Analytical Scientist S P E C I A L S E R I E S : Food Safety and Analysis



#### UPFRONT

# The Secrets of Liquid Sunshine

Do environmental conditions influence the more subtle flavors of whiskey?

The seasoned wine quaffers among you will already know the importance of "terroir" when it comes to the distinct flavors found in your glass. But whiskey is not wine – and here, the impact of soil, climate, and sunlight has been largely overlooked.

Now, a team of industry and public sector researchers (presumably whiskey lovers) decided to investigate whether the environment in which barley is grown can contribute to the unique flavor components of new-make (unmatured) single malt whiskey. To do this, they used two different "analytical" methods: i) a sensory panel, consisting of six highly-trained whiskey enthusiasts who evaluated the spirit based on "holistic aroma and taste perception" and ii) GC olfactometry (GCO).

"In GCO, molecules within the spirit samples are separated via vapor point and polarity, then simultaneously identified via molecular fragment shape and abundance using MS," says Dustin Herb, co-author of the paper. "The molecules are then further characterized by another trained panel who assign an aroma and intensity."

After much separating, sniffing and tasting, the team found that barley variety certainly plays a key role in the flavor of single malt whiskey, but so does the terroir – when and where the grain is grown and how it is managed by individual farmers. Indeed, chemometric analysis of both datasets suggested that the environment and season had more of an effect on aromatic sensory perception than variety alone.



The take home? "Using a terroir model for whisky production creates a niche among local and regional distillers to capitalize on the individual 'terroir' of their local environments - soil types, microclimates, and crop management practices," says Herb. "Furthermore, by placing a value-added label on the barley, farmers are given a profitable rotational crop to help break prevailing monocultures while increasing weed suppression, disrupting pathogen and pest cycles, improving soil health, and overall benefiting subsequent crops."

The team plan to further their research by validating their findings under commercial production scale conditions and looking at other factors that could impact whiskey flavor.

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#### UPFRONT

# Scouting for **Antimicrobial Morsels**

Researchers develop new LC-MS method to track down traces of multiclass antibiotics in different foods

Even before the first antibiotic – penicillin – was used for therapeutic purposes, scientists were aware of the dangers of microbial resistance to these "wonder drugs." Their increased use in the agricultural industry has led to trace levels of these compounds in our food, which could increase the risk of pathogens' developing resistance to them.

Until now, it has been difficult for scientists to analyze a large number of antibiotics in a variety of foods, largely because of their vastly different chemical structures and properties. However, researchers recently published a new approach based on HPLC-MS that enabled them to detect 77 distinct antibiotics in cereals, meat, eggs, milk, vegetables, and fruits – a breakthrough that should help further research on this pressing topic.

REFERENCES AVAILIABLE ONLINE

# UPFRONT Weeding Out Whiskey Wannabes

How spectroscopy helps authenticate whiskey – without wasting a precious drop

According to the European Union's Intellectual Property Office, counterfeit drinks cost the EU €1.3 billion in lost revenue each year. Kishan Dholakia and his team from the University of St Andrews in Fife, Scotland, set out to find a way to catch the crooks in a nondestructive manner – saving the liquor for the whiskey lovers.

They used fluorescence and Raman spectroscopy to collect the unique spectral signatures of different whiskeys. Importantly, the new method allows the spectra of the golden liquid to be directly collected from within the glass bottle.

"We generated a unique beam that looks like a ring on the glass surface and then focuses to a concentric set of rings with a small spot inside the liquid," says Dholakia. The approach is more straightforward and accurate compared with similar techniques. And it can be applied to other alcoholic beverages (including gin, vodka, and wine) or olive oil – another commonly counterfeited commodity. In the future, the team hopes to use the same principle for collecting Raman spectra at depth in biological tissues.





