

Abstracts

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The particle size paradox

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Particle size is an important piece of information for research and development, quality control, and quality assurance, as well as understanding the small physical details in a manufacturing or brewing process. These details can contribute to potential desired and undesired products. With recent technological advances, particles are now measured using various analytical techniques and instrumentation. Different analytical techniques seldom provide the same value for particle size. The “paradox” of particle sizing is that all the different values are the correct value. The four analytical particle sizing instrument techniques discussed and compared include dynamic image analysis, sedimentation, light-scattering, and electric sensing zone. The result of the comparison is that particle size data are specific to the analytical technique.

Jack G. Saad earned his ACS Certified B.S.Chem. degree in chemistry from the University of Georgia in 2002. As an undergraduate, he worked with a variety of tungsten compounds to induce photo-initiated polymerization of cyanoacrylate using specific wavelengths of visible light, an application used in the adhesive industry. He began his career at Micromeritics Instrument Corp. as a particle size analyst for their contract laboratory service, where he developed protocols to obtain particle size distributions using light scattering, x-ray sedimentation, and electro-zone sensing techniques. Beginning in 2004, he worked in the pharmaceutical industry for seven years at Kiel Laboratories and Élan Pharmaceuticals as a laboratory analyst for raw materials testing, manufacturing support, research and development, and quality control. He rejoined Micromeritics in 2011 as an applications specialist. In this position, he is responsible for applications support for particle size instrumentation and conducting research on potential applications for instruments and techniques.

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The feasibility of near-infrared spectrometry for predicting lautering performance of 100% pilsner malt

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In brewing, lautering is known to be a critical temporal step within the production process. Barley malt quality and, therefore, its seasonal fluctuations have a high impact on lautering performance. Although malt has to meet tight specifications, there are numerous variations inside those boundaries. Also, single quality specifications do not provide sufficient information about potential problems during individual brewing process steps, especially lautering performance. Therefore, the usage of near-infrared (NIR) spectra of malt in combination with multivariate statistics offers a new possibility to meet the demands of predicting lautering performance. As part of the study two barley varieties were malted under varied germination time, resulting in six malts with different malt qualities from which NIR spectra were taken and standard laboratory analyses were performed. Brew trials were done with these malts at the institute’s 60 L pilot brewery. Grinding and mashing processes were kept constant. Lautering was performed with a constant program to reduce technological influences and to

emphasize the impact of malt quality on lautering performance. For each malt five brews were carried out. Experts evaluated lautering quality in categories of good, normal, and bad performance. Those evaluations were correlated with the token NIR spectra by using multivariate data analysis techniques to build a calibration. Also, a strictly mathematical model of prediction was developed and validated by expert evaluations of lautering quality. Both methods were adapted to predict lautering performance from malt batches and showed a high predictability in an industrial brewing scale. With this study the feasibility of NIR spectrometry in combination with multivariate statistics to provide a fast and simple application to predict lautering performance by analysis of malt was proven.

Christopher Holtz was born in 1984 in Munich, Germany. He studied brewing and beverage technology at the Technische Universität München, Weihenstephan. Since 2010 he has been a Ph.D. student at the Lehrstuhl für Brau- und Getränketechnologie, TU München-Weihenstephan. Christopher works in the field of NIR applications for analysis, evaluation, and prediction of malting and brewing process steps according to raw material or malt spectra.

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Development of a method for quantifying hop aroma compounds in a dry-hopped beer using HS/SPME-GC-MS/O with aroma extract dilution analysis

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A method was developed for measuring hop aroma compounds in heavily dry-hopped commercial ale. Compounds to be quantified were determined by aroma extract dilution analysis (AEDA). AEDA utilizes an olfactory port to evaluate the aroma of column effluent. The beer of interest was serially diluted, and the compounds with the greatest aroma impact were determined as they persist at the highest dilution levels. The identities of the compounds were confirmed using mass spectral data with NIST library identification, as well as retention index matching. The concentration range of each compound to be quantified was determined, as well as optimization of the internal standard. Other parameters optimized included solid phase microextraction (SPME) extraction and desorption times, inlet split ratio, GC separation, and single ion monitoring (SIM) parameters. Method validation was conducted to demonstrate method robustness. Work in progress includes the positive identification of additional compounds that were aroma active but could not be profiled with sufficient resolution by the mass spectrum detector.

Grant Ruehle received his B.S. degree in chemistry with honors from the University of Denver in 2011. He received several distinctions as an undergraduate student, including the Goldwater Foundation Scholarship. Grant began working as an intern at New Belgium Brewing in Fort Collins, CO, shortly before graduating and was hired as a full-time chemist upon completion of his degree. Grant received the Institute of Brewing and Distilling Diploma in Brewing in 2012. While at New Belgium, Grant has focused on GC-olfactory work and GC-method development, as well as process improvement and optimization. Grant has been an ASBC member since entering the brewing industry and is currently chairing a technical subcommittee. Grant spends his free time trail running and back-country skiing in the mountains of Colorado.

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Combining and aligning analytical evaluation improvements with sensory in global beverages

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This paper recounts and predicts the progress achieved in advancing sensory results and how these are aligned with analytical evaluation techniques. Until recently this has been regarded as unreliable. Recent and ongoing developments to improve analytical evaluation methods for key flavors are described, and their importance is explained for quality control and training protocols. In parallel the requirement for GMP quality flavor standards has driven the development of new flavor preparation methods and new clinical blister packaging for each flavor capsule. The combined significance of these analytical and sensory improvements in accuracy, consistency, and repeatability is demonstrated through examples of subsequent and ongoing training and proficiency testing programs. The authors thank the global customers participating, ASBC, and U.S. regulatory bodies for close cooperation with the Supply and GMP Flavour Centre teams.

Boris Gadzov is director of global sensory management at FlavorActiV. Boris joined FlavorActiV in 2006, where he began as a global sensory manager. Boris is fluent in eight languages, and his language skills have helped develop the business overseas. Before being appointed director of global sensory management Boris presented numerous sensory taster-training programs on-site at the customers' locations, as well as in the customers' local languages. Boris obtained a Ph.D. degree in food molecular microbiology before joining the FlavorActiV team.

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A systematic search for novel, hybrid yeast strains with improved aroma profile and fermentation characteristics

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Fermented foods and beverages have been consumed by humans for over 8,000 years. Originally, these fermentations were spontaneous processes, without any control or knowledge of the microbial driving force behind them. This led to irregular and often inferior end-products. It was not until the late 19th century that scientists proposed to use a well-defined microbial starter culture, consisting of one pure yeast strain. Although this greatly increased the reproducibility of the fermentations, the main difficulty was the selection of a strain with all the beneficial characteristics necessary for an efficient and high-quality fermentation. Until recently, the appropriate tools and knowledge were lacking to make a well-considered and scientifically sound choice about which strain to pick. This is especially true in the beer industry, where brewers often use a particular yeast because of historical rather than scientific reasons. In this study, we screened a wide variety of yeast strains that are particularly suited for industrial purposes such as beer brewing. This resulted in a large collection of industrial yeast strains (>600), with each strain characterized to an unprecedented level of detail (genetic relatedness, fermentation characteristics, aroma production, stress resistance, flocculation, etc.). This high-throughput screening and subsequent data analysis revealed enormous geno- and phenotypic diversity among different *Saccharomyces cerevisiae* strains. Our data allow us to rapidly select yeast strains for specific industrial purposes. Additionally, this dataset provides an excellent platform to select strains for breeding of novel yeasts with beneficial traits from both parents. Using several different breeding approaches (all non-GMO), hybrids with superior beneficial characteristics were constructed. These hybrids were tested and compared to their ancestors. Finally, the dataset also

allows us to dig deeper in the genomes of yeast strains and unravel the underlying genetics of industrially relevant traits like ethanol tolerance and production of aromatic esters using high-quality whole-genome sequencing.

Jan Steensels received a B.S. degree in bioscience engineering from the University of Leuven, Belgium, in 2008 and an M.S. degree in bioscience engineering, with a major in cell and gene technology and minor in industrial microbiology, from the same university in 2010. He did his master's thesis in the Centre for Malting and Brewing Science in 2009–2010. In 2010, Jan joined the VIB laboratory for Systems Biology led by Kevin Verstrepen as a Ph.D. student.

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Large-scale systems biology approach to select and create novel yeast strains with superior fermentation characteristics

KEVIN J. VERSTREPEN (1)

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We present a resource that allows us to select and create superior brewing yeasts. Compared to certain other fermentation industries (bread, wine), the beer industry has given relatively little attention to the selection and optimization of brewing yeasts. This is at least partly due to the fact that each brewery often uses only one or a few particular, proprietary yeasts, whereas wine and bread yeasts are often produced by large, specialized companies. This implies that many breweries are using sub-optimal yeasts and that there is a vast potential for selection and breeding of superior beer yeasts. In past years, our research team has gathered a large collection of more than 500 different industrial *Saccharomyces* yeasts. Each of these yeasts was screened for more than 100 different industrial properties, including such traits as fermentation capacity, ethanol resistance, temperature tolerance, flavor production, and flocculation. In addition, we have also assessed the genetic background of each of the yeast strains. Together, this large set of data (500 yeasts × 100 properties × genetic background) allows us to select yeasts with specific properties to accommodate specific beer types and fermentation properties. Moreover, using our database also allows us to select ideal parents to generate novel yeasts (through crossing, protoplast fusion, genome shuffling, or directed evolution) with improved or combined properties. Last, but not least, advanced data analysis (including biclustering methods) allows us to find correlations between specific traits and/or genotypes.

