the Analytical Scientist

SPECIAL SERIES:

Food and Beverage Analysis

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UPFRONT

Fare Share

We explore the stats behind food fraud across the globe

WHAT IS FOOD FRAUD?

There are a number of definitions out there, but the European Commission defines food fraud as "any suspected intentional action by businesses or individuals for the purpose of deceiving purchasers and gaining undue advantage." It encompasses adulteration, tampering, product overrun, theft, diversion, simulation and counterfeiting.

MOST COMMONLY ADULTERATED FOODS

Fish/ seafood
Dairy
Meat
Alcoholic beverages
Oils/fats

MOST NOTORIOUS FOOD FRAUD CASES

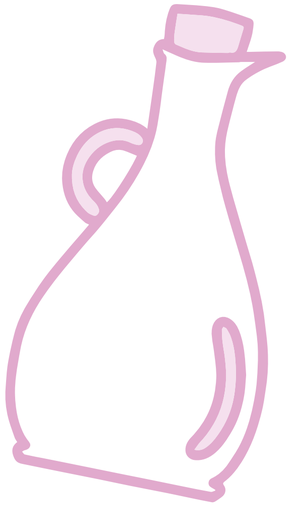
1981 Olive oil
Industrial rapeseed oil was contaminated with the toxic compound, aniline, and sold as "olive oil" to unwitting street vendors across Spain. This led to more than 1000 deaths.

2007 Pet food
Melamine would make a bigger splash in a year's time, but it first hit the papers after thousands of pets in the US died because they'd been given food tainted with the compound.

2008 Melamine milk
A number of Chinese companies were found to be watering down their milk and adding melamine to bypass protein tests. This led to over 50,000 children being hospitalized.

2009 Peanuts
Seven people died and hundreds fell ill when the Peanut Corporation of America knowingly shipped salmonella-contaminated peanut butter across the US.

2013 Horsemeat
Mostly affecting the UK and Ireland, the "horsemeat scandal" arose when equine DNA was found in frozen beefburgers sold in several supermarkets.



UPFRONT

Spotting the Bad Apples

To all honorable athletes out there, be careful where you source your supplements – they could be laced with anabolic steroids! But fear not, because Xia Xu and her team at Zhengzhou University have developed a 2–5 fold more sensitive detection method, which she discusses here.

Why are people adding anabolic steroids to foods!?

Anabolic-androgenic steroids (AAS) are a class of chemical synthesis derivatives, similar to testosterone in structure and activity, and can enhance physical conditioning, including body mass and muscle strength. Banned for the first time at the 1976 Montreal Olympics, they're often abused by athletes to increase muscle quality and improve their overall performance.

Some health foods claim to have strength-enhancing properties, which are attractive to bodybuilders and athletes, but the actual effects are usually not significant. But this interest can tempt some unscrupulous manufacturers to add AAS to otherwise healthy foods to enhance their pharmacological effects – and thus increase sales. However, AAS can cause a range of adverse effects, including brain and cognitive abnormalities – not to mention bans from sporting competitions. To ensure food safety and maintain market order, we set out to develop a quantitative analytical method for detecting AAS in health foods.

What sorts of foods are being adulterated with anabolic steroids?

Foods that claim to have strength-enhancing properties may be adulterated with anabolic steroids – so dietary sports supplements are certainly on the list. In China, AAS may also be illegally added to some traditional Chinese medicines. In addition, because AAS can reduce the fat ratio and improve the feed conversion rate, AAS may also be added to animal feed for the purpose of improving the economic benefits of animal breeding.

What methods are usually used for this kind of analysis?

Numerous detection methods for AAS analysis have been developed, including high-performance liquid chromatography with ultraviolet (HPLC-UV), gas chromatography-mass spectrometry (GC-MS), LC-mass spectrometry (LC-MS) and enzyme-linked immunoassay (ELISA). The methods that combine chromatographic separation and mass spectrometry (LC-MS and GC-MS) provide sensitivity and specificity, and for that reason have become the most widely used methods.

What were your most important findings?