#### IN MY VIEW

# Partnering Up for Food Harmony

We cannot underestimate the power of international collaboration, when it comes to ensuring food security

China is now Europe's largest source of imports, and the second most important export market overall (behind the USA). Bilateral European Union (EU)–China trade amounted to €520 billion in 2015, and this is expected to reach €700 billion by 2020. Part of this – of course – includes the trade of food items. Yet, consumer trust in the food industry across both the EU and China has been damaged by numerous accidental and deliberate food contamination and adulteration incidents, such as the 2008 Chinese melamine incident, and the 2013 EU horsemeat scandal. The ability of EU companies to export foods to and import foods from China has been hampered by these safety, traceability, regulatory, and fraud issues. And Chinese companies trying to export to Europe face similar obstacles.

Twenty-first century food supply chains are increasingly complex and highly vulnerable to safety and fraud threats. Increasing demand and growing markets then enhance the likelihood of food safety incidents and deliberate contamination, which in turn ruins consumer trust and undermines legitimate trade at domestic and international levels. Furthermore, laboratories in Europe and China are often working to different quality standards and using different analytical methods for data production towards certification and confirmation purposes, which can lead to protracted trade disputes and embargoes. An analytical partnership between the two bodies is needed to ensure consumer safety and fruitful trade.

The EU-China-Safe project aims to satisfy this need. The project is looking to develop a partnership between European and Chinese organizations involved in food control, with the aim of delivering a shared vision for food safety and authenticity based on "mutual recognition" in food standards and testing and certification (as has been achieved in other areas between these regions). There are 16 participants from the EU and 17 from China, including key research organizations from both government and industry, that will work together to combat issues with food safety and fraud known to exist between the two trading blocks.

The collaboration will be enabled through the development of an EU-China Joint Laboratory Network, operating through a state-of-the-art virtual laboratory (the virtual "Reference Laboratory 2020") with interchangeable staff from two continents, and using shared data systems to enable cooperative method development. The lab will be used as a "showcase" to communicate and demonstrate best practice



# "I believe that working in partnership is always preferable to working alone. Sometimes corporate or other barriers can get in the way, but breaking these down is well worth the effort."

through a "twinning model" that promises alignment between the two bodies. Innovative traceability tools will strengthen the most vulnerable supply chains, while improved detection of chemical and microbiological hazards and food fraud will be implemented through standard operating procedures, validation, quality control measures, and laboratory web conferences for best practice examples. Trade barriers caused by food safety and fraud issues will be analyzed, and recommendations on how to predict and prevent future events disseminated.

Where are we so far? We have started by collecting reference documents, such as a laboratory inventory, the regulations of both regions, and standard analytical methods. We identified that both regions had partners who were interested in validating GC-MS/MS methods for dioxins analysis (as a more economical alternative to sector instrumentation), and so we have been working jointly to validate one. Major instrument manufacturers are

(thankfully) lending their support, so we can work with confidence that identical technologies will be available to both regions for such exercises. The next stage will be to test the virtual laboratory where there is more variation in analytical approach.

I believe that working in partnership is always preferable to working alone. Sometimes corporate or other barriers can get in the way, but breaking these down is well worth the effort. Sometimes we have preconceived ideas or prejudices about what to expect when working with others, but my experience is to be prepared to have those illusions shattered! There are great partners out there, and there is much to be gained for all involved if you are prepared to put in that extra effort to form an effective and functioning partnership.

Martin Rose, Martin Rose, environmental contaminants and food integrity, Fera Science Ltd, York, UK.

#### ONLINE

# Citizen Science and Food Safety

Public food safety testing is in our sights – but we must prepare accordingly to ensure that faith in experts persists.

Michel Nielen is Principal Scientist of Wageningen Food Safety Research (WFSR) and professor of Analytical Chemistry at Wageningen University, the Netherlands. He is coordinator of the H2020 MSCA ITN project FoodSmartphone and Co-Chair of the Recent Advances in Food Analysis (RAFA) symposium series.

