01

THE ROLE OF ANALYTICAL CHEMISTRY IN THE FOOD AND BEVERAGE INDUSTRY

INTRODUCTION

Food science is vital in today's society. Basic research allows manufacturers to better understand ingredients and how they interact, while product development enables them to enhance their range or introduce novel products. Both require analytical techniques to characterize properties, such as flavor and shelf-life, to determine the role of each ingredient, and to study how processing and storage conditions affect properties. A whole host of food industries rely on analytical science, ranging from producers of meat, seafood, dairy products, oils, cereals, and confectionery, to beverages such as beer, wine, and juices.

Manufacturers must comply with voluntary and mandatory standards concerning food composition, quality, and inspection, and label their products with nutritional information. Analytical techniques must also reliably detect even low levels of contaminants such as bacteria and pesticides. Research also focuses on developing methods to detect allergens in food and drink.

Analytical science allows them to monitor properties and control variations in processing. By analyzing raw materials, it is often possible to predict their subsequent behavior during processing so that conditions can be altered to produce the desired properties.

Quality control is another area where food manufacturers require analytical techniques so they can produce food and beverages with the same properties, no matter when or where they are made.

To meet the changing demands of consumers, manufacturers often turn to food scientists to develop novel products and improve existing ones. Analytical techniques are also needed to test the authenticity of specific food components, as there are many past examples where manufacturers have made false claims about their products. Food fraud is challenging to detect, and scientists are working to develop more effective chemical methods.

The following chapters will outline the research presented at Pittcon about the importance of food science and recent trends. They will also discuss how analytical chemistry has impacted food science and continues to affect its growth. A range of analytical techniques will be considered in detail. Developing these technologies to make them even more efficient and effective will help manufacturers respond to changing consumer trends and maintain the highest standards of safety and consistency. Pittcon will illustrate how researchers are working towards these goals and showcase the latest advances in technology development.

The brewing industry is a thriving sector, which has seen the resurgence of small craft brewers as well as the global expansion of multinational companies. As beer is a complex mix of many constituents, it is a challenge for brewers to produce consistent products that will last over time. Chemical reactions can affect the beer's taste, tendency to foam, smell, appearance, and whether it is safe to consume. The brewer needs to understand and control these reactions to prevent unwanted characteristics in the final product.

At Pittcon, speakers described how analytical chemistry and brewing science have evolved together, allowing the industry to develop and flourish. Technological developments have ensured that modern beermaking is tightly controlled, leading to more consistent, higher quality and safer beer.

1.1 HOW ANALYTICAL CHEMISTRY AND THE BREWING INDUSTRY HAVE EVOLVED TOGETHER

Food science involves studying foods and their constituents using analytical techniques that give information on a range of properties such as composition, structure, and sensory characteristics. Food analysis has a vital role to play in today's society for many reasons.

Manufacturers rely on analytical chemistry to allow them to comply with voluntary and mandatory standards concerning composition, quality, inspection, and labeling, to ensure food safety. Food and drink may contain harmful microorganisms, industrial organic pollutants, or pharmaceutical residues such as antibiotics. Analytical techniques must be able to identify allergens and reliably detect even low levels of harmful materials. Food scientists play a significant role in protecting public health by developing more advanced analytical techniques.

Quality control is another area where food manufacturers require analytical techniques so they can produce food and beverages with the same properties, no matter when or where they are made.

Analytical science allows them to monitor properties and control variations during processing. By also analyzing raw materials, it is often possible to predict their subsequent behavior during processing so that conditions can be altered to produce the desired properties.

To meet the changing demands of consumers, manufacturers often turn to food scientists to develop novel products and improve existing ones. One recent trend favors replacing artificial food additives with natural alternatives, and manufacturers are turning to food scientists to investigate the chemistry of natural ingredients and develop ways to improve their stability.

As there are many past examples where manufacturers have made false claims about their products, analytical techniques are needed to test the authenticity of specific food components. Food fraud is challenging to detect, and scientists are working to develop more effective chemical methods, often by improving the current gold standard methods based on techniques such as liquid chromatography (LC) or gas chromatography coupled to mass spectrometry (GC-MS/MS).

For all these reasons, food science is highly valued, whether it is undertaken in industry, government research laboratories, or universities. Basic research, designed to better understand ingredients and how they interact, is just as important as product development for food companies and ingredient suppliers working to enhance their range or introduce novel products. Both require analytical techniques to characterize properties, such as flavor and shelf-life, to determine the role of each ingredient, and to study how processing and storage conditions affect properties.

References

- McClements, J., *Analysis of Food Products* *https://people.umass.edu/~mcclemen/581Introduction.html*
- He, L., (2019) *Food Chemistry as a Vital Science: Past, Present, Future, ACS Symposium Series Vol. 1314* *https://pubs.acs.org/doi/abs/10.1021/bk-2019-1314.ch016*
- FDA (2019), *Food Safety Modernization Act (FSMA)* *https://www.fda.gov/food/guidance-regulation-food-and-dietary-supplements/food-safety-modernization-act-fsma*

1.1a THE IMPACT OF CHEMISTRY IN FOOD SCIENCE

Analytical chemistry techniques can provide essential details on the chemical composition of food and drink, processing, quality control, and contamination, allowing manufacturers to comply with food and trade laws.

As the food industry is a global business, tracing the origins of ingredients is becoming increasingly important. Food scientists employ a range of analytical methods, including capillary electrophoresis, infrared spectroscopy, nuclear magnetic resonance spectroscopy, and mass spectrometry coupled to liquid or gas chromatography.

This chapter presents some examples of techniques and how they are used in food science. However, more efficient and powerful tools are constantly in development.

Mass spectrometry

Mass spectrometry (MS) separates the components of a sample by mass and electrical charge, and is one of the most versatile technologies for food and beverage analysis. The technique offers high sensitivity, high selectivity, and rapid response times.

It is particularly suited for contaminant detection and can be combined with other methods such as liquid chromatography (LCMS) and gas chromatography (GCMS). It can also be coupled to a high-temperature ionization source in inductively coupled plasma mass spectrometry (ICP–MS).

