new allergies in susceptible individuals. In this case individuals needs to become sensitized first. Currently there are no methods to predict de novo sensitization for novel proteins. In our study we found 4 mealworm sensitized individuals amongst mealworm breeders and entomologists working with mealworm. Two breeders had food allergy to mealworm confirmed by DBPCFC and were not shrimp allergic and the other two had a suspected respiratory allergy to mealworm. The responsible allergens were identified as larval cuticle proteins using HPLC-MS. Furthermore, they were not responsible for cross-reactive reactions in shrimp allergic patients. This indicates that de novo sensitization to mealworm is possible and new allergies can arise when novel foods are introduced on the food market.

In conclusion, Shrimp allergic patients are at risk for food allergy to mealworm. However, there might be a risk also in HDM allergic and other atopic populations. Although the percentages of patients sensitized to mealworm in these latter groups are lower than in the shrimp allergic group, on a population level these groups may concern substantially larger at risk populations. De novo sensitization to mealworm is possible for those not exposed and the cuticle protein represents characterization of an otherwise unknown allergen in a new-use food organism. Unfortunately there are no tools available predicting these new allergies. New initiatives are going on such as ALLPreT to predict for sensitization.

Presentation 6*: Overview of Allergenicity Assessment of Novel/Newly Expressed Proteins in Genetically Modified Foods*

Tao Geng, Ph. D, Bayer Crop Science, *tao.geng@bayer.com*

Abstract: The safety of genetically modified (GM) foods is a sensitive topic in global food markets, notably in European Union, and North Asia. As one of the key steps to ensure the safety of GM foods, allergenicity assessment is to determine the allergenicity potential of novel/newly expressed proteins. Since there is no single test to predict allergenicity potential of proteins, the weight of evidence (WoE) is a consensus strategy in Codex (2003) to determine if a novel/newly expressed protein is safe in term of allergenicity.

This presentation will give an overview of WoE of various endpoints for allergenicity assessment of novel/newly expressed proteins, including source organism, history of safe use, protein expression, bioinformatics, heat stability and in vitro digestion. Several case studies, such as an allergy claim of a post-market product, bioinformatics hits, and allergic source proteins, will be discussed as references to address the future potential findings on allergenicity of novel/newly expressed proteins. In addition, this talk will brief the increasingly challenging regulations from the European Food Safety Authority, which requires additional assessments, such as celiac disease, physiological in vitro digestion, and adjuvanticity.

LUNCH (SPONSORED WATERS AND THERMO FISHER): 12-1 PM (THE MARQUIS TENT)

WEDNESDAY || JUNE 05, 2024 || AFTERNOON

WEDNESDAY

ADVANCES IN DIETARY SUPPLEMENTS

2-5.30 PM, BALLROOM F-J (6 Presentations)

Session 1: ***Challenges and Advancement of In-House Microbiological Testing for Dietary Supplements.***

Chair: Maria Mendres, Microbiology Manager, NOW Health Group, maria.mendres@nowfoods.com

Abstract: *The in-house microbiology laboratory plays a critical role in the dietary supplement manufacturing processes, ensuring product quality, safety, and regulatory compliance in a timely manner. A proficient in-house laboratory can identify the microbial quality and safety of the finished product at every step of the process, from raw materials to packaging, allowing timely corrective actions. This session will discuss the advantages of setting up an in-house microbiological laboratory, the adoption of automation and rapid methods, the challenges of validating methods on different dietary supplement matrices, maintaining proficiency, and collaborating with kit manufacturers in the development of methods that will improve the accuracy and efficiency of testing.*

Presentation 1: *An In-House Laboratory Approach for Validating Rapid Microbial Methods Used for Dietary Supplements*