We developed a novel stable isotope labeling-flow injection analysis-tandem mass spectrometry (SIL-FIA-MS) based strategy for detecting AAS in foods, which used 3-nitrophenylhydrazine (3-NPH) to label the AAS prior to mass spectrometry analysis. The 3-NPH labeled AAS showed dual-polarity property, observing chloride adduct ion ($[M+Cl]^-$) in negative ion mode and proton adduct ion ($[M+H]^+$) in positive ion mode. This simultaneous monitoring $[M+H]^+$and $[M+Cl]^-$ guaranteed 2–5 fold improvements in detection sensitivity.

Could your research have any implications for anti-doping analysis in sports? Or for athletes?

Our validated method provides very specific and high throughput screening of AAS illegally added to healthy foods, which means anti-doping tests could also be done more quickly. Thus, cheating athletes could be caught on the day of the race – or even before. This would be a huge deterrent to athletes taking AAS.

What are your plans for future research in this area?

In the future, we will explore ways to automate the specific and high-throughput methods we've established here, as well as develop screening methods for other illegal adulterants.

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APPLICATION NOTE

Multivariate Analysis of Tomato Varieties Using Smart Metabolites Database

The sample classification filtering function improves the efficiency of data analysis by only analyzing the data for components with a high probability of detection

Metabolomics refers to technology used for the comprehensive analysis of all metabolites in organisms. In medical fields, metabolomics is used as an effective way to search for biomarkers that indicate the physiological changes in diseases. In recent years, metabolomics has also been used in a wide variety of food-related applications, such as analyzing differences in the percentage of ingredients, searching for components with functional benefits, and predicting degradation over time. GC-MS/MS is used in metabolomic analysis to target hydrophilic metabolites such as amino acids, fatty acids, and organic acids because it can analyze such components simultaneously. Detecting trace quantities of these components is best performed using SIM or MRM, which require optimization of the MS parameter settings. Smart Metabolites Database has been compiled specifically for the analysis of metabolic components using GC-MS/MS. This article describes the use of Smart Metabolites Database with multivariate analysis to identify differences in the components in different tomato varieties and to compare the concentrations of sugars across samples.

Smart Metabolites Database Ver. 2

Smart Metabolites Database Ver. 2 includes the analytical condition settings, optimized SIM and MRM transition settings for metabolic components. The number of registered components in the simultaneous analysis method has been increased to 627 components for SIM and 540 components for MRM. In addition, compound-specific quantitative analysis methods for analyzing sugars have been added to those for amino acids and fatty acid methyl esters (FAMEs). The database also includes a new sample classification filtering function. By selecting a filter suitable for the sample being measured, measurements can be limited to metabolic components predicted to be in the sample. This can significantly shorten the time required for data processing. Furthermore, LabSolutions Insight, a software for multianalyte quantitative analysis can be used to easily create lists for multianalyte or multivariate data analysis. These lists can then be output and loaded into the Multi-Omics Analysis Package for principal component analysis (PCA) or hierarchical cluster analysis (HCA).

Multivariate Analysis Results

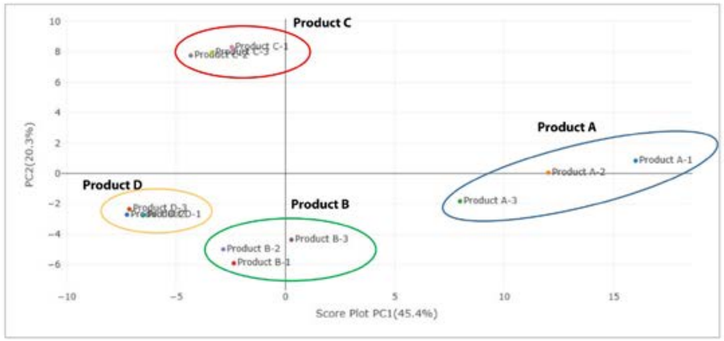
The workflow and the results obtained using the Multi-omics Analysis Package for principal component analysis (PCA) and hierarchical cluster analysis (HCA) of the data obtained from the four tomato varieties (n = 3) are shown in Figure. 1. The samples are clearly differentiated in the PCA score plot and the HCA tree diagram. In addition, the heat map from hierarchical cluster analysis shows that Product A tends to contain more components than the other three tomato varieties, including particularly high amino acid levels. The results also show that Product C contains more sugars including inositol, glucose, and fructose.