#### ONLINE

## Moore's Law and **Crystal Balls**

Can we predict our analytical capabilities 20 years from now? How we could deliver the complete food metabolome by 2041

Michael Rychlik, School of Life Sciences, Technical University of Munich, Germany







APPLICATION NOTE

# How to make wine production more sustainable?

Learn how to reduce water, energy, consumables and waste while maintaining cleaning efficiency and eliminating microbiological contamination

The business case for sustainable manufacturing has never been stronger, with reducing water and energy usage at the heart of the challenge. Winemakers must consider all parts of their production processes when looking to control costs and reduce their environmental footprint.

Filtration is used to clarify and stabilize wine before bottling. The filtration process must be controlled, repeatable and must not affect the body, aroma or taste of the wine. It can be a water and energy intensive process, with systems requiring cleaning between production batches.

Filtration with Kieselguhr filters based on diatomaceous earth (DE) is a widespread technique in the world of wine, particularly in the roughing and polishing phases of musts and wines. Not being an automated system, Kieselguhr filtration requires a qualified operator, and the exhausted DE must be disposed of in a responsible way, e.g. by composting.

Crossflow filtration was introduced to winemaking in the 1980s and is now the most frequently used technique for the preparation of wines in the cellar, before bottling. It uses a selective porous membrane to filter wine.

In recent years, more environmentally aware consumers and an increase in international competitiveness within winemaking have driven new technological advances in wine filtration, enabling further reductions in cost and waste.

This white paper compares Sartorius wine filtration systems with other widely used systems in terms of consumption of energy, water and consumables.

#### READ THE WHITEPAPER NOW



Technical solutions to control water consumption and waste









#### FEATURE

# Piracy in the Pantry

Food adulteration puts public health – and lives – at risk. We spoke to scientists from the US Food and Drug Administration's Center for Food Safety and Applied Nutrition to hear how they're keeping citizens safe.

For most people, fraud is a term more often associated with forged cheques or insurance scams than the contents of their kitchen cupboards. In fact, public awareness of food fraud is almost non-existent, despite infamous incidents across the globe. Our readers in Europe, for example, will remember the horse meat lasagne scandal of 2013...

But equine pasta is just the tip of the iceberg. Today's consumers are faced with hoax honey, counterfeit coffee and bogus booze – amongst countless further imposters, each of which carries economic costs and potential risks to health. To complicate matters further, fraudsters are becoming increasingly sophisticated – in many cases, adulterated or mislabeled foods are not identifiable by appearance or taste.

The task of detecting fraudulent foods has thus fallen to the analytical community. Laboratories across the world are playing the role of detective, piecing together molecular clues to identify the criminals tampering with our food chains. We caught up with the USA's ultimate defenders of public safety, the Food and Drug Administration (FDA), to explore how we can protect the consumers of tomorrow.



#### Death in the pot

The manipulation of food and beverages is an ancient practice, evidenced by the sweetening and preservation of wine with additives such as honey, herbs, seawater and lead in both ancient Rome and Greece. And, though it's difficult to pinpoint the exact origins of food adulteration, it is known that imported spices (very valuable at the time) were mixed with various seeds and berries – and even dust – throughout the Middle Ages. King John of England passed a law in 1202 outlawing the adulteration of bread with dried pulses, and the first US food safety law (the Massachusetts Act Against Selling Unwholesome Provisions) was passed in 1785.

It was in 1820 that the analytical chemistry community began to take aim at the issue, starting with the publication of "A Treatise on Adulterations of Food, and Culinary Poisons" by German chemist Friedrich Accum. Printed with a foreboding cover featuring spiders, snakes, and skulls, and inscribed with the words "There is death in the pot" (2 Kings 4:40), the book tackled issues from coffee bulked out with dried peas to the adulteration of beer with opium.

In the preface, Accum states, "To such perfection of ingenuity has the system of counterfeiting and adulterating various commodities of life arrived in this country, that spurious articles are every where to be found in the market, made up so skilfully, as to elude the discrimination of the most experienced judges." Though Accum's habit of "naming and shaming" individual suppliers meant the book was met with a degree of controversy, his warnings about the dangers of food fraud are still very relevant today.