Krista Chapman*, Maria Mendres, NOW Health Group, maria.mendres@nowfoods.com

Abstract: *Establishing a lean quality management process for an in-house microbiology laboratory requires consideration of the use of "rapid microbial methods." Rapid Microbial Methods (RMM) allow users to obtain microbiology test results faster than traditional culture-plate methods. Instead of waiting days or weeks, RMMs provide results in a timely manner, often within hours. These methods offer several advantages: speed, precision, sensitivity, and automation. Rapid Methods streamlines the testing process to meet the demands of modern industry. While rapid methods in microbiology offer several advantages, they also come with certain disadvantages. One of such disadvantages is that validating the performance of rapid methods can be complex. Ensuring accuracy, precision, and reliability requires rigorous testing and comparison with established reference methods. Raw materials used in dietary supplement formulations are often not in the scope of a rapid method standard validation. The industry is required to conduct an in-house validation of the rapid microbial method before it can be used in dietary supplement matrices. The presentation will provide an overview of the challenges of testing dietary supplement matrices using different rapid method technologies. We will also provide different validation approaches to meet the challenges efficiently.*

Presentation 2: ***Overcoming Challenges in Dietary Supplement Analysis for Microbial Pathogens: A Case Study***

John Mills*, Associate Director, Scientific Affairs, Biomerieux, John.MILLS@biomerieux.com

Abstract: *Microbial safety of dietary supplements presents unique challenges. Testing for dietary supplements and their ingredients is often performed by traditional culture procedures and is time consuming. The increased consumer demand for health and wellness products has fueled the need for effective, rapid and accurate pathogen testing methods. Dietary supplements and nutraceuticals have complex inherent natural properties that can pose challenges to pathogenic testing. Therefore, it is vital to have reliable, rapid testing methods and streamlined workflows to meet the demand for safe products in the market. This session will focus on a case study of working with rapid pathogen testing methods and*

overcoming the challenges that can be associated with the complex matrices in the dietary supplement market. This case study will detail proof of concept work and mitigations that were implemented for this category of products.

Presentation 3: *Verification of an Alternative Technology for Quality Indicator Testing*

Patrick Bird*, Sr. Manager, Scientific Affairs, BioMerieux, patrick.bird@biomerieux.com

Abstract: *Probiotic products are becoming increasingly popular worldwide. When administered in appropriate amounts, probiotic organisms are expected to provide a beneficial effect to the end users' health. As this market grows, the diversity in the genera and species used has expanded. Ensuring these product contain accurate label claims is challenging as current methodologies used by industry are not able to distinguish between closely related species, especially in multi-strain blends. In an effort to resolve this issue, leading global dietary supplement and probiotic provider NOW Foods partnered with bioMérieux to develop a custom probiotics assay. bioMérieux, through its XPRO program, developed the GENE-UP Probiotics Species ID solution, a real-time multiplex PCR Assay that can detect and differentiate 28 different species of probiotics. This session will focus on the development of the assay through the partnership between NOW Foods and bioMérieux.*

Session 2. ***Method Enhancements and New Approaches to Fulfill Regulatory Requirements for Dietary Supplements***

Chair: Chair: Edgar Grigorian, Merieux Nutrisciences, edgar.grigorian@mxns.com

Abstract: *The dietary supplements industry is dynamic and constantly at the forefront of product innovations. Evaluating these products to meet regulatory requirements for potency and stability considerations requires on-going enhancements to existing methods and introduction of new approaches to address growing need for highly sensitive and selective methods. Attendees will gain insight into the work done in this area by presenters who represent the diverse ecosystem that supports the dietary supplement industry.*

Presentation 1: *Adapting Industry Standard Amino Acid Analysis Workflows to the Modern Dietary Supplement Laboratory*

Donald A. Trinité* , Sr. Principal Technical Support Specialist, Waters, Donald_A_Trinite@waters.com

Abstract: *Amino acids are common ingredients in dietary supplements, especially those designed for sports and active nutrition. These products, including protein powders, drink mixes, and tablets, provide detailed information on amino acid content through Supplement Facts labels. To meet US regulatory requirements, manufacturers and testing labs must employ scientifically valid analytical methods for product labeling. Advancements in sample preparation, automation, and liquid chromatography have made amino acid analysis straightforward and relatively quick. Notably, the Waters AccQ•Tag TM Ultra Derivatization Kit has earned recognition by AOAC INTERNATIONAL stakeholders as fit-for-purpose when applied to infant formula and adult nutritionals testing. This presentation will introduce the Waters AccQ•Tag Ultra Amino Acid Analysis solution, showcase its applicability to modern dietary supplements, and highlight best practices for accurate quantitation.*