READ MORE ONLINE

Creating Method Files (Smart Metabolites Database Ver. 2)

Process data from multiple samples (LabSolutions Insight)

Process by multivariate analysis (Multi-Omics Analysis Package)



IN MY VIEW

Beverage Analysis: Just Press Go?

To fast-track beverage analysis, laboratories should explore technology that specifically addresses the pain points of routine testing

Behind every refreshing glass of craft beer, cider, fruit juice, or red wine, there is a team of skilled operators using complex beverage analysis workflows. To ensure customer satisfaction (and subsequent business profitability) there is a critical and constant need for high-quality and desired taste – at scale. Given its crucial role in guiding quality and taste, it's fair to say that beverage analysis lies at the heart of successful beer and wine production.

In fact, accurate and reproducible measurements are also needed to guide production and product release as well as to ensure that alcoholic beverages are safe for consumption. But beverage analysis also faces a number of challenges that make it difficult for laboratories to work efficiently – not least the need to analyze multiple parameters at different time points under a heavy workload.

Many characteristics are routinely monitored; in wine, for example, free and bound sulfur dioxide (a regulated allergen) must be measured alongside pH, volatile acidity, total acidity, residual sugars, residual malic acid, and other wine spoiler indicators that can affect flavor and product stability.

And analysis is not a "one and done" affair. In beer production, for example, changes that affect the later characteristics can occur at any time – some of which alter shelf life. To detect such changes, beer samples must be tested at all process stages – from feed water, malting and fermentation through to the addition of flavoring agents and bottling.

Obtaining data across numerous parameters over time requires the use of different wet chemistry methods, each of which must be calibrated and conducted by skilled operators. But, given the volume of testing, it can be difficult for labs to find staff who are sufficiently proficient at titration, HPLC, spectrophotometry, and all of the other methods required for beverage analysis. And staff shortages aren't the only issue these labs face… There are time pressures, too. The use of numerous multi-step analytical approaches means the overall process is not as fast or as automated as it could be. Indeed, traditionally-used techniques are often tedious, time-consuming, and prone to manual errors. Together with the need for enzymatic reagents and standards preparation, analysts often face workflows that are difficult to execute.

In my view, routine workflows should facilitate walkaway efficiency – you should be able to simply put the samples in, add reagents, and press go…with discrete analyzers (DAs), you can. DAs make use of unique, discrete, and disposable cuvettes to gauge multiple characteristics from a single sample, drastically reducing analysis time. Tedious and time-consuming processes are replaced by automated calibration and sample dilution, thereby reducing the risk of manual error. And each sample is analyzed in a fresh cuvette, minimizing the risk of contamination and giving laboratory staff peace of mind.

But, as with any analytical technique, there are a few drawbacks to DAs that would be remiss of me to avoid. For example, the precision and accuracy of results are highly dependent on the type of photometric setup, direct read or fusion, type of cuvette (single time disposable or reusable), and rinse cycle between measurements. To ensure accurate analysis time after time, operators must therefore be trained to complete this all-important step. This could be more challenging for microbreweries and small wineries, which can face more resource-centric challenges than their larger counterparts. What's more, some DAs are optimized for use with proprietary reagents, which can limit the possible applications using inhouse or third-party reagents, making careful instrument selection key.

For beer and wine producers, having confidence in analytical results is critical to delivering delicious, high-quality beverages for consumers to enjoy. Behind the scenes, laboratories continue to navigate an array of challenges in their quest to ensure integrity of results and support businesses in these industries. DAs offer a way to address some of the difficulties associated with current workflows, while improving reproducibility (1, 2) and reducing time invested. By implementing easy-to-use DAs and ready-to-use reagents, laboratories involved in beverage analysis can look toward a simplified, more efficient future!

Hari Narayanan is the Product Marketing Manager, Thermo Fisher Scientific

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IN MY VIEW

Green Food Analysis: The Missing Link?

Green analytical chemistry principles could play an important role in helping us hit key sustainability goals – so what are we waiting for?

I'd wager that most people have thought about the 2030 Agenda for Sustainable Development adopted by all United Nations Member States in 2015, as well as its 17 Sustainable Development Goals (SDGs) and how they can impact our world and humanity's future. Six years on, UN reports agree that we are not on track to achieve these SDGs by 2030. We can all make individual contributions, but strong and cooperative commitment at all levels is needed, including the upper echelons of science (1). The question I'd like to ask is: how can we push toward the SDGs in our everyday scientific work?