Advion's family of expression compact mass spectrometers integrates with nearly every chemistry technique from simple direct-probe analysis to ultra-high-performance compound separation with LC integration. This gives laboratories the ability to analyze a range of samples using more than one method for complete identification and characterization.

Advion also offers the family of SOLATION ICP-mass spectrometers, which are particularly well-suited for multi-element analysis of trace elements in complex liquids.

Ionization techniques at atmospheric pressure power ambient mass spectrometry (AMS). Since AMS techniques are simple, cost-efficient, and operate high-throughputs, they are increasingly applied in the food and beverage industry. AMS detects trace amounts of analytes and handles almost all types of food samples, including solid, liquid, viscous, and bulk. Sample preparation times are significantly shorter than other techniques, such as LCMS and GCMS.

There are now more than 40 kinds of ambient ionization techniques, including direct analysis in real-time (DART). Agilent, which produces a wide range of mass spectrometers, describes the benefits of using DART as speed and ease of use. It is possible to obtain very accurate data when using DART with a high-resolution instrument such as an Agilent TOF MS, or Q-TOF MS. Agilent has shown how the technology can analyze fungicides, such as thiabendazole, on an orange peel in less than a minute with no sample preparation.

Nuclear magnetic resonance (NMR)

Bulking out food or adulterating beverages with cheaper ingredients remains an issue in the industry. NMR is an ideal tool for quality control monitoring and testing for purity and authenticity. As it provides a characteristic peak for each compound, it can be used to identify and quantify the composition of a mixture.

Bruker has tailored this technology for food analysis. Its FoodScreener™ provides the NMR fingerprint specific to an individual sample, which is compared to spectra databases for authentic samples, allowing identification of missing or unexpected ingredients. The company has recently developed a wine-profiling module for the FoodScreener. It enables the verification of wine variety, origin, and vintage.

Chromatography

One of the most critical methods for analyzing residues and contaminants is high-performance liquid chromatography (HPLC), which separates, identifies, and quantifies components in a mixture

A column filled with solid adsorbent materials pumps through a pressurized liquid solvent containing the sample. To separate each sample component, they interact in different ways as they flow out of the column.

Reversed-phase liquid chromatography with ultraviolet detection is one of the most common HPLC techniques for pesticide analysis, and is essential for ensuring that levels are below accepted limits. HPLC is also well-suited to test for vitamins, sweeteners, and preservatives used as additives, as well as natural food ingredients, such as organic acids.

Knauer has developed a suite of HPLC instruments, as well as its AZURA® GPC Cleanup System for automated sample cleaning of multi-pesticide residues in complex samples. It also has another system that can simultaneously separate and analyze sugars, organic acids, and alcohol. This has proved especially useful for the quality control of alcoholic beverages.

Laser Scanning Confocal Microscopy (LSCM)

Newer microscopic techniques, such as laser scanning confocal microscopy (LSCM), offer various advantages to food scientists. LSCM microscopes, which use a focused laser to scan three-dimensional samples, allow researchers to examine the microstructures within food without the need for time-consuming sample preparation.

LSCM produces images of the sample's internal structure with high contrast and high resolution. For extra detail, samples can be stained with a range of fluorescing chemicals (fluorochromes) that attach themselves to particular constituents, such as proteins or fat.

LCSM has proved useful to study food with high-fat content, showing how fat globules are distributed and how they behave under certain conditions. Other applications include biopolymer food materials, such as skim milk-gelatin mixtures, and emulsions, such as mayonnaise. It allows food scientists to visualize and characterize the composition of their different phases by using specific fluorophores.

Zeiss has developed its LSM 900 series to deal with the most challenging three-dimensional samples. Its new LSM 9 family undertakes confocal 4D imaging with high sensitivity and spectral flexibility. It images dynamic processes in larger fields of view or fixed samples with higher throughput and less bleaching. Its latest models can produce data eight times faster with super-resolution.

Raman Spectroscopy

Raman spectroscopy provides information about chemical structure and identity, phase, and contamination of powders, solids, and liquids by determining vibrational modes of molecules. It is gaining popularity as an analysis technique in food and beverage applications as it is quicker than chromatography techniques and does not require solvents or lengthy sample preparation.

While Raman spectroscopy's use was once limited to the detection of significant compounds in a mixture, surface-enhanced Raman spectroscopy (SERS) has proven to be very useful in identifying trace materials such as pesticides in fruits. Gold or silver nano-substrates enhance the Raman signal.

Horiba has been strongly involved in innovations in Raman technology. As well as SERS, these include stimulated Raman scattering (SRS), top-enhanced Raman scattering (TERS), integration with electron microscopes and atomic force microscopes, hybrid single bench systems, and transmission Raman (for true bulk material analysis). Its machines detect adulterated oils, bacteria, pesticides in food, and melamine (used to dilute milk but give the appearance of normal protein levels), as well as to characterize the structure of grains and crops.

References

- *Advion, Integrated solutions for food authenticity, characterization and safety https://www.advion.com/applications/food-and-ingredients/*
- *Advion, expression® High-performance compact mass spectrometer https://www.advion.com/products/expression-cms/*
- *Agilent, Direct, Rapid Analysis of Orange Peel using the DART Ion Source https://www.agilent.com/cs/library/flyers/public/5990-6638en_lo%20CMS.pdf*
- *Bruker, Food screening by NMR https://www.bruker.com/products/mr/nmr-food-screening.html*
- *News Medical Life Sciences (2018), Life Science Applications of Chromatography https://www.news-medical.net/life-sciences/Life-Science-Applications-of-Chromatography.aspx*
- *Knauer, Liquid chromatography solutions for the food industry https://www.knauer.net/en/Food-Analysis*
- *Zeiss, Laser Scanning Microscopes https://www.zeiss.com/microscopy/int/products/confocal-microscopes.html*
- *Azo Materials (2019), The Role of Laser Scanning Confocal Microscopy (LSCM) in Food Analysis https://www.azom.com/article.aspx?ArticleID=17752 -*
- *Horiba, Raman Imaging and Spectroscopy https://www.horiba.com/en_en/products/by-technique/molecular-spectroscopy/raman-imaging-and-spectroscopy/?author=22&cHash=c867b8d83f4e3e4201038e4420a46df1*

1.1b THE EVOLUTION OF ANALYTICAL CHEMISTRY OF BEER AND BREWING ANALYSIS

Beer remains the most consumed alcoholic drink in the world, with people drinking it for thousands of years. Beer is a complex mix of compounds, and its flavor and other properties come from a delicate balance of these constituents. It is a challenge for brewers to produce consistent products that will maintain this balance for as long as possible.