"Food fraud or economic adulteration is a difficult issue to quantify," says John Callahan (co-chair of the FDA's Foods Program Economic Adulteration Working Group). "In 2010, the Grocery Manufacturers Association estimated that the yearly cost of food fraud in the US is between \$10 and \$15 billion" (2). More recent estimates have been as high as \$50 billion globally. Yet, the economic costs of the issue pale in comparison to the human costs.

#### A moving target

In a famous case from the late 2000s, attempts to fraudulently increase the apparent protein content of infant formula by adding nitrogenboosting melamine and cyanuric acid led to kidney disease - and death - among infants across China (3).

The tragic melamine incident highlights some of the challenges faced by analytical chemists trying to stamp out food fraud. "It is a constantly evolving situation," explains Shaun MacMahon (the Office of Regulatory Science). "As techniques are developed to address specific fraud issues, people intent on adulteration often shift their approaches to avoid detection. The melamine situation, for example, arose because total nitrogen analysis by classical techniques was too blunt to detect nitrogen boosting. More specific techniques, in this case GC- or LC-MS, were needed. But these targeted approaches could not detect other nitrogen-boosting methods."

How did the FDA combat this? "First, we developed MS methods with a wider range of targets," Shaun says (4). "More recently, we have witnessed a shift towards non-targeted methods comprising GC or LC linked to high-resolution MS (HRMS). Such approaches streamline the identification of new adulterants."

It's a similar story for another common target of adulteration: honey. Honey tends to be comprised of nectar from C3 plants (plants that produce the three-carbon compound 3-phosphoglycerate through photosynthesis), and is characterized by a specific range of C13:C12 ratios. Historic honey adulteration used corn syrup as an additive, but corn utilizes C4 photosynthesis, not C3, and thus produces a different range of C13:C12 ratios. Stable carbon isotope ratio analysis (SCIRA) can differentiate these plant products with relative ease.

#### ONLINE

# The FDA versus food fraud

Most readers will be familiar with the FDA, the agency of the United States Department of Health and Human Services concerned with the protection of public health by ensuring the safety of drugs, foods and other biological products.



#### ONLINE

# Classic Cases from the Food Files

Stefan Tordenmalm, Market Manager of Processed Food at PerkinElmer, shares some of the most common types of food fraud reported by customers in food safety labs, and how analytical chemistry is helping to unmask even the most sophisticated scammers.



To evade detection, fraudsters now supplement honey with syrups from other C3 plants, such as rice. "It is much more difficult to apply SCIRA to detect this type of adulteration," says John Mangrum (the Office of Regulatory Science). "This has prompted the evaluation of techniques such as NMR and LC-HRMS to assess the true nature of honey samples."

#### Tools of the trade

GC and LC (in particular, high-performance LC) play central roles in the detection of fraudulent foods. LC is particularly useful for the identification of naturally-occurring chemical markers that characterize botanical materials and juices. And, as outlined above, the combination of LC or GC with one- or two-dimensional MS detection affords a powerful method for both targeted and untargeted analysis. In particular, advances in HRMS in the form of Orbitrap and time-of-flight instruments and electrospray ionization are making untargeted approaches an increasingly valuable weapon in the fight against fraud.

"Untargeted analyses do not require a preidentified target. Instead, HRMS enables researchers to use accurate-mass measurements to narrow down the range of potential answers to a small number of putative chemical entities," explains Ann Knolhoff (the Office of Regulatory Science). "This is having a significant impact on the development of screening approaches that could identify chemical adulterants before they become widespread." Such screening will prove key for products such as dietary supplements, which may be adulterated with pharmaceutical drugs to amplify the apparent effects of the product. With a huge variety of prescription or over-thecounter drugs used by fraudsters, untargeted analyses could save lives.