Our group at the Foodomics Laboratory has made attempts to implement the "Green Foodomics" framework in our research for some time (2). A lot has changed since we decided to integrate Green Analytical Chemistry (GAC) principles in our laboratory. We have modified our analytical methods to fulfill the GAC requirements, but preserved key qualities for method success, such as accuracy, sensitivity, reproducibility, simplicity, cost, efficiency, flexibility, and speed. In this way, we achieve results of consistent merit, but with much reduced environmental cost.

In sample preparation, we have implemented new solvents and integrated extraction processes that are more benign in terms of energy and solvent consumption. We have also searched for solutions across omics platforms to help us determine food constituents and nutrients at molecular levels while protecting the planet. Green chemistry and GAC principles not only promote food security, but also help us strive for many of the individual SDGs, such as SDG 2 (Zero Hunger), SDG 3 (Good Health and Wellbeing), SDG 6 (Clean Water and Sanitation), SDG 7 (Affordable and Clean Energy), SDG 8 (Decent Work and Economic Growth), SDG 9 (Industry, Innovation and Infrastructure), SDG 11 (Sustainable Cities and Communities), SDG 12 (Responsible Consumption and Production), SDG 13 (Climate Action), SDG 14 (Life Below Water), and SDG 15 (Life on Land) (3).

Without any doubt, food analysis can drive renewed approaches in agricultural development, food processing, food security, nutrition, and health, thus promoting the sustainable development of nations. Moreover, GAC strategies promote not only safer, cheaper, and more sustainable analytical methods for food analysis, but also more affordable analytical procedures that can benefit society. Surely we would all benefit from these strategies in our day-to-day research!

Awareness of green chemistry and GAC principles in food analysis has increased exponentially in the last 10 years, with more than 80 percent of the publications including the terms "green chem*" and "food analys*" since 2010. I hope this awareness expands as we move forward. As Paul Anastas mentioned in his inspiring 1999 publication (4): "With knowledge comes the burden of responsibility. Chemists do not have the luxury of ignorance and cannot turn a blind eye to the effects of the science that is created. Because there is the ability to develop new chemistries that are more benign, chemists are obligated to do so."

Therefore, researchers have a responsibility to be aware of the valuable links between green chemistry and success in food analysis. My take-home message: we know how we must proceed and we know what changes we must make. It is not only our duty, but also an invaluable opportunity to give a future to the generations that will come after us.

The Sample Preparation Study Group and Network belongs to the Division of Analytical Chemistry of EuChemS (DAC-EuChemS) and includes three working groups: Science and Fundamentals; Automation, Innovation and Entrepreneurship; and Information Exchange and Networking.

The Sample Preparation Network welcomes new European and non-European regular members. Membership is open to individuals who subscribe to the objectives of the network and who are professionally engaged in or associated with sample preparation.

For more information please visit: https://www.sampleprep.tuc.gr/en/home

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IN MY VIEW

Tackling the Other Pandemic: Food Fraud

The COVID-19 pandemic has been the focus of scientists and politicians for the past 18 months, but not enough is being done to combat another widespread issue with serious health implications: food fraud

It is not scaremongering to suggest that the problem of food fraud has reached pandemic proportions. For those unfamiliar with the topic, food fraud can be defined simply as the intentional adulteration of food (or food ingredients) for economic profit (1). According to PWC's Food Fraud Vulnerability Assessment (2), it is a threat that costs the global food industry between $30 - $40 billion per year. Not only does food fraud have a significant economic impact, but, more concerningly, it has serious health implications for those who ingest adulterated products.

There's no doubt food fraud is a global issue, but my personal focus is tackling the issue in West Africa and bringing it to the forefront of policymakers' agendas. I'm on a mission to foster international collaboration between food academics, chemists, engineers, and scientists to tackle the problem head on.