Kevin Verstrepen studied biological engineering at the University of Leuven. For his M.S. thesis, Kevin joined Isak Pretorius' group at Stellenbosch University to study flocculation in wine and beer yeasts. Kevin subsequently focused on yeast genes involved in flavor formation during fermentation. After obtaining his Ph.D. degree, he joined the lab of Gerald Fink at M.I.T. Revisiting the topic of his M.S. thesis, Kevin discovered that the genes responsible for yeast flocculation contain arrays of highly unstable repeats in their DNA sequence. After spending two years at MIT, he joined Harvard University as a Bauer Fellow. In 2007, he was promoted to lecturer and started teaching industrial microbiology to undergraduate students. Meanwhile, he headed a research team dedicated to studying fundamental genetics using yeast cells as a model system. In 2009, Kevin moved his team to Leuven University, where he holds a dual appointment as an associate professor and research director at the Flanders Institute for Biotechnology (VIB). His team continues to investigate eukaryotic genetics and epigenetics, with specific interests in industrial microbiology.

A method for bioprospecting geographically unique *Saccharomyces* and *Brettanomyces*, including methodology for sampling, propagating, characterizing, and preserving samples to be maintained for geographically unique brews

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There is currently a resurgence of traditional brewing methods incorporating contemporary ingredients among craft brewers. This includes regionally unique varieties of grains and hops, as well as inclusion of yeast and beneficial microorganisms. In a brewer's pursuit of "terroir" (flavor of the earth), varieties of brew such as saison, lambic, cider, meade, and strong ale are achievable with local species of yeast to complement local grains, hops, and fruits in these ales and other "estate" type ales. For this reason Clemson University researchers have developed a method that breweries may follow to propagate regionally specific varieties of yeast to then characterize and refine for brewing. Wild-yeast communities were isolated from Clemson's Musser Experimental Fruit Research farm in Seneca, SC, by in-field whole-fruit mashing techniques and developed for easy repeatability in craft brewing applications. Propagation and storage techniques were investigated for maintaining community fidelity and fermentation characteristics of wild-yeast communities. Nine pure and poly-cultures were selected based upon initial aroma and developed by acid and alcohol enrichment (pH 4, 10% ABV) to limit bacterial presence and stored at -80°C and 4°C. Striking, top-cropping, layered yeast washing, and centrifugation were evaluated as isolation and propagation techniques. Genetic drift and fermentation characteristics were monitored by FAME analysis, DGGE DNA fragment sequencing, and brewing application with varied beer styles to monitor attenuation, aroma, and colony morphology characteristics. This report outlines the methods used and data collected by Clemson researchers so that it may be replicated by interested parties.

Yancey Appling will receive a B.S. degree in microbiology in May 2013 from Clemson University and will enter the biotechnology field as a synthetic biologist. Yancey's past and present work includes interning in the Ajo-Franklin synthetic biology group at Lawrence Berkeley National Laboratory, engineering microbes for production of industrial chemicals, and investigation of yeast domestication and characterization for brewing at Clemson University. After completing a semester of "The Science of Beer" at Clemson, Appling expanded his understanding of zymology through collaborative research with the Clemson Biosystems Engineering Department to develop an experimental plan for the selection of regionally unique brewing strains from wild cultures.

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Beer—To be or not to be? It's really a question of food safety!

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Unlike other food industries, the beer industry is unique, as beer manufacturing has many food safety benefits. Some of them include the microbiologically stable properties of worts due to boil time and the addition of hops; the antibiotic effect of hopped worts, which favor the activity of brewer's yeast over less desirable microorganisms; and the low pH of beer, and lack of fermentable sugar and oxygen, which inhibit growth of pathogenic and most non-pathogenic bacteria. Nevertheless, biological, chemical, allergenic, and physical food safety hazards can potentially be present during the beer manufacturing process at various stages. By adopting a systematic approach to food safety throughout the supply chain, breweries can eliminate these hazards or control them to acceptable levels. The Food Safety and Modernization Act (FSMA) was

passed in 2011. It aims to ensure the U.S. food supply is safe by shifting the focus of the Food and Drug Administration from responding to food adulteration to preventing foodborne illnesses. Therefore, breweries, although believed to be "low risk food producers" due to the inherently safe beer manufacturing process, will be required to comply at some level with FSMA 2011. One way of achieving this is through hazardous analysis critical control points (HACCP). HACCP is a system that identifies and evaluates food safety hazards using risk assessments and designs control measures to reduce these hazards to safe levels. It can be used from raw material production, procurement, delivery, receipt, storage, handling, manufacturing, distribution, and consumption of the finished product. A HACCP-based approach to food safety includes pre-requisite programs that enable the food manufacturer to reduce the number of food safety hazards, resulting in fewer critical control points (CCP). One pre-requisite program that breweries have implemented is the supplier management program, as often times breweries do not have the resources to perform the required quality assurance monitoring. This program places the majority of the food safety responsibility on the suppliers. Agricultural commodities such as barley and hops are normally covered under such a program. With the recent number of foodborne illnesses in United States due to contaminated food products, the Food and Drug Administration has recently published two proposed rules that address produce (fruits and vegetables) safety and hazard analysis and risk-based preventive controls. Consequently, as the brewing industry continues to create novel beer brands that contain interesting and exotic ingredients, it is paramount that much thought be given to food safety and the implementation of a food safety management system, as such a system will enable breweries to both consistently produce safe products and meet food safety regulatory requirements. This paper will explore one brewery's journey to implement a food safety management system.

Patricia L. Pratt received her Ph.D. degree in biochemistry and microbiology from Heriot-Watt University, in Edinburgh, Scotland. Her brewing career commenced in 1993 with Heineken International, Commonwealth Brewery Limited in Nassau, Bahamas. She is currently employed with MillerCoors, Golden, CO, in the Corporate Technical Quality Division. One of her responsibilities includes food safety. She serves as an Advisory Board member for the International Scheme—Brewery Analytes Proficiency Scheme (BAPS), which is administered by the Campden BRI Brewing Division (United Kingdom) and LGC Standards (United Kingdom).

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Investigations on the perfect draught beer

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For years, people have talked about the quality of draught beer and the potential for improvement. Developments in dispensing systems are, however, in most cases, just to improve the appearance of the equipment. The most important aspect for a brewer, hygiene, is often neglected. As a consequence, the quality of beer is destroyed in the last few meters, just before the consumer enjoys it. In recent years in Weihenstephan a series of tests was carried out to enhance the quality of draft beer. They were partially presented already, but since then have been extended with a number of other studies. The investigations of the former tests focused on the handling of tap cleaning. The newer tests now include the hose integration into the beer lines, hose integration to different draught equipment, treatment of beer lines such as by cleaning or cooling, and regular cleaning of the keg coupler. From these findings, a

strategy can be developed to build a beverage dispensing system at the highest level of hygiene. It is also possible to use hygiene guidelines to deal with defining beverage dispensing systems. Additionally, tastings were conducted with beers that were defined as polluted with beer spoiling bacteria. Since the permissible bacterial count in draught beer is discussed again and again, these investigations revealed at what bacterial counts sensory changes occur in draught beer. This will be used in the future as a reference point for the last date of pipe cleaning.

Johannes Tippmann graduated from university in 2004 as a diploma engineer for brewing sciences and beverage technology. In 2005 he started work on his Ph.D. thesis with Karl Sommer at the Lehrstuhl für Verfahrenstechnik Disperser Systeme, TU München, on solid handling in the brewhouse. In 2012 he changed his affiliation and is now working as group leader for the Brewhouse Processing and Beverage Dispense Technologie work group at the Lehrstuhl für Brau- und Getränketechnologie, TU München. Since 2000 he has worked as a student research assistant with dispensing systems and collected lots of experience in this subject area. Since 2006, he has been responsible for research issues in dispensing systems. He is also a member of the Dispensing Systems Technical Committees of the government Association for Food and Catering Industry (BGN) and of the DIN German Institute for Standardization. He is working for the MEBAK Dispense Work Group and has published a number of papers.

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Application of Si₃N₄-microsieves for a rapid detection of trace contamination in beverage industries

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A market study performed by the institute of brewing and beverage technologies (TU Munich, Weihenstephan, Germany) showed that detection of CFU (colony forming units) is still the state-of-the-art method for controlling trace contamination in filtered beer. Detection of CFU is a two-step process consisting of membrane filtration and incubation on nutrient media, and it is used by >90% of the German breweries with capacities above 10,000 hL/annum. Major drawbacks of this method, among others, are a long incubation time (3 to 14 days depending on the growth rate and type of microorganisms) and a high potential for misjudgment and -interpretation. Alternative methods such as PCR or fluorescence techniques are costly and, hence, only used in <10% of the breweries. Consistently, the market study also showed that the current time-consuming and labor-intensive analysis methods are a major concern of the industry. The scope of this project is the development of a rapid and robust detection method for microbiological analysis that avoids incubation at every stage. It consists of dead-end and cross-flow microfiltration with silicon nitride (Si₃N₄) microsieves based on silicon wafers from the semiconductor industries. The major advantages of these microsieves are low filter resistance, high chemical and thermal stability, as well as a defined and small pore size distribution. Moreover, microsieves are cleanable and therefore reusable. This study mainly focuses on the implementation of microsieves in an automated microfiltration system and on the assessment and evaluation of possible analyzing techniques. Thus, the pure surface filtration with 1 µm thin membranes facilitates “on-the-chip” analysis with optical fluorescent techniques. First results showed that laboratory filtration based on silicon nitride microsieves is procedurally reproducible, and by utilizing the fully automated cross-flow filtration procedure, it was possible to improve the laboratory dead-end filtration of beer by over 60%. Furthermore, silicon nitride microsieves showed much higher fluxes than state-of-the-art polymeric membranes. Thus, even for microorganisms with a low cell count, the developed “on-the-chip” method enables reproducible detection directly after filtration by utilizing no staining and fluorochrome staining in combination.