There are more than 3000 different components in beer, including carbohydrates, proteins, ions, microbes, organic acids, and polyphenols. Some of these originate from the raw materials, but others form during brewing or as the beer ages. Such chemical reactions can affect the beer's taste, smell, the tendency to foam, appearance, and whether it is safe to consume. Over the years, analytical chemistry has helped brewers increase the efficiency of the brewing process and become more innovative.

The science of beermaking

Making beer requires starch (usually malted barley), yeast, hops, and water. Starch provides sugars for the yeast to grow and gives body to the beer. Barley's mix of carbohydrates, saccharides, amino acids, proteins, and vitamins combine to affect the beer's taste, aroma, and color. It also contains polyphenols that contribute to antioxidant activity.

Brewing involves many stages. First, barley malt and brewing water are mixed (mashed) and heated, converting starch to sugars and forming a liquid called wort. By controlling the temperature during mashing, brewers can alter the ratio of these sugars. Higher temperatures produce higher proportions of non-fermentable dextrins, which contribute to body and 'feel', while lower temperatures produce more fermentable sugars and higher alcohol content.

The wort is then filtered and boiled with hops. Boiling converts α-acids found in hops into iso-α-acids, which provide bitter flavors. The hops also contain a complex mixture of terpenes to give beer its aroma. The boiling process is critical to the final product. For example, it affects the beer's color and the concentrations of malt components, such as dimethyl sulfide, that can lead to 'off-flavors'.

The next stage is fermentation, where yeast is added to convert carbohydrates into ethanol and carbon dioxide. During fermentation, various volatile phenolic compounds are formed (such as esters and aldehydes), depending on yeast strain and fermentation conditions. This enables the brewers to create unique flavors. Fermentation can last a few days or several weeks. When the yeast is removed, the beer is left to mature to develop more flavor.

All brewing ingredients contain a wide variety of chemical components which interact at each stage. The brewer needs to control these reactions to produce the desired beer. Water is another key factor in the final product, as beer is 90% water (by volume). Factors such as pH, alkalinity, ion, microbial content, and disinfection by-products can affect the beer.

Analytical techniques

The brewing chemist requires information for many standards. These include color, bitterness (defined by International Bittering Units), alcohol content, calorie, and carbohydrate content. A range of techniques can provide detailed information on the final product as well as its components, how they change over time, and how they affect the beer. These techniques include chromatography, mass spectrometry, Raman spectroscopy, matrix-assisted laser desorption/ionization, capillary electrophoresis, and flame ionization detection.

One of the most useful pieces of equipment for a brewery's laboratory is the spectrophotometer, which tests bitterness, color, and other factors. Atomic absorption spectrophotometry detects inorganic contaminants, such as arsenic and mercury, absorbed by barley and hops during growth. Organic contaminants, such as pesticides, may also find their way into beer from plants, and these are usually detected with chromatography techniques, such as gas chromatography-mass spectrometry (GCMS). As minerals such as calcium carbonate and sulfate can affect taste, ion chromatography can be used to test for ions in water used in the process.

Testing raw materials for contaminants, such as heavy metals and pesticides, can be done with inductively coupled plasma mass spectrometry (ICP-MS). Shimadzu has developed a series of instruments for this purpose as well as a screening system for mycotoxins, which are hazardous metabolites produced by molds. It can detect ten mycotoxin components in 14 minutes at concentration

levels specified by EU standards.
In other developments, Shimadzu has reported a new GCMS method using multiple reaction monitoring for simultaneous detecting and quantifying six N-nitrosamines. These compounds, which can be carcinogenic, are formed when barley is fire-dried to produce malted barley.

Another variation on MS - proton transfer reaction mass spectrometry (PTR-MS) – is proving a rapid and sensitive method to analyze volatile organic compounds (VOCs) such as terpenes. These compounds, given off by hops, combine to produce the distinctive flavors and aromas of different beers. PTR-MS can be combined with gas chromatography to detect low concentration VOC isomers, which often trace constituents within complex mixtures.

The impact of analytical chemistry on the brewing industry and beer analysis

Advances in analytical science have fed into scientific progress within the brewing industry. Technological developments have ensured that modern beermaking is tightly controlled, leading to more consistent, higher quality and safer beer. Professor Matt McCarroll addressed Pittcon to discuss how analytical chemistry and brewing science have co-evolved, allowing the industry to develop and flourish.

Professor McCarroll, who is the director of the Fermentation Science Institute at Southern Illinois University, Carbondale, explained how scientific developments, such as the thermometer and the saccharometer (which measures sugar content), have had a profound impact on brewing, allowing brewers to control and monitor the efficiency of the mashing process.

Other significant inventions include the development of the pH meter and the spectrometer, which form the basis of many of the standard testing methods for quality control. Furthermore, problems arising within the brewing industry in the past have led to scientific advances that are still applied today. One example is how the study of alcoholic fermentation led to the use of the term enzyme in 1877 by Wilhelm Kuhne. Another is the process of pasteurization, which arose from Louis Pasteur's studies into the spoiling of beer and wine. Emil Christian Hansen also developed yeast propagation methods while working at the Carlsberg Brewery laboratories in 1883.

In modern times, there is an ongoing need for well-trained scientists in the brewing industry. Craft brews are growing in popularity, and there are now around 7000 craft breweries in the US. However, estimates suggest that less than 10% of the breweries use advanced instrumental analysis in their processes. The cost and need for trained technicians are significant factors, although small breweries can use centralized quality control laboratories or satellite stations.

The industry must guarantee quality and consistency by having access to analytical techniques to enable brewers to improve quality control, produce better tasting beers, devise novel marketing approaches, and develop innovations in craft brewing.