Why West Africa specifically? Firstly, Ghana is my home country and where I have established my career and expertise – I lecture at the University of Cape Coast's School of Agriculture in Ghana, and I lead the Africa Centre for Food Fraud and Safety. Secondly, the combination of various factors makes the problem of food fraud in West Africa particularly prevalent. Africa imports $35 billion in food annually (3), so communities can be flooded with fraudulent foodstuffs from abroad. The complexity of our global supply chain, inconsistent regulations, lack of robust testing frameworks, and a lack of support from policymakers all serve to amplify the problem. Consequently, the need for innovative and accessible solutions to test food authenticity is vital.

Recent examples of food fraud reported in local and regional media include palm oil laced with banned food colorant Sudan IV, and meat and fish treated with an embalming agent (formalin) to keep it unnaturally fresh. Despite growing reports, awareness of such fraud within the community and its negative effects on human health remains limited. And that leaves the door wide open for dishonest trade, ultimately limiting the right of African consumers to clean, healthy food – instead, exposing them to the risk of illness or even death. Since the COVID-19 pandemic, there has been a further spike in food fraud due to panic buying, price inflation, and food shortages. Climate change is also compounding the problem: over 100 million people in Africa are at risk (4). In short, the need to eradicate food fraud grows greater with time.

To provide solutions to the issue of food fraud, I have been fortunate enough to collaborate with researchers from the Institute of Global Food Security, Queen's University Belfast (IGFS-QUB) on Agilent-funded research led by Professor Chris Elliott.

We developed a two-tier approach for screening food, specifically rice in this project. Handheld near-infrared spectrometers can scan large numbers of samples in the field, with suspected fraudulent foodstuffs being sent for confirmatory analysis in the laboratory using LC-MS, GC-MS, and ICP-MS. My goal is to use these tried and tested methodologies across West Africa to mitigate food fraud. We're already looking at the use of portable sensor devices for rapid onsite and non-destructive screening of palm oil, with 15.7 percent of palm oil imported worldwide being delivered to Africa (5).

The opportunity to share our expertise across borders has helped us develop practical, effective long-term solutions to combat food fraud in Africa and across the globe. Together we have hosted workshops that showcase our findings, and in turn revealed how uninformed stakeholders in Africa were about the problem of food fraud. And that led us to establish the Africa Centre for Food Fraud and Safety.

The collaboration between the Africa Centre for Food Fraud and Safety and IGFS-QUB has created an important platform. We have been able to generate awareness and attract talent to combat food fraud in West Africa and beyond. African communities should not be a dumping ground for harmful and fraudulent foodstuffs. Our mission is simple but essential: To make Africa's food safe.

Ernest Teye is the Senior Lecturer, School of Agriculture, University of Cape Coast, Ghana

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APPLICATION NOTE

Drinking Water – the Most Important "Food" on Earth

Shimadzu´s complete solution for drinking water testing

Picture 1: HIC-ESP ion chromatograph for anion detection in drinking water

Picture 2: LCMS-8060 NX for determination of PFAS in drinking water

There is 1.4 billion km^3 of water available across the globe. But 97.5 percent is saltwater, and another 2 percent is bound in ice and snow. Less than 1 percent is freshwater on the continents, of which the global population is currently using roughly one third per year, with an increasing trend. Safe drinking water supply is still very difficult in some regions in the world, but the situation in European countries appears to be very good.

In 2020, the European Parliament has adopted the revised Drinking Water Directive (DWD). In force since January 2021, it replaces the 20-year-old directive (98/83/EC). Key features of the revised directive are reinforced water quality standards, which are more stringent than the WHO recommendations. From analytical point of view, the tighter limits for contaminant testing are important – e.g. the heavy metals and anions. Newly included are emerging pollutants, such as endocrine disruptors and per- and polyfluoroalkyl substances (PFAS), as well as microplastics.

Heavy metal monitoring, anion detection, and "PFAS total"

For the simultaneous quantitative determination of heavy metals in drinking water, inductively coupled plasma mass spectrometry (ICP-MS) is the most preferable tool for quality control. Meeting all the requirements of the new DWD, the Shimadzu ICPMS-2030 is an easy-to-operate and fast system.

ICPMS-2030 is able to save all masses recorded during sample measurement. The LabSolutions ICPMS development assistant software proposes optimum parameters for each element in the sample.

According to the new DWD limits, ion chromatography is a method of choice for high sensitivity measurement and detection of anions such as bromate, chlorate, and chlorite. The Shimadzu HIC-ESP ion chromatography system is an ideal tool for sensitive measurements of these anions in drinking water samples, according to DIN EN ISO 10304-1:2009.