Roland Novy graduated in 2010 from Technische Universität München with a master's degree in engineering for brewing sciences and beverage technology. In the same year he started work on his doctoral thesis with Thomas Becker at the Institute of Brewing Science and Beverage Technology. His research project is titled “Development of a Microbiological Rapid Detection Method Based on Silicon Nitride Microsieves.”

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Dry hopping: Myths versus realities

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(1) Orval Brewery; (2) KULeuven-FBIW-M²S-MbS-LIBR; (3) Barth-Haas Group/Hops Academy; (4) University of Liège, Gembloux

For many years, we have worked with colleagues and researchers to understand the origin, issue and fate of different molecules solubilized by the dry-hopping process. The target is to understand and explain the extraction dynamics and to show the complexity of evolution of volatile and non-volatile fractions of noble hop varieties. It is obvious to say that “dry hopping” is more than just introducing some solid matter, such as leaves or parts of them, into a small or large reactor, such as an extractor or fermenter. Indeed, it is already well known that the complexity of the molecules present confers to the system a high level of chemical instability. Further, the susceptibility of some compounds to oxidation by molecular oxygen is so strong that it creates tremendous problems for brewers who wish to keep a nice natural hoppy flavor in their beer. In this work, we considered not only the different fractions regarding their volatility, but based our research on the solubility criteria used in the perfumery and reported in Franchomme's diagram. In this way we were able to screen between the compounds solubilized taking in account their polarity and their electric charge. Depending on the dry-hopping technique applied, static or dynamic, under air or inert gas, the issue of each molecule will be different and sometimes surprising. Indeed, under oxidative conditions it's known that β-myrcene is unstable, while some dry-hopping conditions as in closed stainless-steel kegs can extend the shelf life of this molecule for a very long time (several weeks). In the same way, the key place of *R*-linalool is discussed in its function in the environmental conditions of the technique. It will be explained by exact science why and under which conditions these typical molecules evolve in the different ways to form other more or less odorous molecules. Finally, taking into account industrial aspects, practical examples will be shown concerning the influence of crop year, variety and essential beer chemical characteristics regarding some oxidative aspects such as flavanoids content and oxidation rate. In conclusion, we try to shed light on the reasons the art of hopping is successfully applied by various brewers, while it is ineffective for others.

Guy S. Derdelinckx is the holder of the Chair of Specialized Aspects of Brewing Microbiology at KULeuven-Leuven-FBIW-M²S-LIBR-site Arenberg 33. He obtained an M.S. degree with a specialization in tropical and subtropical agronomy (1979) and defended a Ph.D. thesis in the brewing field on the stability of oligomeric flavanoidic structures (1985). In addition to intensive consulting activity in breweries in Belgium, Europe, and Africa (1989–2004) he developed research to improve the quality of top-fermented, bottle-fermented, and sour beers as lambics. In this way, he tried to help Belgian specialty beer brewers to make their beer more consistent and to improve resistance to different microbial and chemical reactions involved by oxidation. In the same way, he has been active for many years in the quality of the dry-hopping process. During the five last years, his research team has developed, with the help of colleagues and through international cooperation, University of Cork (J. Titze) and Weihenstephan-Triesdorf (V. Ilberg), a model explaining the mechanism of primary gushing of beer (J. ASBC, 70:249-256, 2012).

Hops as a regulator for the red coloration of beer

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In the early Middle Ages, beer brewers in some German cities (e.g., Hamburg and Nuremberg) converted from red beer technology to white beer technology (*Weißbiertechnologie*). Up to now one could only say with confidence that this did not represent the advent of wheat beer production (in Germany white beer [*Weißbier*] or wheat beer [*Weizenbier*] is used synonymously). Could it be that this actually meant that the brewers were attempting to produce beer of a certain color? On the whole, it is quite difficult to brew a beer with a distinctly red color using only barley. However, in field experiments conducted in Syria with the black barley native to the area and using Bronze Age brewing practices (a cold mash process and mixed fermentation), unhopped beers exhibited a distinctive red hue. If the wort was hopped, however, the finished beer was not red. This poses the intriguing question of whether individual hop fractions suppress red coloration stemming from barley. And, with reference to historical accounts from the Middle Ages, is the description of the shift from red beer technology to white beer technology a clear indication of the advent of the collective use of hops in brewing? This would be entirely consistent with many other clues from that period. In the proposed experiments, individual hop fractions will be added in specific concentrations to a standardized black barley malt mash and fermented with yeast and strains of lactobacillus. The lactic acid bacteria are important, because comprehensive preliminary testing has shown that a pH of 3.8 is necessary in order to bring about a noticeable red pigment in an unhopped beer. If a fraction or fractions are described, ways need to be identified regarding how, after removal of this fraction or these fractions, a natural red hue arises in the beer, while of course retaining all other necessary quality characteristics for which the hops are responsible.

*Martin Zarnkow apprenticed as a brewer and maltster from 1989 to 1991 at a small brewery in Frankonia. Martin finished a Dipl.-Ing. (FH) degree, option brewing technology, in 1996 at TU München in Weihenstephan and worked as a brewmaster for one year in a medium-sized brewery in Germany. Since 1997 Martin has been the head of the research group for beer and beverage technologies at Lehrstuhl für Brau- und Geträketechnologie (Institute for Beer and Beverage Technology) at TU München in Weihenstephan. In 2010 he finished his external Ph.D. research at the University College of Cork, Ireland, on the subject "Proso Millet (*Panicum miliaceum L.*) a Sustainable Raw Material for the Malting and Brewing Process."*

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Hop-derived water-soluble contributions to aroma in beer

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While a significant source of hoppy aroma in beer comes from the oil fraction in hops, researchers have hypothesized that water-soluble compounds found in hops, such as terpenoid glycosides, may contribute to the aroma and flavor of beer. This study examined the origin of the aroma contributed by the water-soluble components in hops. Spent material from supercritical CO₂ extraction of Simcoe hops was boiled in a pH 4.2 buffer solution (50 g of spent hops/L of buffer) for 3 hr. The supernatant from the cooled and centrifuged mixture was treated separately with a lager yeast, ale yeast, acid addition (pH 2.7), or 1 of 4 individual enzyme treatments (β -glucosidase + pectinase mixtures). Volatiles arising from the treatments with aqueous extracts of the spent hop materials were measured using a stir bar sorptive extraction

(SBSE)-GC-MS. Linalool, nerol, garaniol, and other 20 terpenoids, as well as β -damascenone and β -ionone were analyzed. Different treatments generated unique volatile aroma profiles. The descriptive sensory analysis of the extracts identified distinct differences among the treatments. The treatment at pH 2.7 produced the most intense overall and pine aromas. In addition, herbal/tea aroma was most intense for pH 2.7 and one of the enzyme treatments. Ale yeast produced greater hop aroma intensity than the lager yeast. The results confirm that the water-soluble components left behind in the spent hops could contribute unique hop aroma to the final beer.

Thomas Shellhammer is the Nor'Wester Endowed Professor of Fermentation Science in the Department of Food Science and Technology at Oregon State University (OSU), where he leads the brewing science education and research programs. His brewing research investigates hops and beer quality, hop-derived bitterness and its quality assessment, and the origins of hop aroma and flavor in beer. He directs the brewing education component of the Fermentation Science program at OSU and teaches courses on brewing science and technology, beer and raw material analyses, as well as an overview of the history, business, and technology of the wine, beer, and spirits industries. Tom received his Ph.D. degree from the University of California, Davis, in 1996. During the 2008–2009 academic year, while on sabbatical leave from OSU, he worked at the Technical University of Berlin as a Fulbright Scholar and Alexander von Humboldt Fellow. Tom is the International Section chair and Board of Examiners member for the Institute of Brewing and Distilling, London, England; a member of the Editorial Board of the Master Brewers Association of the Americas' Technical Quarterly; and ASBC vice president.

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The mathematics of microbiology

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Although many microbiologists do not enjoy math, there are situations in microbiology where math can be usefully applied. Among these are sampling to detect contamination and identification of contaminant microorganisms (both bacteria and wild yeasts). The likelihood of detecting a contaminant depends on its concentration, the volume basis sampled, and the number of replicate samples taken. In situations where a large volume of sample can be passed through a membrane filter, the chances of detecting contamination are fairly good. Where difficulty in filtration limits the volume that can be filtered (such as occurs with high viscosity or the presence of a significant amount of solids in a sample), direct plating may be needed. This limits the volume to the amount that can be taken up by agar, and the likelihood of detection is much less. Simulation modeling can be used to compare the efficacy of various sampling schemes (numbers and volumes of samples) for different levels of contamination. Selective media for detecting wild yeast contamination of culture yeast can be based either on substances that inhibit growth of culture yeast or on sole carbon or nitrogen sources that culture yeast can't use. In the latter case multiple carbon (or nitrogen) sources can be combined to detect a higher proportion of wild yeast species, as long as none of the components can be used by the culture yeast. A database of 83 characteristics for 469 yeast species was used to produce combinations of carbon sources or of nitrogen sources that should detect 84 or 88% of non-*Saccharomyces* species, respectively. Microbial contaminants can be identified in a number of ways. For some of these approaches (decision tree and similarity matching coefficients) the outcomes of each test must be scored either positive or negative, and an incorrect result is often fatal to successful classification. This binary scoring compresses (and so discards) the information from intermediate responses (such as delayed, weak, or slow growth). Very specific identification of unknowns is possible with antibody- or DNA-

based methods, but this requires a battery of probes, including each possible (or likely) contaminant, and can be expensive in time or money. With multivariate pattern recognition, the information in intermediate responses can be preserved, and single incorrect results are less damaging. Variation in responses among species can occur due to gene swapping between species or strains or because of different individual responses between different strains of a species. Fuzzy clustering holds particular promise for this application because it represents responses for an organism in a probabilistic way.