Spectroscopic approaches also play a key role – namely NMR, UV, infrared and near-infrared. The combination of these approaches with chemometric techniques for data analysis enhances their utility. Of note, the (relatively recent) advent of cavity-ringdown spectroscopy has also reduced the cost of SCIRA, improving its accessibility for labs around the world. And, on the topic of isotopic techniques, specific isotope natural fractional NMR is making waves in the detection of suspect wines and spirits.

When asked about game-changing techniques in this space, Jonathan Deeds (the Office of Regulatory Science) highlighted the significant impact of DNA analysis. "Detecting fish species substitution (for example, selling farm-raised Asian catfish as wild-caught grouper) was heavily reliant on outdated protein electrophoresis, but we can now apply DNA-based techniques such as DNA barcoding for species identification," Jonathan explains. "The FDA has thus adopted this approach for identifying species substitution. We have applied this technique in investigations of octopus substitution with lower-priced and more easily obtained jumbo squid, and also the substitution of domestic blue crab with meat from lower value swimming crabs."

The approach is also applicable to plant-based samples. "With plants, we witnessed an initial focus on chloroplast sequencing and identification within these sequences, but movement towards genome skimming (a next-generation sequencing method that targets portions of the entire genome) is helping the community identify plant substitution much more efficiently," says Sara Handy (the Office of Regulatory Science).

## A brighter future?

Looking forward, the FDA is using every weapon at its disposal in the fight on food fraud – including recalls, seizures, injunctions and import refusals when warranted. Import alerts represent a valuable tool on this front and firms who trigger an import alert (and their products) are now subject to Detention Without Physical Examination.

Analytical advances are another key pillar of the FDA's strategy. As John says: "Food is a complex matrix, and there is seldom one method

or technique that can single-handedly characterize every aspect of a sample." The solution? "Methods that integrate multiple approaches to provide full fingerprints of food will likely represent a major focus." This will likely constitute combining broad screening approaches like NMR with untargeted HRMS or inductively coupled plasma-MS to give total profiles of food products. Advances in targeted screening, DNA analysis, chromatographic separations, and MS and NMR libraries will also be crucial.

"The development of portable devices to move methods out of labs and towards processing plants and docks would also make a huge difference, as would advances in automated sample preparation technologies," says John. Robotics approaches could facilitate greatly improved sample throughput, and portable devices could even allow consumers to assess the safety of food products in shops and markets. One thing is for sure: scientists working in this field will be kept busy.

Indeed, analytical chemists across the globe are hard at work to put consumers' minds at rest. Between new international alliances like the EU-China-Safe project and ever-growing collaborative databases to track confirmed cases of fraud, our defensive arsenal grows each day. And, with the European Commission Knowledge Centre for Food Fraud and Quality outlining such databases as critical for future success (alongside early warning systems and the creation of centers of competence), it would seem we are taking confident strides towards improved food safety.

But there is no place for complacency, with experts predicting a rise in food fraud as supply chains are disrupted by COVID-19 (5). When it comes to stamping out food fraud entirely, the words of Friedrich Accum seem as relevant today as when they were written in 1820: "The eager and insatiable thirst for gain, is proof against prohibitions and penalties; and the possible sacrifice of a fellow-creature's life, is a secondary consideration among unprincipled dealers."

REFERENCES AVAILIABLE ONLINE





#### APPLICATION NOTE

# Automate Standard **Preparations for Food** Analyses – A Real World Evaluation

Andrew+ and the cloud-native OneLab Software platform have been evaluated in our food analysis lab for routine liquid handling methods involving mixing and serial dilutions for sample preparation and standards.