Newly included in the DWD is the determination of PFAS. The parameter "PFAS total" refers to totality of PFAS with a maximum concentration of 0.5 µg/l. Shimadzu has developed a full package of state-of-the-art analytical methods for monitoring of PFAS using the LCMS-8060 NX triple quadrupole mass spectrometer.

Monitoring provides confidence

During water treatment, problems may occur which can be highlighted by so-called indicator parameters. One of these is TOC (Total Organic Carbon), which can be considered as a cautionary warning in case of abnormal changes.

Another indicator is the oxidizability parameter, as a measure of the sum of all chemically oxidizable organically bound compounds present in water. While not a direct cause for concern, it can lead to re-germination or undesirable disinfection by-products.

For drinking water analysis, the NPOC (non-purgeable organic carbon) method is used. Sample preparation is done automatically in the TOC-L analyzer, a series of instruments used in laboratory applications.

Total solution for drinking water analysis

Rigid controls and continuous monitoring according to strict guidelines make drinking water the most-closely monitored "food" in the world. Shimadzu offers the complete toolbox for fully automated and sensitive analysis of contaminants in drinking water according to the new European drinking water directive.

READ MORE ABOUT SHIMADZU'S TOTAL SOLUTION FOR DRINKING WATER ANALYSIS

FEATURE

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

"Our study thus provides not only a way to understand beer in previously impossible depth, but also a way to retrace the evolution of brewing means – and even of civilization itself."

(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.

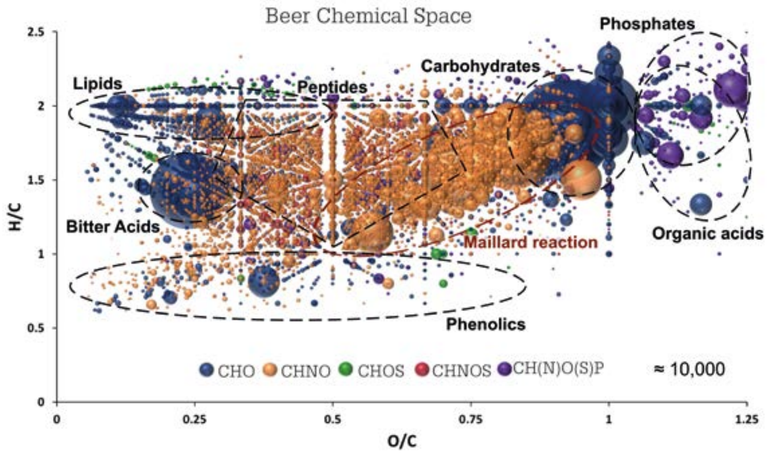
In contrast, the basic building block of fatty acid synthesis, acetyl-CoA, is obtained via an oxidative decarboxylation of the pyruvate. Another dehydration step during chain expansion leads to less oxygenated lipid species. These can be found on the top left of the van Krevelen. Polyphenols are significantly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

A complicated concoction

Our FIA-FTICR-MS approach was able to resolve thousands of yet unknown metabolites in the beer matrix and assign them to possible structural families in the van Krevelen diagram. The definite compositions then enabled us to integrate these molecules into a network via distinct mass differences. These conversions of sum formulae mirror chemical and biochemical reactions and give us information about the processes happening during brewing (and even inside the raw materials used) on a molecular level.



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.

On this basis and through statistical data mining (OPLS-DA), we extracted deep metabolic information reflecting the types of beer analyzed (lager, craft, wheat, abbey). The holistic picture obtained through FIA-FTICR-MS indicated that the hop components in particular differ between these classes. As craft beers are dry hopped (adding hop umbels after the fermentation), these hop components are more oxidized and more phenolic compounds can be extracted.

Far less hops are used when brewing wheat beer, which consequently demonstrates reduced richness of these compounds. On the contrary, the metabolic signature of wheat beer is characterized by the additional grain used; wheat secondary metabolites (phytoanticipines) define the unique metabolic profile of wheat beers. And we were also able to describe a previously unknown derivative of those plant defense molecules, which shows the capability of our approach to investigate hidden metabolites.