Karl Siebert received a Ph.D. degree in biochemistry from Penn State in 1970. He then joined the Stroh Brewery Company in Detroit, MI, where he spent 18 years and held positions from research associate to director of research. In 1990, he joined Cornell University as a professor of biochemistry in the Department of Food Science, where he has continued to work on beverages, particularly beer. He received two MBAA Presidential Awards, and with his colleague, Penny Lynn, received the Eric Kneen Memorial Award (for the best paper published in JASBC in the prior year) three times. He received the ASBC Award of Distinction in 1999 and the MBAA Award of Merit in 2011.

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Detection and identification of beer spoilage bacteria using T-RFLP

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Beer is considered a safe beverage because of its high microbiological stability. However, a few kinds of bacteria that belong to the genera *Lactobacillus*, *Pediococcus*, *Pectinatus*, and *Megasphaera* can grow in beer, and the spoiled product causes serious economic losses for brewers. In order to brew high-quality beers, detection and identification of these spoilage bacteria are very important. In this study, we developed a novel detection and identification method of beer-spoilage and related bacteria using terminal restriction fragment length polymorphism (T-RFLP). After in silico analysis, we successfully designed primer sets to amplify specific fragments of 16S rDNA in each genus (*Lactobacillus*, *Pediococcus*, *Pectinatus*, *Megasphaera*). After amplification, purification and restriction enzyme digestion were performed to obtain species- or group-specific terminal restriction fragments (TRFs). TRFs were separated and detected using a genetic analyzer. As a result of T-RFLP analysis with target species, the actual TRF size matched the predicted TRF size well, while no amplification products or TRFs were observed with non-target species tested. The detection limit of the T-RFLP method was determined for the two *Lactobacillus* species (*L. brevis* and *L. paracasei*) and was found to be 2×10^4 CFU/test and 6×10^4 CFU/test, respectively. We conclude that the T-RFLP method described here provides sufficient reproducibility and sensitivity to detect beer-spoilage and related bacteria in complex environments.

Hajime Kanda received a master's degree from the Department of Agricultural and Environmental Biology, Tokyo University, Japan. He joined Sapporo Breweries, Ltd. in 2006 as a microbiologist in the Frontier Laboratory of Value Creation.

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Determining the level of microbial contamination causing haze formation in bottled craft beer by inoculating with a variety of brewery bacteria and wild yeast

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With the exception of some styles of beer (*Weissbier*, *Witbier*, and some sour beer varieties), haze (turbidity) is usually an undesirable characteristic. The formation of haze in beer can be linked to filtration issues, an intricate protein-polyphenol complex, microbial contamination, or a combination of the three. This study focused on the levels of microbial contamination that can cause haze formation in bottled craft beer. While the presence of microbial contamination of any type shows issues within the process stream, not all levels of contamination will yield undesirable characteristics (off-flavors, ropiness, and/or turbidity). Three types of bottled craft beer ranging from 4.8 to 6.5% alcohol by volume with <250 ppb dissolved oxygen, <69 ASBC haze units, and 0 colony forming units per 100 mL sample were used in the study. Fifteen species within six groups of bacteria (*Lactobacillus*, *Pediococcus*, *Acetobacter*, *Gluconobacter*, *Pectinatus*, and *Megasphaera*) and five wild yeast species (*B. lambicus*, *B. bruxellensis*, *P. fermentans*, *S. bayanus*, and *S. pastorianus*) associated with brewery contamination were chosen for analysis. To mimic the occurrence of a post-fermentation, bottling line (packaging) contamination, bottles were inoculated at varying levels of contamination (3–100 colonies/mL) and re-sealed for aging. Inoculated bottles were incubated and analyzed for three durations (14, 28, 84 days) and at three temperatures (5, 20, and 30°C). A turbidity meter was used to assess the haze level, and traditional microbiological techniques were used to validate and monitor contaminant levels throughout the experiment. As expected, higher temperature incubation was linked to higher levels of haze formation in most of the samples when compared with the controls ($P < 0.05$). Nine of the fifteen bacterial species tested created a significant haze level increase ($P < 0.05$) when incubated at 5°C. Out of the nine bacterial species causing a significant increase in haze, only the highest inoculation levels (50–100 colonies/mL) created a strong haze (>276 ASBC haze units) in the beer after 84 days of incubation. The samples inoculated with wild yeasts created strong haze levels when incubated at high temperature; however, only *B. lambicus* and *S. pastorianus* created a significant haze increase through all incubation temperatures. *Pediococcus* and *Pectinatus* genera produced the quickest haze ($P < 0.05$ when compared to the control) and strongest haze (>500 ASBC haze units) formation out of the contaminants tested. Increased alcohol level diminished the haze-forming potential of samples containing *Acetobacter* and *Gluconobacter* genera of bacteria. The survival rate of the contaminants shows divergent results with regard to haze formation. Low survival (<10% inoculation level) of some wild yeast samples formed a significantly high haze level ($P < 0.05$), whereas most bacteria with low survival did not produce a significant haze level increase ($P > 0.05$).

Joshua Adler received a B.S. degree in biology from Dalhousie University in Halifax, NS, Canada. While pursuing his degree he became very interested in food science and was the first Dalhousie student to obtain a minor in the discipline. His undergraduate thesis focused on problems encountered in wheat beer production, which he presented at the 2011 ASBC Annual Meeting. Josh is continuing his brewing research as an M.S. candidate at "Dal" where his research is focused on using various techniques to detect the premature yeast flocculation potential of malt, which he presented at the WBC 2012. In 2011, Josh became the quality manager at Propeller Brewery. At Propeller Josh strives to routinely produce beers of the highest quality, as well as create innovative and useful brewing research. When outside the laboratory, Joshua can usually be found training at the boxing club, at the movie theater, or enjoying a pint with his friends. One of his life's ambitions is to visit as many of the worlds' brewing and distilling regions as possible.

***Pediococcus claussenii* plasmid copy number when grown in beer**

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Pediococcus claussenii ATCC BAA-344^T (Pc344) is a beer-spoilage bacterium with a sequenced genome that consists of eight plasmids and a 1.8 Mb chromosome. Considering the nutrient limitations of the beer environment and the energy required to replicate plasmids, we were interested in the copy number of each mobile element when Pc344 is growing in beer. We analyzed a non-ropy variant (Pc344NR) that had lost plasmid pPECL-7, as no difference in beer-spoilage ability (i.e., hop resistance, ethanol tolerance, growth rate) is seen between the ropy and non-ropy isolates, and DNA extraction is more efficient and reliable from the variant that cannot produce exopolysaccharide. Plasmid copy number was assessed via quantitative PCR on DNA extracted from Pc344NR growing mid-exponentially in beer and MRS (a nutrient-rich environment for comparison). Plasmid-specific primers were used to determine the absolute copy number of each mobile genetic element in three biological replicates from each growth environment. Plasmid copies per bacterial cell were then established based on the concurrent detection of two single-copy chromosomal genes. Very little change in copy number was found for pPECL-4 and the two small cryptic plasmids (pPECL-1 and -2) in Pc344NR growing in beer compared to MRS. In contrast, plasmids pPECL-3, pPECL-5, and pPECL-6 all showed higher copies when Pc344NR was growing in beer. Lastly, the *horA*-containing plasmid pPECL-8 was barely maintained in cells growing in MRS, while approximately one copy was kept in all cells growing in beer. The finding of higher copy numbers being maintained in the energy-limited beer medium led us to believe that these plasmids are very important for Pc344NR growing in this environment. We, therefore, sought Pc344NR plasmid-variants and isolated several that maintained the same ability to grow in beer, despite lacking one or more plasmids. Plasmid copy number qPCR analysis was broadened to include select plasmid-variants and demonstrated trends that were mostly similar to those found for Pc344NR. We can conclude that the *horA*-plasmid pPECL-8 is very easily lost in Pc344 isolates growing in MRS, whereas the plasmid is maintained (although not essential) in cells growing in beer. It also appears that pPECL-3 is required for growth in either environment, as no variants have ever demonstrated the ability to lose this plasmid. Further investigation of these plasmid variants is ongoing, with the goal of elucidating the role specific plasmids (and genes contained on each) play in the ability of Pc344 to survive in the harsh niche posed by beer.