References

- *Schug K.A. et al. (2019) A review of the analytical methods used for beer ingredient and finished product analysis and quality control. Analytica Chimica Acta https://www.sciencedirect.com/science/article/pii/S0003267019308992*
- *Elsevier SciTech Connect (2015) The Chemistry Behind Beer Flavor http://scitechconnect.elsevier.com/chemistry-behind-beer-flavor/*
- *Shimadzu, Beer Analysis https://www.shimadzu.eu/beer-analysis*
- *Shimadzu, ICPMS-2030 https://www.shimadzu.eu/icpms-2030*
- *Shimadzu, Analytical and Testing Instruments for Beer https://applicationstation.ssi.shimadzu.com/sites/default/files/total-support-beer-analysis.pdf*
- *Pittcon 2020, Is that a Chemist in the Brewhouse? Coevolution of the Analytical and Brewing Sciences with a Look to the Future https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=612&SessionDateID=4*
- *Azo Materials (2019), Fast Detection of VOCs in Beer Aroma https://www.azom.com/article.aspx?ArticleID=18096*

1.1c ADVANCED ANALYTICAL METHODS FOR BEER ANALYSIS AND BREWING MONITORING

While beer consists mainly of water, malted grain, hops, and yeast, modern craft brewers are experimenting with many more ingredients, including fruits, vegetables, and spices. With brew-pubs and the traditional major brewing companies also keen to diversify, the result has been an abundance of new beers on the market, but also new challenges in beer analysis and process monitoring.

With this in mind, a symposium at Pittcon titled Analytical Chemistry of Beer and Brewing presented recent developments in analytical chemistry methods for the brewing industry. Analytical techniques provide brewers with an extensive toolkit allowing them to develop and design new beers, monitor all stages of the brewing process, ensure consistency, and detect issues such as infection.

Common techniques for beer analysis and brewing monitoring

The range of analytical methods used for beer analysis and brewing monitoring is extensive. It includes gas chromatography, liquid chromatography, matrix-assisted laser desorption/ionization, capillary electrophoresis, mass spectrometry, and flame ionization.

High-performance liquid chromatography (HPLC) is a handy technique for separating, identifying, and quantifying each component in a mixture. It can be used to analyze amino acids, polypeptides, and proteins in beer.

ThermoFisher Scientific has experimented with HPLC methods combined with a spectro-electro array platform to measure polyphenols and bitter acids in beer samples. Brewers wanting extra bitterness in their beers - a recent trend in US microbreweries – add extra hops during brewing to increase these compounds. However, other hop-derived compounds, such as proanthocyanidins, can cause cloudiness in beer (haze) and degradation during storage. Measurements of polyphenols and bitter acids enable brewers to differentiate between beers and aid studies on beer stability.

Ion chromatography is a versatile technique that meets many of the analytical requirements of brewing. By monitoring proteins, carbohydrates, and alcohols, the method allows the brewer to determine the extent of fermentation. It also gives the brewer information on the concentration of added preservatives and colorants. For example, ThermoFisher Scientific has developed a cost-efficient and rapid new system to test for carbohydrates, alcohols, organic acids, and inorganic anions and cations.

The brewing industry has embraced the use of spectroscopy methods for routine analysis of the chemical composition of beer. Spectroscopy is based on the interactions between light and molecules in different regions of the electromagnetic spectrum (such as the ultraviolet, visible, Raman, and near infra-red (NIR)). Raman spectroscopy is particularly suitable for water-containing samples.

Recently, measuring the Raman spectra of complex samples such as beer has become easier thanks to the release of high resolution and portable instruments with excellent detection capabilities.

NIR spectroscopy is a useful tool to analyze barley and malt crops in the field to assess ripeness and plant health. It can determine levels of starch in the crop, allowing brewers to predict ethanol production, and can also help analyze yeast samples and ground hop samples to determine their acid and oil content, moisture levels, and predicted storage lifetimes. Armed with this information, brewers can choose the best strains for their beer styles and control the fermentation process more tightly.

Another useful technique for analyzing hop strains is gas chromatography-mass spectrometry (GCMS). Each strain consists of a different composition of volatile components and offers distinct aroma characteristics. GCMS is particularly beneficial for identifying essential oils.

Many extraction and concentration methods have been developed for sample preparation, but most recently, the headspace-trap process has been shown to reduce sample preparation time and analyte losses. The sample is placed in a closed vessel and heated to a specific temperature to release the volatile compounds that can be sampled for analysis. This system analyzes a large number of samples in a relatively short time and is easily automated.

Proton transfer reaction mass spectrometry (PTR-MS) is also well-suited to identify volatile compounds. However, analyzing alcoholic beverages by PTR-MS can be challenging because signals from ethanol can obscure compounds that appear in lower concentrations. However, TOFWERK has developed a system that combines a gas chromatogram, which can separate isomers, with its Vocus 2R PTR-TOF machine. This removes ethanol well before most aroma compounds and provides accurate analysis of terpenes in a beer sample in 15 minutes.

Beer contamination is a key issue for brewers, as microbes found on barley, such as bacteria and fungi, can survive the brewing process. A technique called matrix-assisted laser desorption/ionization e time-of-flight e mass spectrometry (MALDI-TOF-MS) has been used to detect microbes in the mash instead of using expensive and time-consuming traditional culture methods. It involves using a laser to ionize samples into charged molecules that can then be separated based on their mass-to-charge ratio. The method generates 'protein fingerprints', which are identified by database comparison, and has proven to be a powerful identification tool due to its high throughput and versatility.

Other applications include detecting microbes in water samples, replacing real-time polymerase chain reaction (PCR), or culturing methods. It has also been used to create a database of common brewing yeasts, allowing brewers to select the best yeast for their product, and identify strains that could cause beer spoilage.

New challenges in beer analysis

Analytical techniques can prove indispensable for brewers developing new beers. For example, a new trend is for fruit-flavored beer. Researchers are employing analytical chemistry to demonstrate how adding fruit to beer can significantly improve nutritional quality, an aspect that could lead to novel marketing approaches.

A recent study has shown that adding fresh fruit during the fermentation process appears to boost levels of bioactive compounds as well as antioxidant activity. Researchers used HPLC to examine the total polyphenol and flavonoid content of 10 fruit beers compared with conventional beers. They found that all fruit beers contained higher levels of phenolics, with cherry beers containing the highest.