Authors: Kai Liu, Ben Pointer, Jinchuan Yang, Nigel Skinner, Scott Toerber, Dennis Karote

The evaluation was focused on the automation of the simple operation in sample preparation, the serial dilution and mixing for the standard and sample solution preparation. The scope of the evaluation was designed to test with a wide range of assays for different analytes, involving different techniques and solvents. The analytical techniques that were involved included chromatography-based techniques, such as ion chromatography-conductivity detection (IC-CD), liquid chromatographyfluorescence detection (LC-FLR), liquid chromatography-ultraviolet/visible spectrometry (LC-UV/Vis), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and non-chromatography

	Analyte	Technique	Diluent	Operations	Total dilution ratio	Accuracy (dilution ratio)	
						Andrew+	Manu
1	Sodium	IC-CD	Water	Serial dilution: 5mL standard solutions mix with 5ml water and mixing	1:64	-0.5% to 3.0%	N/A
2	Retinol	LC-FLR	Hexane	Serial dilution of 1- 10mL standard solu- tions with hexane and mixing	1:250	-2.8% to 2.9%	-0.5% to
3	Vitamin D3	LC-UV/Vis	Methanol	Serial dilution of 0.4- 10mL various standard with diluent and mixing	1:250	0.2% to 1.7%	-0.3% to
4	Galactose	Electrochemical detec- tion	Water/Methanol	Serial dilution of 1- 3mL standard solu- tions with diluent mixing	1:500	-0.63£ to 0.65%	N/A

	Analyte	Technique	Diluent	Operations	Total dilution ratio	Number of samples	Difference % (compared results)
1	Folic acid	Microbiological turbidity	Water	Serial dilution of 0.4- 10mL standard solutions with diluent and mixing	1:125	5	-3.8% to 5.7%
2	Cysteine and methionine	LC-FLR	Water	Single dilution from 10 $\mu$ L to 500 $\mu$ L and mixing	1:50	3	Cysteine: -0.5% to 2 Methionine: -0.7% to
3	Folic acid	LC-UV/ Vis	Water	Single dilution from 100 $\mu$ L to 500 $\mu$ L and mixing	1:5	4	Within -1.2% to 2.5% fo no acids in four sam
4	Folic acid	LC-MS/MS	ACN/Water	Single dilution from 150 $\mu$ L to 1500 $\mu$ L and mixing	1:10	4	Within -1.1% to 0.4% samples and 6.7% in on

Table 2. Relative difference in food analysis results between robot vs. human operations in standard sample-prep

based techniques. The solvents included those common to food analytical labs including hexane which is volatile and difficult to pipette. The evaluation was carried out in two main steps: Evaluating the performance of the Andrew+ Robot in standard solution preparation and then evaluating of the robot in real-world food analyses. Key findings: Andrew+ Robot performance is consistent with rigorous requirements in accuracy

and precision in sample preparation. The accuracy of automated robot operation ranged from -2.8% to 3.0%, as compared to -5.0% to 4.2% by a human operator, enabling technical staff to be freed for higher level tasks, whilst ensuring full traceability and reducing risk of repetitive strain injury and error.

Table 1. Accuracy of automated serial dilution and mixing of standard solutions in different assays

Analyte	Technique	Operations*	Precision
	IC- CD IC- C	10 $\mu$ L sample mixed with 490 $\mu$ L water	2.0%
$C_{1}$		250 $\mu$ L sample mixed with 250 $\mu$ L water	0.3%
Choline		0.7%	
		2500 $\mu$ L sample mixed with 2500 $\mu$ L water	0.6%

Table 3. Precision of automated dilution and mixing of sample solutions in water (n=8)

## **Benefits**

- Accuracy and precision of the Andrew+ Robot for solution preparation is equal or better than manual preparation
- Analysts were liberated from repetitive time-consuming operations, which leads to increased productivity and quality of analytical work
- Andrew+ and OneLab Software is easy to set up









#### SOLUTIONS

# The Dark Metabolome in Your Glass

Unmasking the metabolome of beer to protect the quality of pints and empower archeological sample analysis

Beer is one of the oldest known beverages – and it remains a firm favorite today. In fact, beer development – and the processes involved – have paved the way for many facets of modern science. An example: fermentation for brewing led to the discovery of microorganismal metabolic regulation through the cultivation of single yeast cells.