Barry Ziola received a B.S. (with honors) in botany from McGill University, Montreal, QC, Canada, in 1970. After completing a Ph.D. degree in biochemistry at the University of Alberta, Edmonton, AB, Canada, in 1975, he undertook a three year postdoctoral stint at the University of Turku, Turku, Finland. He has been at the University of Saskatchewan, Saskatoon, SK, Canada, since 1978, with promotion to professor coming in 1986. His interest and continuing research in brewing spoilage bacteria dates to the mid-1980s.

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Introduction of a new beer tasting and evaluation method and its effects on quality

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Among its eight domestic breweries, Asahi Breweries, Ltd. produces ≈14 million hL of its flagship product, Asahi Super Dry, annually. The production of beer with uniform quality at each brewery is essential

both for achieving target flavor and for meeting or exceeding customers' expectations. Beer samples from each brewery have routinely been evaluated according to items on the beer flavor wheel; the overall evaluation is then graded based on a scale of five separate levels. However, in real practice, if off-flavors are present, the evaluation score is determined based on only two grades (presence or absence of off-flavors). Thus, overall scores tend to be similar and only minimally reflect the positive side of the flavor wheel. In addition, the overall evaluation score is based on sensory profile analysis, which varies according to the taster's experience and sensitivity and is strongly influenced by the presence of a single off-flavor, rather than by the total flavor balance. Therefore, to improve upon this method, we developed a novel concept-based approach to beer tasting and evaluation. In this approach, we set five concrete sensory characteristics that each represent the product's target taste profile. During tasting, the overall evaluation score is graded according to the results of the five characteristic scores (graded into three levels). Principal component analysis (PCA) is then used to analyze the overall evaluation score, and the five characteristic scores and their relationship are plotted on a two-dimensional graph using the PCA scores obtained. The analyzed data are then assembled into a common file and shared electronically among the tasters and brewmasters in each brewery. This visualization of both the sensory characteristic and overall scores enables trends in the flavor of each brewery's beer samples to be easily understood. Both scores have already proven useful for drafting and verifying new measures aimed at improving flavor. After all eight breweries introduced the concept-based tasting approach in 2010, variations in quality decreased, and the average overall evaluation score of Asahi Super Dry increased by 4%. One of the major contributing factors to these improvements was that the concept-based tasting scores were effectively utilized for the plan-do-check-act cycle of various flavor improvement projects. Therefore, we anticipate that this novel tasting and evaluation method will act as an effective indicator and contribute to uniform quality production.

Shoji Iguchi received his B.Agric. degree in 1997 from Kobe University, Hyogo, Japan, in agricultural engineering. He began employment with Asahi Breweries, Ltd. in 1997 as an engineer in the Ibaraki Brewery. In 2008, he was awarded an M.S. degree in brewing and distilling from Heriot-Watt University, Edinburgh, UK. Since November 2012, he has functioned as the deputy manager for the Production Technology Center R&D Promotion Office at the Ibaraki Brewery.

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Establishment of a control technology for irritating-mouthfeel with bentonite

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Irritating-mouthfeel, including that induced by carbonation and bitterness, is one of the major characteristics of beer, together with flavor and taste. Although intensive research has been conducted on controlling the flavor and taste components of beer, little has been reported on the factors influencing such feeling. Therefore, the objective of this research was to establish a control technology for irritating-mouthfeel. We first investigated the key flavor of beer that contributes to irritating-mouthfeel and found a negative correlation between protein concentration and mouth irritation. We then searched for a material that selectively removes proteins from beer and determined that bentonite, which is widely known as a protein adsorbent and to have cation exchange ability, was most efficient among the tested materials. To examine the effect of bentonite addition in the storage tank of beer, we analyzed beer treated with 1,000 ppm bentonite using GC-MS, HPLC, and spectrophotometer. The results of the analysis showed that bentonite selectively removed proteins and

alkaloidal constituents, with the exception of those related to the favorable flavor and taste of beer, such as amino acids, organic acids, sugars, and hop esters, and that the removal efficacy increased at higher bentonite levels. We confirmed that both the NIBEM value and color of beer treated with bentonite were decreased by $\approx 10\%$ compared with those of untreated beer and that the physical stability was unaffected. In addition, the results of a consumer research survey showed that beer treated with bentonite provided stronger mouth irritation than untreated beer.

Seiko Miyashita is an analyst in the Department of Flavor and Chemical Analysis Research Laboratories for Brewing at Asahi Breweries Ltd. She graduated from the Department of Material and Life Science in the Graduate School of Engineering at Osaka University before joining Asahi Breweries Ltd. in 2009. She has been engaged in the research and development of analytical technology since 2011, with a particular focus in the area of brewing science.

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In-process taste panels—Benefits and challenges

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The fast-paced and 24/7 environment of a brewery requires a robust quality system. With the cost of resolving quality issues increasing for each stage of the process, it is of utmost importance to ensure that prior to packaging the beer it is within specification and free of off-flavors. The most efficient and powerful method to test finished beer quality is to utilize a team of trained tasters capable of checking off each batch before packaging. Here we discuss the potential roadblocks and benefits of starting and nurturing an in-process tasting regime. Case studies will be presented to demonstrate that with adequate training, technology, and commitment it is possible to achieve a functioning and useful in-process tasting program—one which is capable of catching and flagging anomalies, determining blend ratios, and, most importantly, preventing out-of-specification beer from entering the package.

Lindsay Guerdrum received a B.S. degree in biochemistry and molecular biology from the University of New Mexico in Albuquerque and an M.S. degree in food science and technology from the University of California, Davis. While at UC Davis she focused on malting and brewing science under Charles Bamforth, working on gluten-free beer research. She began employment at New Belgium Brewing Company in 2011, where she is currently a sensory analyst. She has served on the ASBC Sensory Technical Subcommittee for two years and is currently the gluten-measurement subcommittee chair. In her free time she enjoys the Colorado mountains on her mountain bike and skis.

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Towards holistic flavor stability models and predicting beer flavor stability

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The stability of small-pack beer is still a perpetual problem for beer brewers, especially those with long distance–time distribution networks. However, the pathways of beer flavor instability are complex and highly inter-related, which is a fundamental problem when applying traditional experimental approaches to study one variable at a time. Our approach here has been to attempt to create a holistic framework that encompasses the chemical entities of beer flavor stability—in essence a “Bohringer map” of beer flavor. Here we present the elements of the framework we have constructed. Specifically, by creating sub-elements such as sulfite, aldehydes, amino acids, reducing sugars, etc., made up of discrete chemical equations, we have used multi-response kinetic modeling to estimate the time-dependent changes in all of the components

considered. In this paper, we outline the principle of the framework presented and explore the specific example of sulfur dioxide, including its various forms in beer, as a function of pH and its activity, which is highly dependent on the presence of, for instance, free carbonyls and reactive carbon-carbon double bonds. There are undoubted challenges to this approach, not least of which is the parameterization of the kinetic models themselves and the assumptions implicit in some of these kinetic models. Nonetheless, we contend that such an approach can be helpful in both identifying future research activities (to estimate model parameters) and in understanding the connectivity of apparently disparate parts of the network. Finally we explore two model outputs based on previous research carried out at Heriot-Watt: a) the derivation of analytical concentrations, and b) the derivation of flavor intensity. A comparison of the two models reinforced the view that fluctuations in flavor components at or around their flavor threshold has a disproportionately large impact on the overall sensory perception of the resulting beer. Such observations can aid in the prioritization of fresh beer compositional control. We note that, in agreement with existing literature, a potent approach to managing beer flavor instability is the management of non-proline FAN in fresh beer. Going forward, we contend that mining and parameterizing such models can provide additional valuable insights for management of flavor stability in trade.

An organic/analytical chemist by training, Paul Hughes joined BRF in Nutfield in 1990. After nine years working on research problems in raw materials, beer quality, and analysis, Paul moved to Heineken International in the Netherlands as principal scientist, where he focused on product quality research and global product safety and integrity issues. In 2005 Paul returned to the United Kingdom and joined Heriot-Watt as professor of brewing, before assuming the role of ICBP director in 2006. Paul is currently leading strategic projects to enhance further Heriot-Watt activities at the forefront of distilling education and research, as well as leading a research program on a wide range of quality and production challenges in both the brewing and distilling sectors. These include the sensory interaction of bitterness and hoppy aroma, valorization of distillery waste, modeling whisky maturation, and sensory integration of distilled gin volatiles. Paul holds a B.S. degree and Ph.D. qualification in chemistry from the University of London, an MBA from the University of Surrey, and the IBD brewing diploma. His research has been recognized by both IBD (Cambridge Prize) and ASBC (Eric Kneen Award). He serves on the editorial boards of several journals. In his spare time, Paul runs his own publishing company, (with a primary focus to increase access to important historical brewing works), and he has a growing interest in all aspects of botanicals.