Numerous empirical studies suggest that the molecular composition of a beer is very diverse. In brewing literature, rough estimates of the exact number of molecules it contains circulate continuously. We have shown that these estimates are clear underestimations – even without isomeric compounds and molecules inaccessible with ESI and sensitivity limitation.

At last, the typical molecular composition of this unique beverage has been revealed.

This base of knowledge may be used to monitor, control, and guide brewing processes to ensure authenticity, and also to investigate unknown samples (such as historical beers) on a molecular level. By showing the potential of extracting certain molecular patterns from this diversity, our work adds value to modern food safety and quality control. Integrating our foodomic approaches into nutrition and health studies could, for example, help correlate a defined molecular pattern with specific outcomes or observations.

The beer of yesteryear

The history of beer accompanies that of our culture and civilization – by no means a run-of-the-mill story of antiquity and tradition. It all started thousands of years ago, when mankind set out to produce durable beverages from domestic cereals. Since then, the tale has evolved alongside jurisprudence (such as the Bavarian Purity Law, which was introduced more than 500 years ago), technology (refrigeration by Linde is a great example), and science (including the discovery of fermentation by Pasteur and single yeast cell isolation by Hansen, as well as today's cell and gene editing and single-cell metabolomics).

Our study thus provides not only a way to understand beer in previously impossible depth, but also a way to retrace the evolution of brewing means – and even of civilization itself. Different methods of brewing inevitably leave imprints on the metabolic profile of the final (delicious) product. And, for very old but well-preserved samples, it will be possible to trace the methods of brewing used, as well as some of the raw materials and additional processes used.

It may come as no surprise that information of cultural importance can also be extracted from archeological samples with our method. Which type of beer was brewed in a specific region at a specific time? Was it already possible to continuously brew with bottom-fermenting yeast (refrigeration)? Are there any clear indications for or against complying with the purity regulations? How was the grain malted (as is reflected in its darkness and dark metabolome)? Answers to all of these questions can be found within.

Admittedly, samples of beer or beer-like finds from earlier human history are rarely so well preserved that one can work with a liquid that has remained behind. In these cases, the residue crust must be examined, and its molecular pattern compared with the beer we know today. We are not limited to individual potential marker molecules, which often are ambiguous. We can offer whole metabolite profiles from hops, used cereals, yeast and fermentation or Maillard processes that offer an extensive base of knowledge about the beer's metabolome.

As for the future, we see this study as a starting point. We hope to open and answer many more questions about the brewing industry – and our society – as time progresses. So let's raise a glass to the future – cheers!

We would like to acknowledge Marianna Lucio and Michael Rychlik Prof for their valuable contributions to this work and Martin Zarnkow and Patrick McGovern for their stimulating discussions in the last decade on beer and civilizations - we couldn't do it without them!

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SITTING DOWN WITH

A Selective Food Directive

Sitting down with… Michel Nielen, Principal Scientist of Wageningen Food Safety Research (WFSR) and Professor of Analytical Chemistry at Wageningen University, Wageningen, the Netherlands

Tell us about your current position!

I'm currently professor of analytical chemistry at Wageningen University, and I'm also the principal scientist at the applied research institute Wageningen Food Safety Research (WFSR). As principal scientist, I'm responsible for the strategy of the R&D program – so I am involved in things like chairing our research committee, coaching talent and advising the board on strategic R&D decisions. It's a multidisciplinary institute, so our activities range from analytical chemistry to toxicology, microbiology, and data science. In addition to these roles, I am also coordinator of the European MSCA ITN project FoodSmartphone and co-chair of the Recent Advances in Food Analysis (RAFA) symposium series.

What are some of the highlights from your career in analytical chemistry?

I have a long history in analytical chemistry. I started out four decades ago doing my master's research at Leiden University. At the time, we were developing low-dispersion post-column reactors for HPLC – this was before LC-MS was invented of course – and the idea was to address some of the selectivity challenges with current HPLC-UV methods by performing chemical reactions after the column. Alas, these reactors had issues of their own. We ended up solving a lot of them by developing segmented-flow reactors that enabled longer reaction times and, eventually, even performed on-line immunoassay detection after HPLC for the first time. That was a highlight of my early career that has influenced my later research, combining instrumental analysis with biorecognition assays.