Nowadays, food, its safety, and nutritional value are crucial factors regarding our health and well-being. And that's why we study so-called "dark metabolites." These are components of food and beverages that are as-of-yet unidentified. In the case of beer, these metabolites persist in spite of the empirical knowledge accumulated through brewing research over the years.

Our mission is to describe the richness of beer compositions – including these hidden metabolites. This knowledge could also enhance our understanding of archeological beer samples and the people who drank them. We'll toast to that!

#### To the metabolome - and beyond!

Metabolite profiling of food provides extensive and valuable data

regarding food safety and quality. Advanced analytical strategies can decipher complex biological and chemical systems and fathom their interaction with each other and our bodies. But, in addition to already well known molecules, a flood of uncharacterized compounds from food and beverages (the dark metabolome!) influence our metabolism and our health. Our work simply stimulates an awareness of that which remains unknown.

Beer just happened to be the perfect matrix for applying our approach. This is in part because of its rich molecular diversity; there is a wide range of raw materials present in beer, as well as additional thousands of compounds produced through processes such as malting, boiling, and fermentation.

We set out to characterize this highly complex system on a compositional level by extracting metabolic profiles, which in turn drive certain attributes of the drink the world loves so much. The aim: to produce a fundamental base of knowledge about the beer metabolome and its origin beyond common databases. Using such a database, old beer and beer-like beverages (archeochemistry) as well as modern industrialized beer (quality control and inspection) can be put into context.



#### A visualized approach

We needed a holistic approach to decipher the diversity, plurality, and complexity of beer samples. But extraction methods and chromatographic pretreatment can limit what can be made analytically visible in terms of polarity and physicochemical properties. Another approach was needed...

A flow-injection analysis (FIA) approach used in clinical metabolomics and for further food samples was our weapon of choice. By diluting beer and then directly and continuously injecting it into the MS-system, we can analyze beer with minimal changes to its chemical composition. With FIA, characterizing much of beer's molecular diversity becomes a tangible task; however, it's not all "sunshine and roses." Such approaches require the highest possible mass resolution to avoid overlapping signals and to differentiate all possible elemental compositions.

The most advanced mass spectrometers in terms of mass resolution and mass accuracy are high magnetic field Fourier transform ion cyclotron resonance (FTICR) instruments. Thus, FIA-FTICR-MS approaches have the power to resolve not hundreds, but tens of thousands of features that might otherwise remain hidden in a very short window of time. The magic happens with a measuring time of 10 minutes and as little as half a drop of beer.

Because of its unmatched mass accuracy, which amounts to 0.1 ppm, it was possible to assign a sum formula and concrete elemental compositions to each mass signal. Or, in layman's terms, the MS method can assign a compositional name to previously uncharacterized molecules!

But the approach isn't without its drawbacks... FIA-FTICR-MS lacks information about isomers and concrete molecular structures, which requires a second analytical technique. Another weapon was needed! After raiding our analytical armory for a second time, we decided to

characterize the most important molecules on a structural level using UPLC-ToF-MS – and some trusty van Krevelen diagrams!

### A pint of van Krevelen, please!

The van Krevelen diagram makes sense of the compositional information that a molecular composition provides. By plotting the ratio of hydrogen to carbon atoms of a molecular formula against the O/C ratio, we can identify regions in the diagram that reflect the compositional nature of respective molecules and associated biochemical origins.

The tentative classification of metabolite compositions of beer into substance classes lies in their biosynthetic pathway. In gluconeogenesis (glucose production by metabolic processes), the addition of water to the pyruvate gives the carbohydrates very saturated and oxygen-rich compositions, which are located in the upper right region of the diagram.

In contrast, the basic building block of fatty acid synthesis, acetyl-CoA, is obtained via an oxidative decarboxylation of the pyruvate. Another dehydration step during chain expansion leads to less oxygenated lipid species. These can be found on the top left of the van Krevelen. Polyphenols are significantly more unsaturated and have lower H/C ratios.