Antioxidant activity in fruit beers was also about two to three times higher than conventional beers. This is good news for beer drinkers as polyphenols and flavonoids have known health benefits such as protecting against inflammation, cancer, and viruses. It is also good news for brewers as research indicates that beer containing high levels of phenolic antioxidants has a more stable flavor and aroma, as well as a longer shelf life.

As the consumer landscape evolves, brewers need to ensure that they can rise to the challenge of creating novel brands. Pittcon heard from Mark Yocum, Technical Director of Anheuser-Busch, the world's largest brewing company, on how the firm has exploited analytical chemistry to respond to changing consumer demands and how science has shaped its business. He described how AB uses the latest analytical methods to create a culture of quality and consistency, how it has contributed to technology advances into brewing, and how it uses its analytical expertise to support all areas of production, from barley- and hop-breeding to packaging innovations.

References

- *Pittcon 2020, Analytical Chemistry of Beer and Brewing https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=26&SessionDateID=4#*
- *Schug K.A. et al. (2019) A review of the analytical methods used for beer ingredient and finished product analysis and quality control, Analytica Chimica Acta https://www.sciencedirect.com/science/article/pii/S0003267019308992*
- *ThermoFisher Scientific, Beer testing information https://www.thermofisher.com/uk/en/home/industrial/food-beverage/food-beverage-learning-center/beverage-testing-information/beer-testing-information.html*
- *Cozzolino D. (2019) Spectroscopic approaches for rapid beer and wine analysis, Current Opinion in Food and Science https://www.sciencedirect.com/science/article/pii/S2214799319300670*
- *News Medical Life Sciences (2019), Using Near-Infrared Analysis in the Brewing Industry https://www.news-medical.net/whitepaper/20190410/Using-Near-Infrared-Analysis-in-the-Brewing-Industry.aspx*
- *TOFWERK, Vocus PTR-TOF https://www.tofwerk.com/products/vocus-ptr-tof/*
- *Garaguso I. et al. (2020) Characterization of bioactive compounds and antioxidant activity of fruit beers, Food Chemistry https://www.sciencedirect.com/science/article/pii/S0308814619315523*
- *Pittcon 2020, A Brewmaster's Approach to Analytical Chemistry, Analysis, Quality, and Taste https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=648&SessionDateID=4*

1.1d ANALYSIS OF HOP COMPOUNDS IN BEER

Hops are a vital ingredient in brewing. Brewers use the hop flower, a delicate, pale-green cone which contains compounds that give bitterness to beer when used early in the brewing process, and aroma when added at the end. Hops are also a preservative and extend the beer's life.

Brewers are particularly interested in hop essential oils, 'bittering' acids, and polyphenolic compounds. Essential oils provide scents that range from fruity to spicy, while α-acids are the primary source of bitterness in beer, although other components also contribute. Polyphenols give beer its 'body' and offer antioxidant properties. The levels of these components vary depending on the hop variety, stage of ripening, and climate.

Hops contain around 250 essential oils (terpenes and sesquiterpenes). Approximately 22 of these are known to give aroma or flavor, but the key oils are myrcene, humulene, and caryophyllene. Most are partially evaporated during wort boiling, while some remain in the wort and can react to form compounds with anti-inflammatory properties (α-terpineol) and sedative properties (linalool).

The major α-acids are cohumulone, humulone, and adhumulone. During brewing, up to half of all α-acids are converted into iso-α-acids. These are the main bittering agents in beer, and they also influence foam stability and show some antiseptic properties. However, if they are oxidized or degraded by light, they can produce compounds with bad odors. Hops also contain β-acids, such as lupulone. It is thought that lupulones could also contribute to beer flavor.

Phenolics, including benzoic acid and flavonoids, are mainly formed from tannins, and play a complicated role in beer quality, contributing to mouthfeel, astringency, color, and bitterness. High molecular weight polyphenols tend to precipitate with proteins during mashing. Associated with beer haze, they are usually removed. Some low molecular-weight phenols are thought to be responsible for unwanted flavors. However, some phenolics are beneficial to health, for example, the flavonoid xanthohumol has antibacterial and anti-inflamatory properties.

Measuring hop compounds

Various analytical methods measure hop compounds. As part of the symposium on Analytical Chemistry of Beer and Brewing at Pittcon, John Paul Maye of Hopsteiner, one of the largest and oldest hop companies in the world, discussed these techniques (focusing on high-performance liquid chromatography). His talk also covered different approaches to making beer, such as dry hopping, where hops are added directly to the fermentation vessel, usually to add aroma.

Techniques well-suited to measure essential oils include mass spectrometry methods, including gas chromatography-mass spectrometry and proton transfer reaction mass spectrometry. Spectrophotometry was traditionally used to measure hop acids, which involves extracting a beer sample with iso-octane and measuring the absorbance of the organic phase (at 275 nm). However, this approach measures all extracted compounds, not just the ones causing bitterness.

HPLC is a more specific method to determine the quantities of hop-derived iso-α-acids. It is also a valuable tool for the detection and measurement of phenolic compounds. Agilent Technologies brought its next-generation, ultra-high-performance 1290 Infinity II LC System to Pittcon. This instrument offers high separation and detection performance, allowing brewers to detect a wide range of compounds.

Extraction

Hops can be processed to extract essential oils and bitter acids, which are then added during brewing. Attention is now also focusing on extracting phenolics, which can be added for health reasons. The extraction of oils is often carried out with steam distillation, while acids can be retrieved using organic solvents, such as methylene chloride or hexane, and phenolics with aqueous solvents. Using hop-processed products, which can be bought in different forms, such as dried or frozen, give the brewer more control over the final beer. For example, pre-isomerized iso-α-acids regulate bitterness.

Future of beer technology

> Sustainability is one of the main drivers of technology advances, and brewers are keen to make their processes more environmentally friendly.

Researchers are developing novel ways to make hop extraction 'green'. Recently, extraction processes that cut both operation times and energy consumption have emerged. These 'intensification strategies' include microwave-assisted hydrodistillation, pulsed electric fields, and pressurized extraction. Other efforts are dedicated to moving away from organic solvents, such as using supercritical carbon dioxide technology.