Presentation 2*: Determination of Huperzine A in Dietary Supplements by LC-MS/MS*

Gloria Wang, Zeyu Han* and Wendy Yuan, Merieux NutriSciences, wendy.yuan@mxns.com

Abstract: *Huperzine A is a promising nootropic compound isolated from a species of moss*

(Huperzia serrata). It is claimed to improve memory and concentration and has been proposed as a treatment for dementia. An analytical method has been developed using a methanolic extraction followed by determination by LC-MSMS in MRM mode. Separation was based on a HILIC column and gradient elution with formic acid (0.1%) and water as one mobile phase and formic acid (0.1%) and methanol as the other. Three transitions were monitored, one for quantitation and two for confirmation of identity. The method uses simple extraction and provides a sensitive (LOQ= 0.5 ppm), accurate and specific determination.

Presentation 3: *Global Framework on Dietary Supplements*

Aditi Sharma and Sumeet Narang[*2], Senior Scientist - Analytical & stability[1], Senior Director and Head of Global Excellence[2], R&D Health & Wellbeing, Unilever, aditi.sharma@unilever.com

Abstract: The dietary supplement market has grown in the past decade, as consumers are becoming increasingly conscious of their health and want to proactively take steps to enhance their overall wellbeing. The regulations and compliance standards are getting stricter to guarantee the quality and effectiveness of supplements. Even consumers are becoming conscious of the quality and safety of the products. Internationally, Dietary supplements can be classified by different names, such as Dietary Supplements (DS), Health Food (HF), Food Supplements (FS), Natural Health Products (NHP), Complementary Medicines (CM), evolving categories such as novel & functional foods and each category has an expected standards that needs to be followed for local market compliance. To meet regulatory compliance and consumer expectations for quality products, it is critical to understand the analytical methodologies that can help measure qualitatively and quantitatively the product efficacy and safety claims that we are delivering to the consumers. It is also important to study how the product changes over its shelf life and if it offers the same benefit by end of shelf life. However, the key challenge is the lack of reliable analytical methodologies to study the product attributes at release and over product stability.

In this session, we will cover the international framework of the expectations and some of the key challenges related to analytical methodologies and product stability. We will also address analytical methodology gaps in botanicals and different types of contaminants, that need more research in global scientific literature.

WEDNESDAY

CURRENT STATUS AND PROGRESS IN THE ANALYTICAL LANDSCAPE OF DAIRY AND DAIRY PRODUCTS

2-4 PM, Mallard (3 Presentations)

Session 4: ***Hot Topics in Chemistry: Whey Protein Hydrolysates/Alternative Uses for Whey/Vitamin Analysis***

Chair: Andy Powers, American Dairy Products Institute, apowers@adpi.org

Abstract: *The potential uses for whey continues to grow along with the analytical challenges. Of these challenges is finding standardized methods for protein hydrolysates that can be identified and used across the industry for regulatory acceptance. The AOAC is starting a working group on this task and this session will dive into the complexity of methods. Additionally, alternative uses for whey continue to evolve to provide ingredients that can be used in the food and dietary supplement markets. Discussion on technology used for production of an ingredient that can be used in these markets will be covered.*

Continuing with the theme of new technology, a presentation on Supercritical Fluid Chromatography (SFC) will provide insight into testing for vitamins in infant formulas and how this could be the future for testing certain compounds in the large expanse of dairy based products

Presentation 1: *Standardized Methods for Analysis of Whey Protein Hydrolysates*

Brian McGrath*, Associate Director – Research and Analytical Chemistry, Synergy, BMcGrath@synergytaste.com