A pint of beer can be mapped according to the corresponding sugar phosphate, nucleotide, and phospholipid spheres. Due to the divergent biosynthetic pathways of the amino acids and the associated different residues, a peptide region is difficult to narrow down. Small organic acids usually have a very high O/C ratio that can exceed the value of one.

Due to their special biosynthesis, the hop-specific "bitter acid" compounds in beer have both the phenolic base structure and the compositional characteristics of terpenes, which the prenyl side chains

A van Krevelen diagram, which shows the distribution of 10,000 compounds from the beer metabolome by their H/C and O/C ratios. The diagram maps the compositional space of beer and enables specific patterns to be recognized.



are based on. Accordingly, these "terpeno-phenolics" show a very characteristic positioning in the van Krevelen. Hence, it is possible to visualize the entire holistic variety and complexity of the beer metabolome in one diagram! But it is necessary and extremely important to say that these classifications are by no means fixed boundaries; they merely represent well-founded reference points!

#### REFERENCES AVAILIABLE ONLINE READ THE FULL ARTICLE ONLINE

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#### SITTING DOWN WITH

# **Contaminant Characterizer**

Sitting Down With... Stefan van Leeuwen, Senior Scientist, Wageningen Food Safety Research, University of Wageningen, The Netherlands

### What is the overarching theme of your work?

Environmental contaminant analysis is really the core of my work - particularly substances that persist in the environment for long periods. These so-called persistent organic pollutants (POPs) eventually enter our food chain, so they are of special concern for human health. I started my career studying polychlorinated biphenyls (PCBs) and brominated flame retardants with Jacob de Boer's group at Wageningen Marine Research, but, by the early 2000s, our international coworkers had pointed us towards a new pollutant of interest - perfluorooctane sulfonate. Soon many more per- and polyfluoralkyl substances (PFASs) were detected, and their study became a field in its own right. After 5 years at the VU University in Amsterdam, where I obtained my PhD developing analytical methods for detecting the occurrence of POPs in fish for human consumption, I started in my current position at Wageningen Food Safety Research, where research on POPs in food has continued.

#### Have there been any landmark moments during your time in the field?

In 2006, we published the results of the first international proficiency test for PFASs in environmental and human samples. The data was very scattered and not comparable between labs; it was obvious that these analytes required different analytical approaches than the field was used to. In the following years, enormous effort was made to improve method development – commercial standard providers have devoted considerable time and energy to developing high-quality

standards and mass-labelled analogues. This has resulted in a huge increase in the quality of reported results, which will in turn improve our understanding of the effects associated with PFASs in the years to come. Regarding chlorinated paraffins, a new high-resolution MS (HRMS) and statistical approach from the Bogdal lab has recently provided researchers with a more powerful tool to probe these industrial contaminants in food.

## What are the "tools of the trade"?

We use several techniques including LC-MS/MS and GC-HRMS to conduct targeted analysis of a number of environmental contaminants, including dioxins, PCBs, and PFASs. Chlorinated paraffins in food present a particularly challenging phenomenon, requiring HRMS (Orbitrap) coupled with LC to ensure the resolution necessary for complete analysis.

### What technological developments does the field need?

The OECD has published a list of over 5,000 PFASs; of these, we routinely analyze about 20 using a targeted LC-MS/MS approach. Although not all 5,000 PFASs may be relevant for food or environmental contamination, it's clear that a more holistic approach is needed. The solution lies in complementary approaches: in vitro effect assays, oxidizable PFAS detection, total organic fluorine detection, and untargeted identification. Several groups in our institute are joining forces to develop and combine these approaches, and so far, the results have been promising.

Elsewhere, the accumulation of contaminants in the "circular economy" is gaining considerable attention. When materials are recycled, undesirable substances like brominated flame retardants can be unintentionally introduced into products. We need to better understand how recycling processes lead to contamination and where these substances accumulate, which will – of course – require new analytical approaches.





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