Food and drink containing functional or health-giving properties is another recent trend and is driving analytical developments. Research has confirmed the health benefit potential of specific hop components, and this has provided an incentive for their use in brewing. However, this aim requires the development of novel extraction processes and targeted monitoring of the final product.

The growth of new styles, such as dry hopping (infusing beer with additional fresh hop flavor and aroma) and hop-forward (flavor is hoppier), means hop growers are developing plants with unique aromas and chemical characteristics. Analytical techniques are developing alongside this search for novelty.

As Scott Lafontaine of the University of California, Davis, explained at Pittcon, several studies indicate that understanding the chemical composition of aroma hops helps brewers create more sustainable hop-forward beers as it allows them to work out the best varieties for use throughout the brewing process. Such information can also help hop growers produce premium aroma hops by giving them analytical targets.

Some hop strains are rich in free volatiles (such as geraniol, 3-mercaptohexanol, 4-mercapto-4-methyl-2-pentanone), which are significant in producing hoppy beer aromas, while other strains contain higher amounts of bound volatiles (such as thiol precursors and glycosides).

Hop growers and brewers wanting to create a particular aroma can vary several factors, as Lafontaine explained. These include harvest timing, hop genetics, and yeast enzymatic activity during fermentation. Analytical chemistry in the form of LCMS techniques can then be used to measure aroma components directly.

Technology is also coming to the aid of craft brewers facing challenges with the traditional approach of describing beer bitterness, the International Bittering Units (IBU), which is based on the UV absorption of iso-α-acids at 275 nm.

Bruce Hamper of the University of Missouri, St Louis, and Kurt Driesner of Urban Chestnut Brewing Company described how some modern beers, created using late hopping and dry hopping, contain significant amounts of α-acids and oxidized derivatives. As these also absorb in the UV region such as iso-α-acids, they can obscure IBU measurements.

Spices added to the mix can also have this effect. Hamper and Driesner described a variation of liquid chromatography-mass spectrometry called selected ion MISER (multiple injections in a single experimental run) LCMS. This technique provides a comparative assessment of the amount of α-acids and iso-α-acids. Knowing the relative amounts of the two components can provide information on bitterness levels.

References

- *Hops, All About Beer magazine* *http://allaboutbeer.com/learn/beer/hops/*
- *The Science Behind Hops – Part 2 – Essential Oils (2013), Craft Beer Academy* *https://craftbeeracademy.com/the-science-behind-hops-part-2-essential-oils/*
- *Pittcon 2020, Analysis of Hop Compounds in Beer* *https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=574&SessionDateID=4*
- *Dominguez, H. et al. (2019), What is new on the hop extraction? Trends in Food Science and Technology* *https://www.sciencedirect.com/science/article/pii/S0924224419304704*
- *Agilent, 1290 Infinity II LC Systems* *https://www.agilent.com/en/products/liquid-chromatography/infinitylab-analytical-lc-solutions/1290-infinity-ii-lc-systems/1290-infinity-ii-lc-system*
- *Jaskula, B. et al. (2007), Enhanced Quantitative Extraction and HPLC Determination of Hop and Beer Bitter Acids, Journal of the Institute of Brewing* *https://onlinelibrary.wiley.com/doi/pdf/10.1002/j.2050-0416.2007.tb00765.x*
- *Pittcon 2020, Uncovering and Utilizing the Hidden Potential of Aroma Hops to Make More Sustainable Hop-forward Beers* *https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=604&SessionDateID=4*
- *Pittcon 2020, Comparison of Beer Bitterness using Rapid MISER LCMS Analysis of Hop Derived Humulone Constituents and Sensory Evaluation* *https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=639&SessionDateID=4*

CONCLUSION

Food science makes a vital contribution to society. A wide variety of analytical techniques offer food and beverage manufacturers ways to develop novel products, improve processes, detect contaminants, and maintain strict quality control by providing information on ingredient structure, composition, and contaminants.

Demand for advanced analytical methods is increasing from both industry and regulators who want more efficient and effective technologies to help them respond to changing consumer trends and maintain the highest standards of safety and consistency. Pittcon has illustrated how researchers are working towards these goals and has showcased the latest advances in technology development.

Innovations in analytical science have fed into progress within the brewing industry. Modern beermaking is tightly controlled, leading to more consistent, higher quality and safer beers.

A symposium at Pittcon entitled Analytical Chemistry of Beer and Brewing presented recent developments in analytical chemistry methods to study beer ingredients, the brewing process, and the evaluation of beers. Speakers explained how analytical techniques had provided brewers with an extensive toolkit allowing them to develop new beers, monitor all stages of the brewing process, ensure consistency, and detect issues such as infection.

The wide range of analytical methods used for beer analysis and brewing includes spectrophotometry, mass spectroscopy coupled to liquid or gas chromatography, Raman spectroscopy, infrared spectroscopy, and nuclear magnetic resonance spectroscopy.

The study of hop compounds is a particularly important area of research and development for brewers. Brewers are interested in hop essential oils, 'bittering' acids, and polyphenolic compounds. The levels of these components vary depending on the hop variety, stage of ripening, and climate. Various analytical methods are used to measure the compounds found in hops. Pittcon discussed these techniques, such as high-performance liquid chromatography (HPLC), which has become an increasingly popular method to determine the quantities of hop acids, as well as phenolic compounds.

Looking to the future, analytical methods develop quickly, and sustainability is one of the main drivers for advances as brewers are keen to make their processes more environmentally friendly. Researchers are developing novel ways to make the extraction of hop components more 'green'. Recent trends for food and drink to contain functional or health-giving components mean analytical techniques must be developed that can monitor such compounds. In brewing, research has confirmed that certain hop components have potential health benefits, providing an incentive for their use in new beers. Analytical techniques are also developing as brewers' search for novelty leads them to develop new beer styles with unique aromas and chemical characteristics.

1.2 THE SCIENCE BEHIND THE BEER AND BREWING INDUSTRY

In this interview, Christopher Welch from the Indiana Consortium for Analytical Science and Engineering (ICASE) discusses the new and improving developments in the analytical chemistry of beer and brewing analysis.

Beer consists primarily of four ingredients: water, malted grain, hops, and yeast. Yet, the analysis of beer and the brewing process has changed dramatically over the last decade.