After my master's, I did my PhD at the Free University of Amsterdam, where I became interested in bringing additional selectivity to HPLC-UV via on-line SPE. I then spent 10 years in industry at AkzoNobel. That was an amazing period in my career, because I spent a lot of time exploring novel technologies. I managed to convince them to get one of the first API-MS systems, because we were encountering a lot of impurities -thanks to the separation power of the new technique CZE- and struggled to identify them. This turned out to be quite an achievement; we ended up proving that electrospray ionization could be used for not only small molecules and proteins, but also synthetic polymers. Later on, we combined this with MALDI MS and ended up producing one of the most widely cited review papers on the subject. After my time in industry, I came almost full circle in my career by returning to academia. Now, once more, my focus is on selectivity in analytical chemistry.

"Your position as an expert witness is obviously independent in a court case but, quite often, it turns into a debate about the expert's own integrity and that of their institution. It's something I don't like to see happen."

You refer to "selectivity" as a common thread throughout your career. Can you tell us more?

To me, there are three fundamental questions in analytical chemistry: What's in there? How much is in there? And where exactly is it located? That first question is essentially the issue of selectivity and it's something I've focused on in many different ways. To illustrate what I mean, I'll use an example from my time as an expert witness in a specific court case for the government. Once, the judge asked how I could be sure that there was no other molecule on the planet that would yield a similar LC-MS signature to the banned substance under investigation. I'd never thought about it like that. Until that point, we'd followed the official EU legislation – determining retention time, checking against reference standards, determining the relative abundances of certain ions, and checking that they didn't deviate too much. So this really got me thinking.

In the years following that case, one of my PhD students worked on a way to give a quantitative measurement of the selectivity of an LC-MS result. He derived empirical relationships between all kinds of molecules in databases across the globe and came up with a probability function that we could apply and tell the court, essentially, how (un)likely it was that another molecule could mimic the result given as evidence. This nicely illustrates what we mean by selectivity.

What do you love most about what you do?

I find what I do really fun; that's what keeps me motivated. In general, I would say that I love the design of instrumentation and working toward simplicity. In the 1980s, during the glory days of my PhD, I worked on designing an in-line solid phase extraction cartridge built into the axis of a micro-LC injection valve. That one never made it into a commercial product, though similar designs would later be released. But it was great to come up with a prototype SPE cartridge exchanger that is still on the market in a fourth-generation instrument. In a similar vein, our current team is working on smartphone-enabled ionization by using the USB port of the phone to provide 1.5 volts to an inexpensive transformer board/HV generator that supplies several kilovolts to a coated metal blade for direct electrospray of samples into a transportable MS. Again, it's this combination of instrumental design and simplicity (costs less than 10 USD) that I really enjoy.

What drives you?

I have two main drives. First, I really enjoy the development and growth of young people – my students and PhD students. I like to see them gradually coming up with better ideas than I ever could myself; it's amazing! My second driver is seeing instruments designed with the "KISS" principle in mind – keep it simple, stupid. I love that saying and it has stayed with me throughout my career. I constantly come across new research that follows this principle and it renews my love for the field. For example, one of my university colleagues recently came up with an amplification assay for genetic material that worked in what was essentially an espresso cup. There was no requirement for special heaters or thermal cycling; you just applied the sample to the cup and placed it into hot water. It's incredibly simple, it worked, and I think that's fantastic!

What keeps you awake at night?

Mainly rather crazy ideas and concepts, but in the past court cases kept me awake because they were stressful situations. More generally, I don't like how scientists' integrity is often called into question. Your position as an expert witness is obviously independent in a court case but, quite often, it turns into a debate about the expert's own integrity and that of their institution. It's something I don't like to see happen.

What is your message to today's analytical scientists?

We need to learn how to improve our communication with target audiences. People should be aware – whether the audience is a group of scientists, marketers, industry people, or the general public – of how to correctly communicate the work they are doing. One of the key things we should be looking to develop in young scientists is the ability to deal with the doubts around lab testing. It's not only an issue in court cases; in general society, we see that governments and scientists are being trusted less and less. As analytical scientists, we cannot ignore that situation and we certainly have a role to play in remedying it. From my perspective, it would be beneficial to build aspects such as social science, psychology, and marketing techniques into our analytical education.

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