Abstract*: Commercially, whey proteins are a by-product of the cheese manufacturing process. Depending on the end use application, whey proteins can be hydrolyzed to modify their technological and/or nutritional properties, which is typically achieved by enzymatic means. There are several different analytical approaches for describing the extent or pattern of whey protein hydrolysis. These include spectrophotometric methods, such as OPA and TNBS, as well as chromatographic techniques, such as SEC and HPLC. Unfortunately, many of these approaches can produce different results and there is no globally recognized standard method in place.*

Presentation 2: *Tagatose Production from Whey*

Anil Rajaram Oroskar*, Babu Antharavally, Gautham Oroskar, Orochem, anil@orochem.com
George Huber, PhD*, University of Wisconsin – Madison, gwhuber@wisc.edu

Abstract: *D-Tagatose is an isomer of Galactose which is approximately 90% sweeter than sucrose. Only 20% of the orally ingested tagatose is metabolized completely and mainly in the liver. In 2001, D-tag was appointed by the Food and Drug Administration (FDA) as a generally recognized safe product (GRAS), and subsequently it has been used as a nutritional sweetener or low in calories. After this, the European Union (EU) introduced D-tag as a "new food ingredient," without any restrictions on the amount to be used. Currently, D-tag is used as a sweetener in beverages, yogurt, creams, and dietetic candy [3]. Orochem Technologies Inc., a Biotech Company based in Naperville Illinois and University of Wisconsin Chemical Engineering, Madison WI have developed a process to produce Tagatose from Whey which is a byproduct of Cheese Production. This process involves the production of d-tagatose from lactose after acid hydrolysis to provide a hydrolysate having 1 equiv of d-glucose and 1 equiv of d-galactose for each unit of lactose converted. More particularly, the process involves the isomerization of d-galactose to d-tagatose and the use of a simplified separation scheme based on simulated moving bed (SMB) separation. The isomerization of d-galactose to d-tagatose is carried out in the presence of calcium oxide or calcium hydroxide. The process is useful for providing a simplified processing route to providing pure d-tagatose and glucose as two products from lactose hydrolysate. In an alternate embodiment, a process is disclosed for the production of d-tagatose from fermented lactose hydrolysate to provide a crystallized d-tagatose product. D-tagatose is useful as a food additive, as a sweetener, as a texturizer, as a stabilizer, or as a humectant*

Presentation 3: *Analysis of Fat Soluble Vitamins in Infant Formulas by SFC*

Sydney Kocher and Jacob Snider*, Nestle US, sydney.kocher@us.nestle.com

Abstract: *Fat soluble vitamins (FSVs) A, E, D, and K are heavily scrutinized in nutrient level compliance due to their importance in bodily functions. Accurate and precise analysis of FSVs is critical to ensure levels are compliant in sole-source nutrition, such as infant formula and adult nutritional products. A lack of FSVs can pose health risks such as cancer and immune system disorders. An excessive intake of FSVs has a greater risk of toxicity due to their tendency to accumulate in fatty tissues compared to their water soluble vitamin counterparts. Previous FSV methods at NQAC generated large quantities of hazardous solvent waste, solid waste in the form of SPLE columns and the sample extraction was very time*

consuming. To combat these issues, a UHPSFC-MS/MS method was developed for quantitative determination of vitamins A, E, D, and K in various food products, including infant formulas and adult nutritionals. A variation utilizing a saponification step was also implemented for sample matrices with known interferences. This method preparation is less time consuming, produces less waste, and uses a low volume of solvents. Additionally, super critical fluid chromatography (SFC) produces low volumes of solvent waste and allows a faster run time. We have been running the method for five years. In this talk we will discuss the method development as well as challenges and solutions we have come across regarding sample preparation and instrumentation.