In the last 20-30 years, we have seen a revolution in the brewing industry worldwide. How has the analysis of beer and precursors in the brewing industry evolved over the last two to three decades? What has changed when it comes to the analysis of beer?

The biggest changes have been the emergence of the craft beer industry and a dramatic surge in the popularity of home brewing. These changes have been paralleled by important advances in analytical chemistry, especially the development of instrumentation to allow analysis outside of conventional laboratories by non-experts.

Beer analysis has always been a high-tech enterprise inside the laboratories of the large industrial brewers. However, in the past, homebrewers and smaller craft brewers relied heavily on old-school measurement tools such as thermometers, hydrometers, balances, and buckets.

Over the past few years, I have been collaborating with Professor Bruce Hamper, from the University of Missouri at St. Louis, on applying state-of-the-art HPLC-MS technologies to beer and brewing analysis. Bruce teaches a class on beer and brewing, and notes that beermaking provides these students with an excellent introduction to complex measurement science and fundamental scientific principles. We have noted a growing interest in this subject and thought it would be a fun topic for a Pittcon symposium and beer-tasting event, bringing together experts from large and small breweries with homebrewers, educators teaching classes on beer and brewing, as well as just beer lovers.

Why is the analysis of the brewing process critical?

We think it comes down to what Henry Ford said about automobile manufacturing: "You can't improve what you can't measure". Over the past few millennia, we humans have done a remarkable job using our existing senses to develop amazing beers, but many factors are contributing to success or failure that are just not that easy for us to taste, smell, see or feel. Obtaining consistency in the brewing process is a challenge for large and small brewers.

There is a significant amount of time between the initial brewing of malted grains and the packaging of the final product. Monitoring the profile of ingredients and changes during the brewing process is critical for obtaining a consistent product, and modern analytical technologies can shed light on these situations. We will share several excellent case studies profiled in our symposium at Pittcon.

How has the emergence of the new craft brewery phenomenon impacted beer and brewing analysis?

Firstly, there are now many more producers making a staggering variety of different products. The small size and limited scale of some of these manufacturers means that there is a growing interest in performing analysis of beer and brewing using less costly analytical instrumentation that does not require a high degree of expertise to operate. We think this is one of the most exciting aspects of the current field and an area where Pittcon participants may be able to lend a hand in figuring out next-generation tools for beer analysis.

In a recent study, Bruce, a group of friends, and I sampled more than 75 different beers from around the world, including many standard lagers, porters, and stouts, but also several of the hop-heavy ales that craft beer makers have been producing in recent years.

In addition to tasting, we analyzed the samples for levels of the hops-derived humulones and isohumulones using a high-throughput MISER HPLC-MS method that we have developed. Interestingly, we were able to analyze all the samples in about one hour, with the results showing a remarkable window into the flavor profile created by these two hops-derived compounds. The humulones correlated with a resinous, 'hoppy' flavor and fragrance, while the iso humulones provided the characteristic bitter taste favored in classical lager and bitter beers.

There are only four basic ingredients in beer: water, malted grain, hops, and yeast. However, over the last decade, a new craft beer trend has surfaced, which has expanded the ingredient list to include a wide range of fruits, vegetables, and spices. How has the analytical process behind beer analysis and the brewing process changed with the inclusion of these new ingredients?

We have yet not investigated these other flavorings, but with modern craft beers containing everything from hot peppers to espresso, pumpkin, and grapefruit, there are ample opportunities for expanding analytical testing to analyze these specific compounds arising from these sources. Some of the speakers in our Pittcon session represent craft-brewers who manufacture such beers, and they will be able to shed additional light on this topic.

How do you help brewers remain consistent in the quality of the final product? Why is this important?

We are lucky to have INBEV- Anheuser Busch, a brewmaster from one of the biggest beer manufacturers in the world, as a speaker in our session this year at Pittcon. In 2017, INBEV-AB produced 612 million hectoliters of beer, which is roughly 31% of all beer produced globally. We will see what they have to say on this important question of quality control, batch-to-batch reproducibility, and maintaining product flavor standards from year to year.

Can you tell us about the recent development in analytical chemistry that has been employed to monitor the brewing process and quality of beer?

Modern chromatography is playing an increasingly important role in monitoring the brewing process and product quality. The American Society of Brewing Chemists (ASBC) regularly updates methods of analysis for the brewing industry. These are the 'Standard Operating Procedures' for the industry and have included more emphasis on HPLC, GC, and MS in recent years. We will hear more about these developments in our symposium, but we are also interested in learning what emerging analytical technologies for inexpensive, on-the-spot analysis could be employed in beer and brewing analysis.

What do you hope to gain from attending Pittcon and discussing your research?

We think this is a fascinating topic and an excellent opportunity to bring researchers together from across the entire spectrum of beer and brewing science – from large industrial breweries to craft breweries, homebrewers, and teachers of brewing courses.

We are hoping that this event will stimulate some new collaborations and research opportunities, perhaps furthering opportunities for new handheld devices and sensor technologies to be used in the analysis of one of our very favorite substances – beer.

Where can our readers go to find out more?

We recently published a method for high-throughput analysis of hops derived compounds in beer: Rapid Determination of Humulones and Isohumulones in Beers Using MISER LC-MS Analysis, Bruce C. Hamper, Kerstin Zawatzky, Vivian Zhang, Christopher J. Welch, J. Amer. Soc. Brew. Chem. 75 , 333-338, 2017.

1.3 GAS-CHROMATOGRAPHY -MASS SPECTROMETRY (GC-MS) IN THE FOOD INDUSTRY

GC-MS is widely used in the food industry. It is well-suited to detect and identify contaminants and residues, such as pesticides and pollutants. However, it is equally good at determining levels of additives and colorants, providing food composition data for labels, and analyzing taste and aroma.

One particular application where GC-MS has proved its worth is in detecting fraud. Unscrupulous food and drink manufacturers have always experimented with adulterating food, usually to save costs. As a result, detecting food fraud, tracing ingredients, and authenticating products are becoming increasingly important as consumers demand protection. Food regulations require product labels to show composition, origin, and the process of production. However, as the food industry becomes more global and the range of food items expands, the danger of counterfeit and contaminated food products is growing, with possible far-reaching consequences as adulterated products may cause health problems.