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Beer redox stability conferred by thiol-containing peptides and proteins: A kinetic study of 1-hydroxyethyl radical scavenging ability

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Several studies have suggested sulfite (HSO_3^-) as a central antioxidant in beer due to its ability to decompose hydrogen peroxide, yielding sulfate (SO_4^{2-}) and thereby inhibiting the generation of 1-hydroxyethyl radical (HER). Also, HSO_3^- may trap volatile aldehydes by nucleophilic addition, producing non-volatile adducts. Reduced thiol-containing peptides and proteins have been shown to be consumed during beer storage and aging and well correlated with beer redox stability. HER is known to be the main radical species formed during beer aging processes and responsible for thermal oxidation of beer-sensitive components. Herein we report the reactivity of thiol-containing peptides and proteins

towards HER aiming to provide a basis for a kinetic modeling and to better understand at the molecular level the beer redox balance. The reactivity of the thiol-containing compounds towards HER was evaluated using a competitive kinetic approach, employing spin-trap 4-POBN (α -(4-pyridyl *N*-oxide)-*N*-*tert*-butylnitrone) as a probe and electron paramagnetic resonance (EPR) to detect the HER-POBN radical adduct in model solutions. Thiol-containing compounds were shown to be very reactive towards HER, with apparent second-order rate constants close to the diffusion limit in water and ranging from 0.5 to 6.1 $10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ for the HisCysLysPheTrpTrp peptide and reduced LTP1 protein, respectively. This reaction shows a moderate kinetic isotope effect ($kH/kD = 2.3$), suggesting that HER reduction is governed by hydrogen atom abstraction from the thiol groups rather than electron-transfer. The content of reduced thiols in different beers was determined using a methodology established previously by Lund and Andersen employing the ThioGlo[®]1 labeling reagent and the derivatized thiol monitored by reverse-phase liquid chromatography using fluorescence detection. The total content of thiols in beer (oxidized and reduced) were determined after a reduction step employing TCEP (*tris*(2-carboxyethyl) phosphine) as a disulfide reductant. The amount of thiols and the apparent second-order rate constants for scavenging HER by thiol-containing peptides indicates that proteins are the major scavengers of HER in beer. A good correlation among total protein and total thiol content in beer has been observed, and the content of reduced thiol strongly correlates with beer redox stability.

Natália Ellen Castilho de Almeida received her B.S. degree in chemistry from the Chemistry Institute at São Carlos of the University of São Paulo in 2008 and obtained her M.S. degree in analytical chemistry in 2011 under the supervision of the Daniel R. Cardoso. In March 2011 she started work on her Ph.D. degree in inorganic and analytical chemistry at the Chemistry Institute at São Carlos of the University of São Paulo, spending 6 months at the University of Copenhagen assisted by Mogens L. Andersen and Marianne N. Lund. Natália has been investigating oxidative reaction mechanisms in beer since 2009 using EPR and liquid chromatography coupled to mass spectrometry.

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Advances in solventless sample extraction for beer flavor analysis

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Flavor analysis is extremely challenging due to the low concentration of aroma-active compounds in the sample, and tedious extraction and concentration steps are typically needed to enrich the analytes. The development of non-solvent a flavor extraction technique such as solid-phase micro-extraction (SPME) allows for rapid extraction and concentration of volatile compounds from the matrix. SPME coupled with gas chromatography-mass spectrometry (GC-MS) has proved to be a very valuable technique to analyze volatile aroma compounds in foods and beverages. However, SPME fiber saturation and competitive adsorption of the volatile matrix need to be carefully addressed for reliable quantification. Stir bar sorptive extraction (SBSE) is another solventless extraction technique. A poly(dimethylsiloxane) (PDMS)-based SBSE-GC-MS technique can be used to analyze esters, hop-derived terpene and terpene alcohols, and C_{13} norisoprenoids such as β -damascenone in beer with high sensitivity and reproducibility. A new ethylene glycol (EG)-based SBSE-GC-MS was also demonstrated to be able to analyze polar compounds such as phenols and guaicolins in beer in the presence of high alcohol concentration. This presentation will discuss the practical considerations for solventless flavor extraction/analysis techniques, such as SPME, SPDE, SBSE, and microvial insert thermal desorption, and their applications in flavor analysis in beer and other food systems.

Michael C. Qian is a flavor chemist at Oregon State University. He received his B.S. degree in chemistry from Wuhan University of China, his M.S. degree from the University of Illinois at Urbana-Champaign, and his Ph.D. degree from the University of Minnesota under the guidance of Gary Reineccius. Michael's research interests at Oregon State University have covered aroma/flavor chemical/biochemical generation in dairy products, small fruits (blackberry, raspberry, and strawberry), wine and wine grapes, beer, and hops. He has published more than 50 peer-reviewed original research papers and 12 book chapters in the field of flavor chemistry and analytical chemistry. He is a co-editor of four books, Flavor Chemistry of Wine and Other Alcoholic Beverages, Volatile Sulfur Compounds in Food, Flavor and Health Benefits of Small Fruits, and Micro/Nano-encapsulation of Active Food Components, published by the American Chemical Society and is a frequent speaker at national and international meetings. Before he came to academia, Michael spent 10 years in industry as a research scientist. He is a former chair of the American Chemical Society-AGFD Flavor Chemistry Sub-Division and is currently serving as chair-elect of the American Chemical Society-Agricultural and Food Chemistry Division.

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Making gluten-free beers with barley malt and a proline-specific endoprotease

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Barley, wheat, rye, and oats are commonly used in beer brewing; however, these grains contain gluten. As a result, gluten-free (GF) beers are generally made with grains that do not contain gluten, such as sorghum, rice, buckwheat, etc. These beers are safe to drink for people who display sensitivities to gluten or have been diagnosed as having celiac disease—a potential life-threatening condition. Typically these GF beers display a different flavor and may take longer to ferment compared to beers made with barley malt that contains gluten. However, it is also possible to produce beers that are low in gluten when grains containing gluten are used. Indeed, a large amount of gluten proteins are removed during the brewing process as a result of sedimentation, centrifugation, boiling, and chillproofing, bringing down the levels of gluten to below 100 ppm in most cases of barley beers. Interestingly some commercially available lager beers display very low levels of gluten, which would qualify them as gluten-free according to the *Codex Alimentarius* (<20 ppm of gluten). A proline-specific endoprotease (PSEP) has been suggested as a means to hydrolyze the remaining gluten proteins from barley malt to bring the levels down to undetectable (results vary according to enzyme dosage and method used to measure gluten). This technology offers the possibility of making traditional beers (lagers and ales) gluten-free solely by adding a small amount of enzyme during fermentation without compromising the original characteristics of the beer. PSEP is an enzyme widely used to cleave haze-sensitive proteins and prevent the occurrence of chill haze. Both haze-sensitive and gluten proteins contain a large amount of the amino acid proline and, therefore, represent an ideal substrate for PSEP. More importantly, the enzyme is so specific that it does not impact foam stability. This paper will focus on the nature of PSEP and the process of making GF beers with malting barley, including the recommended procedures to measure gluten accurately and current levels of gluten in commercial beers.

*Sylvie Van Zandycke studied biochemical engineering and fermentation at the Institute Meurice (Brussels, Belgium); she completed her degree in 1996. She obtained her Ph.D. degree on *Saccharomyces cerevisiae* in 2000 from Oxford Brookes University in the United Kingdom. After that Sylvie was employed as a project manager for the brewing consultancy firm SMART Brewing Services until 2004, when she left the United*

Kingdom for lovely Montreal, Canada, accepting a post with Lallemand as project manager for their Genetic Identification Laboratory, focusing on yeast and bacteria used in alcoholic beverage production. In 2007 Sylvie became technical sales manager for Lallemand Brewing, looking after dry yeast and nutrition products on a global basis. At the end of 2011 she joined DSM Food Specialties to occupy her current position as technical service manager for brewing enzymes in North America.

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Infusion mashing opportunities of corn and rice adjuncts for high attenuated beers demonstrated by a thermostable glucoamylase from *Penicillium oxalicum*

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Widely used adjuncts like corn and rice are separately processed in the brewhouse, utilizing a cereal cooking step prior to blending with the malt mash followed by saccharification at 60–66°C. The separate adjunct cooking is needed because these adjuncts contain starch with a gelatinization temperature ($T_g > 68^\circ\text{C}$) above the temperature activity range of malt amylases. From a process simplicity and cost perspective, avoidance of a separate cereal cooking step through infusion mashing of these adjuncts together with malt has for a long time been a desire of the brewing industry. In this paper it is demonstrated, that infusion mashing of such adjuncts is possible by using selective saccharification enzymes that are active at or above the gelatinization temperature of the adjunct starch. Application of thermo-stable saccharification enzymes enables infusion mashing of corn adjunct for both maltose-based wort for ordinary attenuated beers, and also for highly attenuated glucose-based beers. A maltose-based wort with more than 75% fermentable sugars (>65% RDF) can be obtained by infusion mashing of up to 50% corn grits when adding an enzyme blend containing a new thermo-stable saccharification amylase. A glucose-based wort for highly attenuated beers can be produced from infusion mashing of 50% corn grits by an enzyme blend containing an amylase and the thermo-stable glucoamylase from *Penicillium oxalicum*, which has a temperature optimum at 70°C and ≈80% residual activity at 78°C (pH 6). The enzyme is, therefore, active during late mashing and lautering. This provides the basis for an efficient saccharification in infusion mashing applicable to reach high attenuation levels (RDF = 86%), with brewing yields comparable to traditional adjunct cooking. As the *P. oxalicum* glucoamylase works during lautering, the enzyme frees up potential to shorten the mashing time to, for example, only 1 hr without increasing the enzyme dose to the same high levels traditionally used for *Aspergillus niger* glucoamylase. A key challenge of infusion mashing of corn adjunct is that the gelatinization temperature of the starch varies depending on the source of the corn adjunct; the performance of the enzyme blend is demonstrated for corn adjuncts with different gelatinization points of the starch measured by DSC. The *P. oxalicum* glucoamylase provides unique benefits for high attenuated beers, which can be of great value to the brewing industry.

Hans Peter Heldt-Hansen has been the manager for the Brewing R&D Department at Novozymes since 2007. The department is responsible for the development of new and improved enzymatic solutions for the brewing industry. Hans, with a background as a biologist, has been working for Novozymes for 30 years and has been manager for the Microbial Screening Department, as well as different application development departments, including dairy and baking.