WEDNESDAY

ADVANCES IN DIETARY SUPPLEMENTS

2-5.30 PM, CONFERENCE DINING ROOM (4 Presentation)

Session 4. *Understanding Prebiotics, Probiotics, and Postbiotics from Concept to Testing*

Chair: Andrew Morin, Merieux Nutrisciences, andrew.morin@mxns.com

Abstract: *The landscape surrounding Prebiotics, Probiotics, and Postbiotics can be vast and complex. Different types of products face differing regulations depending on the target market. While a wide variety of testing methods are available to meet the different regulatory agency requirements and quality questions surrounding these products. This session seeks to expand the knowledge base around Pre/Pro/Postbiotics, beginning with an overview of their uses, benefits, and market positioning. Then a dive into industry concerns and questions around pre/pro/postbiotics will be conducted to help understand the biggest points of uncertainty and pain for manufacturers and producers working with these products. Chemical testing of prebiotics will be discussed, looking at the different types of Prebiotics and how testing is performed for these products. Finally, the methods of molecular and microbiological testing of Pro/Postbiotics for quantity and identity will be discussed; helping to understand which tests can lead to what information for different types of samples. Overall, the session will broaden the knowledge base around these products and examine the different types of testing and associated results*

Presentation 1: *Unraveling the Relationships between Prebiotics, Probiotics, and Postbiotics*

Mallory Gandhi, PhD*, Hollison LLC, mgandhi@hollison.com

Abstract: *Prebiotics, Probiotics, and Postbiotics are all important biological products that can help support gut health. This presentation describes each product and their relationships in supporting gut health. Current accepted definitions of each will be described in addition to exploring the many roles biotics can play to support a healthy gut environment.*

Presentation 2: *Biotics - Their Future & Challenges*

Brian Schaneberg* , PhD - Director of the Illinois Tech Institute of Food Safety and Health (IFSH), and an Industry Professor of Food Science, Illinois Institute of Technology, bschaneberg@iit.edu

Abstract: *Our health can be impacted, both positively or negatively, and maintained through many different factors, both internal and external. The human microbiota is one key system that has been showing a direct correlation to our overall health. It is a complex mix of microorganisms that can easily become imbalanced. Years of research have indicated correlations between gut health and potential impacts ranging from immunity, brain, and cardiovascular outcomes to name a few. This knowledge has*

led to the billion-dollar category in the functional food industry marketing pre-, pro-, and post-biotics claiming to help support a healthy microbiota or even claiming to cure or prevent disease. The outlook for this category continues to be positive, but it also comes with its challenges as science continues to better understand how the microbiota around and in us directly impact the functions of our body, and how we each can supplement our diet to help support a healthy microbiota.

Presentation 3*: Analytical Testing of Prebiotics: featuring Beta-Glucan, GOS, and FOS*

Rick Jeswein* Research and Development Chemist, Mérieux NutriSciences, rick.jeswein@mxns.com

Abstract: *Prebiotics are compounds in food that act as an energy source for beneficial microorganisms such as bacteria and fungi in the gut microbiota. The benefits are wide ranging and include but are not limited to improved digestion and metabolism, regulating blood sugar and insulin, lowers inflammation and stimulates the immune system, balancing hormone levels, lowers cholesterol, reduce risk of allergies, and reduces risk of colon cancer. This presentation focuses on the prebiotics beta-glucan, galactooligosaccharide (GOS), and fructooligosaccharide (FOS). We will discuss their significance to the human body, structures, sources, and analytical testing methods*.

Presentation 4: *Microbiological and Molecular Testing of Probiotics and Postbiotics*

Andrew Morin, MS*, Research & Development Microbiologist, Mérieux NutriSciences, andrew.morin@mxns.com

Abstract: *Recent history has seen a rapid expansion of the market for probiotics, as well as the emergence of markets for postbiotics. The large number of different organisms, strains, matrices, excipients, and combinations thereof can make finding the best testing method difficult. In addition to the complexity of selecting the best testing for a given sample, consideration must also be given to the information that testing can generate, and in what units that data is collected. A number of different methodologies and technologies exist to identify, quantify, and characterize probiotics and postbiotics: plating, flow cytometry, qPCR, ddPCR, and next-generation sequencing (NGS). Each method has different limitations and benefits, and understanding these nuances is important to selecting methods that are fit-for-purpose. This presentation will discuss the various probiotic and postbiotic testing methods: their strengths, weaknesses, outputs, and use cases, to help guide and inform the process of selecting the best test for a product*