With consumers, regulatory bodies, and the food industry itself demanding strict monitoring and quality control of food, GC-MS offers efficient and effective analysis. Chromatographic techniques detect adulteration and determine authenticity, while the analytical capability of mass spectrometry characterizes a broad spectrum of components in very complex systems.

Specific applications

GC-MS is incredibly versatile. The technique is particularly good at separating single compounds or small groups of compounds from mixtures, as well as analyzing complex mixtures. For example, it has been used to monitor ethyl carbamate (a suspected mild carcinogen) which forms naturally in fermented foods and drinks. It has also been used to detect toxic compounds such as furan, acrylamide, and bisphenol A.

For complex mixtures, the technique has been used to detect and quantify pollutants in foods, such as pesticide residues on nuts and seeds, as well as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated compounds, phthalates, chloropropanols, and mono-aromatic hydrocarbons, including benzene, toluene, ethylbenzene, and xylene.

GC-MS' specific strength is providing volatile compounds profiles. This makes it particularly useful for food manufacturers wanting to test flavors and aromas, even at trace levels, and has been used to study coffee, beer, wine, and soy sauce.

Testing is important for several reasons. For example, it allows manufacturers to differentiate between and select the raw materials going into a product, such as the particular strain of hop that gives beer its distinctive smell and taste. It also helps to determine how storage time impacts aroma and taste.

Researchers from the Australian Wine Institute used GC-MS to study 18 aroma compounds reported to contribute to 'oxidative' flavor in wines. It allowed them to measure trace quantities of various alkenes, aldehydes, furans, and alcohols in the same analysis. They also studied how different bottle stoppers (synthetic and natural corks, and screw caps) can affect the formation of oxidation-related compounds as the wine ages.

Meanwhile, researchers from the Industrial Research Institute of Ishikawa and Ishikawa Prefectural University in Japan reported to Pittcon on how GC-MS can be used to characterize volatile compounds in Hojicha, a traditional Japanese drink with a distinctive flavor. In their talk, Characterization of Flavor Compounds in Roasted Tea Products by Foodomics Approach with GC/MS and GC/Olfactometry, they explained how Hojicha is produced by roasting green tea at high temperatures. It contains more than 100 volatile compounds, such as pyrroles and furans, and pyrazines, which are a significant contributor to its smell.

In their study, the researchers focused on global metabolite analysis (metabolomics) to characterize flavor compounds of 19 types of roasted tea by using GC-MS and GC-Olfactometry. Their analysis showed that shop-bought products contained more 2,4-heptadienal and furfural, producing fatty and burnt flavors. However, products purchased from specialty tea shops contained more pyrazines (roast flavor), geraniol and linalool (floral), and furaneol (caramel). Tea produced from roasting the stems of green tea contained more pyrazines, geraniol, linalool, and furaneol than teas derived from the leaves of green tea.

The application of GC-MS in beer testing was discussed in another talk at Pittcon entitled Metabolomic profiling of Beer using GC-MS and GC-FID. Researchers from Shimadzu Scientific Instruments and Ise Kodoya Brewery introduced a new approach towards classifying and visualizing beer quality, allowing them to determine how specific components influence taste from batch-to-batch and plant-to-plant.

Speakers from LECO Corporation discussed how GC-MS can be used to study food and drink containing cannabis extracts (usually CBD) in their talk entitled the Characterization of Food and Beverage Products Containing Cannabidiol (CBD) by GC-MS Analysis. As CBD has been associated with potential health benefits, the demand for its use in soda, tea, coffee, and candy is growing.

The LECO researchers explained how there is an increasing requirement for analytical methods to improve the understanding of these products, particularly in the areas of chemical composition and associated therapeutic claims. Knowing the quantities of CBD present, for example, is critical for prescribing suitable doses.

It is also essential to monitor for other components that may have been extracted from the plant, or for contaminants.

Such analysis is also useful for developing products that can mask or complement the aromas and flavors associated with CBD. The LECO researchers described how they undertook targeted and non-targeted analyte detection with GC-MS, and reported how they used it to quantify CBD levels and determine aroma profiles for a range of products.

Agilent was one of several manufacturers that exhibited its range of GC-MS instruments at Pittcon. For example, its 5977B single quadrupole GC-MSD includes the Agilent High-Efficiency Source (HES) for handling samples with particularly challenging detection limits. The Agilent 7000D and 7010B GC/TQ systems offer sensitivity for targeted analysis, while the 7250 GC/Q-TOF offers extreme flexibility from untargeted screening through to routine quantitation with Low Energy EI ionization capabilities coupled with high-resolution accurate mass (HRAM) and MS/MS capabilities.

In summary, gas chromatography-mass spectrometry (GC-MS) is a vital tool for the food and beverage industry, helping researchers to monitor contaminants, detect fraud, and develop new aromas and flavors.

References

- *Pastor, K. et al, (2019) Gas Chromatography in Food Authentication, Gas Chromatography. https://www.intechopen.com/books/gas-chromatography-derivatization-sample-preparation-application/gas-chromatography-in-food-authentication*
- *ThermoFisher Scientific, Emerging Techniques: Gas Chromatography Mass Spectrometry for Food Applications https://www.thermofisher.com/blog/food/emerging-techniques-gas-chromatography-mass-spectrometry-for-food-applications/*
- *Mayr, C.M. et al, (2015) Journal of Agricultural Chemistry, Quantitative Analysis by GC-MS/MS of 18 Aroma Compounds Related to Oxidative Off-Flavor in Wines*
- *Pittcon 2020, Characterization of Flavor Compounds in Roasted Tea Products by Foodomics Approach with GC/MS and GC/Olfactometry https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=728&SessionDateID=3*
- *Pittcon 2020, Metabolomic Profiling of Beer using GC-MS and GC-FID https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=1425&SessionDateID=3*
- *Pittcon 2020, Characterization of Food and Beverage Products Containing Cannabidiol (CBD) by GC-MS Analysis https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=1524&SessionDateID=5*
- *Agilent, Extensive range of GC/MS instruments https://www.agilent.com/en/products/gas-chromatography-mass-spectrometry-gc-ms/gc-ms-instruments*