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Occurrence of deoxynivalenol-3-glucoside in barley and malt from North Dakota

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Fusarium head blight (FHB) is problematic within several barley production regions around the world, and as a consequence, samples of barley and malt are routinely screened for tricothecene mycotoxins. Deoxynivalenol (DON) is most frequently detected in North American barley, although much lesser amounts of nivalenol, 3-acetyldeoxynivalenol, and 15-acetyldeoxynivalenol also are detected. The presence of conjugated forms of the tricothecenes, where the toxin is covalently linked to another moiety, was first discussed more than 20 years ago. However, it was only in 2005 that the occurrence of the conjugate, DON-3-glucoside (DON3G), was reported in wheat that was naturally infected with FHB. Since this time there have been numerous reports on DON3G in wheat, maize, and cereal food products. DON3G, which is sometimes referred to as a masked mycotoxin, is of concern, as it is not detected by routine methodology, but nevertheless may be converted to DON during food processing or digestion. While several studies have reported the occurrence of DON3G in commercial beer, there has been very limited information on its occurrence in barley and malt. The current study, reports the levels of DON, other tricothecenes, and DON-3G on barley samples from inoculated nurseries and commercial fields. Commercial samples were collected over multiple crop years in North Dakota. A subset of these samples was micro-malted. Tricothecenes were determined by GC-ECD, and DON3G by HPLC ion-trap MS and UPLC QTOF MS. The biomass of *Fusarium* sp. on grain samples was assessed by real-time PCR with a fluorogenic TaqMan probe. Levels of DON3G on barley were generally below 15–20% of the mol% of DON but were observed to increase during malting.

Paul Schwarz is a professor of plant sciences at North Dakota State University, where he directs malting barley quality research and serves as the director of the Institute of Barley and Malt Sciences. Paul publishes and lectures on barley and malt quality. His current research is primarily in the area of food safety and mycotoxins. He previously has worked at the Kurth Malting Corp. and A. Egger Bierbrauerei and was a visiting scientist at the Coors Brewing Co.

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Factors affecting the formation of dimethyltrisulfide in beer

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Thiols and sulfides give beer an unpleasant aroma even at ultra-low concentrations. Among sulfur compounds, dimethyltrisulfide (DMTS), which has a rotten vegetable taste, is occasionally involved in the unpalatable aroma of regular and non-alcoholic beers (NAB). Although several papers have reported precursors of DMTS such as S-methylcysteinesulfoxide in hop and methionine, the mechanism of DMTS formation and methods for controlling DMTS formation are not fully understood particularly in NAB. In the present work, we examined the factors that affect the formation of DMTS in NAB and regular beer and propose new methods for controlling DMTS formation in these beverages. We collected several NAB samples from three breweries and several beer samples from another three breweries in Japan and analyzed the concentrations of DMTS using gas chromatography mass

spectrometry analysis. In NAB, the amount of DMTS decreased during boiling and increased in the whirlpool process. The concentration of DMTS in the final products varied among the breweries and showed a high correlation with the copper (Cu) level. A Cu spiking test revealed that higher amounts of Cu in the whirlpool process generated increasing concentrations of DMTS. As Cu is eluted from the kettle at low pH during the production of NAB, we increased the pH during boiling of the wort and effectively decreased the elution of Cu by >90% compared with the typical brewing process. As a result of this modification, the DMTS concentration in the final product was markedly decreased. This finding indicates that reducing the amount of Cu during boiling effectively reduces DMTS formation in NAB. In beer, the amount of DMTS also decreased during boiling and increased in the whirlpool process. However, the concentration of DMTS decreased during fermentation, reaching a few parts per trillion in the finished product. We also assessed the effect of beer storage on DMTS levels, which were found to increase during storage at room temperature, although the DMTS concentration varied based on the type of beer. In addition, a stable isotope-labeled DMTS spiking test of the wort revealed that stable isotope-labeled DMTS was not detected after storage. This suggests that DMTS generated during storage is unrelated to DMTS in wort. Additional experiments showed that the fermentation condition affected the amount of DMTS formed during storage. This result suggests that DMTS formation in beer during storage can be minimized by improving the fermentation conditions.

Norio Doi has been a researcher in the Research & Development Laboratories for Brewing at Asahi Breweries Ltd. since 2009. In 2009, he received his M.Eng. degree in polymer chemistry from Kyoto University, where he focused on tissue engineering.

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Green aroma volatiles affecting sensation in the throat when swallowing

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The sensation in the throat when swallowing is important for beer because consumers demand a pleasant sensation in the throat, especially in Japan. We have reported that total nitrogen and bitterness units, nonvolatile compounds, were negatively correlated with a light-finish sensation from the results of the sensory evaluation and the parameters by the biometric system for measurement of the swallowing motion (J. ASBC 67:217-221, 2009). The purpose of this study was to examine the relationship between aromas and the sensation of beer passing down the throat. Therefore, we investigated the effect of some green aroma volatiles, 1-hexanal and *cis*-3-hexenal, on sensation in the throat. 1-Hexanal and *cis*-3-hexenal have a green, leafy odor quality and are the major green odorants in hops. 1-Hexanal is one of the potential contributors to off-flavor in soybeans, while *cis*-3-hexenal is a key compound in fresh tomato, green odor. A “light-finish” was defined as a clean flavor and taste felt in the throat during swallowing, while a “full-finish” was defined as a rich flavor and taste felt in the throat during swallowing. 1-Hexanal and *cis*-3-hexenal were added to beer-flavored alcoholic beverages. The sensory score for “full-finish” sensation in the throat increased as the concentration of 1-hexanal and *cis*-3-hexenal increased. Therefore, the addition of 1-hexanal and *cis*-3-hexenal, even below the odor threshold previously reported, resulted in intensification of the “full-finish” sensation. During swallowing, volatiles are released from the food matrix, and they reach the nasal cavity through the pharynx (upper part of the throat). This pathway for aroma perception is retronasal olfaction. It was assumed that aromas felt retronasally might affect the sensation in the throat. Although 1-hexanal and *cis*-3-hexenal are supposed to have an unpleasant odor at high concentrations in beer, these compounds at an appropriate concentration might positively affect

throat sensation when swallowing. In future studies, understanding the effects of other aroma volatiles on throat sensation when swallowing is expected.

Hidetoshi Kojima is a biochemist in the Frontier Laboratories of Value Creation, Sapporo Breweries Ltd. He graduated from Tokyo University in 1999 with an M.S. degree and then joined Sapporo Breweries, Ltd. He has been engaged in research on antioxidants in barley and the application of the electronic nose and taste sensor. He has worked on the development of a biometric system measuring swallowing motion during drinking and received a Ph.D. degree from Niigata University in 2010. He has been working on wine making at Okayama Winery, Sapporo Wines, Ltd. (2008–2011). He received the Bioscience, Biotechnology, and Biochemistry Paper Award from the Agricultural Chemical Society of Japan in 2000 and the Eric Kneen Memorial Award from ASBC in 2010.

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The effect of protein and carbohydrate levels on the chemical and sensory properties of beer

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Beer is a complex beverage that contains over 400 different compounds, including macromolecules such as proteins, nucleic acids, carbohydrates, and lipids. Of these non-volatile macromolecules, the compounds of greatest interest are carbohydrates and proteins. The objective of this study was to evaluate the impact of different levels of protein and carbohydrates on the partitioning of volatile compounds and the associated perception of flavor and aroma. Beer treatments containing different concentrations of proteins (pro) and carbohydrates (CHO) (low pro/low CHO and high pro/high CHO), and known levels of five volatile compounds (ethanol, myrcene, ethyl hexanoate, isoamyl acetate, benzaldehyde) were created and evaluated using gas chromatography-mass spectrometry (GC/MS) and a trained sensory panel. With the exception of benzaldehyde, the GC/MS analysis showed significantly lower headspace concentrations of the volatile compounds in the high pro/high CHO beer treatment ($P < 0.05$). Trained sensory panel evaluation results revealed that in the high pro/high CHO beer treatment, apple and almond aromas were perceived as more intense, along with an increased perception of apple, almond, and hoppy flavors. A significant decrease in the perception of banana flavor was also observed ($P < 0.05$). The results highlighted the difference between data collected by instrumental and sensory analysis, suggesting the need for caution when correlating results between the two methods. This study provides detailed assessment of important flavor component interactions in beer and contributes to the limited knowledge on the interaction between non-volatile (carbohydrates and proteins) and volatile components in beer.

Luis Castro received a B.S. degree in chemistry from the University of Costa Rica in San José, Costa Rica. After two years spent working in both industry and academia, he moved to Washington State University, School of Food Science, to pursue graduate studies. After obtaining his M.S. degree in food science under Barbara Rasco, working in the field of food safety, he enrolled in the Ph.D. program at the same institution, working with Carolyn Ross. It was here where he started research on the impact of beer matrix components and their interactions in the sensory perception of beer. He is currently a research assistant in the sensory laboratory at Washington State University and is working on his dissertation to obtain a Ph.D. degree in food science.

New approach for the continuous fermentation of beverages

KONRAD MUELLER-AUFFERMANN (1)

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Ever since Henry Ford introduced conveyor belt production, nearly all industries aim for the application of continuous- or semicontinuous processes in order to increase productivity and efficiency and due to ecological benefits. This trend can also be monitored in the beverage industry, where approaches have been documented since the early 1900s. Even though this technology was partly introduced here (especially for bottling/filling plants), the continuous fermentation of beverages has not succeeded in larger scale so far. Whereas such a technology can be applied fairly easy if only one product is being produced (e.g., alcohol or lactic acid), the complexity and synergies of the compounds contained in beverages have impeded the technological implementation so far. A new approach, using a simple technology that may be retrofitted into an existing plant, was developed by the author and will be presented in detail. The introduced plant can be operated with nearly any yeast type and the process can be adjusted flexibly. Besides the functional principle, results of diverse pre- and long-term trials in a specially developed pilot-scale plant will be presented.

Konrad Müller-Auffermann already had two years of international experience before he studied beverage and brewing technology at the Technical University of Munich. During his studies he worked for several major construction companies, some in other countries. In 2009 Konrad was employed at the Research Center Weihenstephan for Brewing and Food Quality as a consulting engineer specializing in brewing, fermentation, and filling technologies, with particular focus on cereal-based beverages and fermentation methods. In 2010 Konrad became head of the Research and Development Department of the institute. His recent projects combine the theoretical knowledge of the university, with the long term experience and ideas of consulting engineers, mainly in cooperation with the industry.

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High-gravity brewing: Effects of aeration on fermenting ability and flavor compound and lipid composition of brewer's yeast

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There are a number of economic and quality advantages associated with high-gravity brewing: increased brewing capacity, efficient use of plant facilities, lower cleaning costs, and improved flavor stability. However, there are several production problems when the wort concentration is raised to certain values. Due to osmotic stress caused by high gravity and increases in ethanol concentration during fermentation, large amounts of extract cannot be converted to ethanol and carbon dioxide, and these remain in the beer. Many approaches, such as adjusting the pitching rate, temperature, and dissolved oxygen concentration, have been attempted to overcome these problems. However, almost all the studies used fermented volumes of <5 L and have not referred to beer flavor. Thus, we investigated the effects of aeration on fermenting ability and flavor compounds and lipid composition of yeast in high-gravity brewing with a 100 L scale pilot plant and 30% original extract. Lager wort (20%) was brewed according to standard production procedures, and then malt extract and sugar syrup were added to give an original extract level of 30%. After cooling, wort was aerated, and lager yeast was pitched at levels of 4.0×10^7 viable cells/mL of wort. Oxygen was continuously sparged for 0, 12, 24, 36, and 72 hr after transfer to the fermentation tank at a fermentation temperature of 15°C. Volatile higher alcohols, esters, organic acids, and lipids were determined at the end of fermentation. Fermentable extracts remained at >5% when air was not

sparged. On the other hand, they decreased to <1% with more than 24 hr of aeration. Succinic acid increased in proportion to sparging time, while the decrease in acetic acid suggests that the aerobic conditions during sparging result in accelerated TCA cycle metabolism. The lipid composition of yeast cell membranes was examined, and ergosterol and unsaturated fatty acid were found to increase in proportion to sparging time. Sensory evaluation of these beers, which are diluted to 5 vol/vol % alcohol, revealed that beer with no sparging is excessively sweet, but beer with >24 hr of sparging has a light flavor that is equivalent to standard lager beer.

Takuya Ohashi is a scientist in the Research & Development Laboratories for Brewing of Asahi Breweries Ltd. He received his M.S. degree in biostudies from Kyoto University in Japan, where he majored in cell biology. He has been at the Kanagawa Brewery since 2009, primarily in the brewing section. Since April 2012 he has worked in the brewing technology section of the laboratories for brewing.

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Solubility, supersaturation, and evolution of carbon dioxide during alcoholic fermentation

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At the onset of wort fermentation, carbon dioxide is generated through the metabolism of fermentable sugars. The gas is dissolved within the media until the saturation limit is reached (as determined by media composition and partial pressure differences between the wort and headspace). Common convention dictates that the wort will reach saturation and the carbon dioxide will subsequently evolve into the headspace at a rate proportional to its generation. This simple model seems to be generally accepted. Through investigation of CO₂ solubility, it was determined that the actual behavior of CO₂ generation and evolution, while complicated, can be easily modeled. An understanding of this process helps to explain several phenomenon such as a previously reported "bump" in CO₂ evolution rate in the latter half of fermentation and the apparent disconnect between CO₂ generation and evolution. It was observed that initial CO₂ evolution was below that predicted, given the known rate of sugar consumption and the equilibrium saturation level. It was also determined that under normal brewing conditions, the rate of CO₂ production exceeded the rate of evolution (driven by differences in CO₂ partial pressure between the wort and headspace) resulting in supersaturation of the wort. The degree of supersaturation is dependent upon many conditions, such as the shape of the tank, temperature, headspace composition, etc. In an unpressurized laboratory setting, the amount of CO₂ dissolved within solution was approximately four times the saturation limit at peak fermentation. Post-peak fermentation, the release of supersaturated CO₂ resulted in a period of CO₂ evolution in excess of generation, explaining the observed "bump" in CO₂ evolution rates. Our laboratory assessed the level of wort CO₂ supersaturation at various points throughout the fermentation through measurement of the total evolved and dissolved CO₂. In this manner the total CO₂ generation rate was determined and confirmed to be accurate with the aid of a carbon balance completed upon the entire fermentation. Utilizing findings of a recent study from our group on CO₂ solubility, the CO₂ dissolved in wort was compared to theoretical and empirical models. This study should provide brewers with more knowledge on how to control their fermentations. It would be especially useful if the rate of CO₂ evolution is utilized as a predictor of fermentation.

Andrew J. MacIntosh has completed bachelor's and master's of applied science degrees in the field of biological engineering. After working in industry as an engineer for several years, he decided to further his academic career at Dalhousie University (Nova Scotia, Canada) where he worked on the scaled-up production of a novel antibiotic. He is now

pursuing a doctorate in brewing science under the supervision of Alex Speers, with whom he has the unique opportunity to closely examine the progression of brewing fermentations. Andrew has recently completed his four year apprenticeship with the Association of Professional Engineers of Nova Scotia and has been registered and licensed to practice as a professional engineer. In addition to his research, Andrew has published and presented with ASBC, is an active member of the Canadian Institute of Food Science and Technology, and regularly serves on the council of the Dalhousie Engineering Graduate Society.

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Refining Balling's theorem: How the ratios of fermentation products change with time

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(1) Dalhousie University

The fermentation process has been scrutinized since the 1700s by microbiologists and food scientists. In 1865, the chemist Carl Balling analyzed brewing operations, focusing on the products of fermentation. Using beer with an original wort extract of 10–14°P, Balling found that from 2.0665 g of fermented extract, the following products were generated: 1.000 g of alcohol, 0.9565 g of CO₂, and 0.11 g of dry yeast matter. This formula, and associated calculation of original extract, are utilized worldwide and endorsed by both EBC and ASBC. That is not to say the formula has remained unchallenged. In the ensuing years since its derivation, the formula has been disputed on multiple grounds. Subsequent researchers have noted that while not perfect, the formula is a good approximation that is well understood and widely utilized. Additionally, most deviations in Balling's theorem can be accounted for; these were well summarized by Henning Neilson and others in 2007. The aforementioned studies have assessed the accuracy of Balling's model at the end of fermentation and the relationship between final values and original extract. However, the ratio of fermentation products is known to vary throughout fermentation. For example, the majority of yeast propagation is completed during the first half of fermentation, whereas the initial CO₂ produced is dissolved within the wort and does not evolve. Modern methods of analysis allow researchers to follow the parameters of Balling's formula over the entire fermentation and examine how the product ratios change with time. This study monitored each parameter in Balling's formula over the course of several fermentations. The generated CO₂ was calculated from evolved CO₂ and dissolved gas. Both the yeast in suspension and total yeast were enumerated, and then gravimetrically measured, while sugars and ethanol concentration were determined using high-pressure liquid chromatography. Under close scrutiny, the production of beer becomes less of a "black box" operation, and phenomena such as bumps in CO₂ production, supersaturation of wort, and yeast growth dynamics can be better understood. Information concerning how product ratios change over fermentation will be reported allowing brewers to make informed decisions concerning the original gravity of partially fermented beer and to more accurately estimate the alcohol, yeast, and sugar content of their final product.

Alex Speers has been appointed professor, chair, and director of the International Centre of Brewing and Distilling at Heriot Watt University in Edinburgh effective this spring. Until then he will continue as a professor in the Food Science program at Dalhousie University. Born in Creston, BC, Canada, he gained B.S. (Agric.), M.S., and Ph.D. degrees in food science at UBC. In the past, Alex has been employed in the Quality Assurance Departments of both Labatt and Molson Breweries. His current research interests include various aspects of the brewing process, including fermentability, yeast flocculation, fermentation modeling, extract calculations, and the properties of (and problems created by) β -glucan and arabinoxylan polymers. He has organized and/

or presented brewing workshops in Australia, China, the United States, and Canada. Alex has spent sabbaticals at CUB/Fosters in Melbourne, Australia, and the Columbia Brewing Company in Creston. He is a past chair of the Editorial Board of the MBAA Technical Quarterly. Alex belongs to several professional societies and is a member of the editorial boards of JASBC, JIB, and TQ. He has published or presented more than 150 papers and is a Fellow of the Institute of Brewing and Distilling. In 2011 he received the W. J. Eva Award from the Canadian Institute of Food Science and Technology.

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Improvement of the brewing yeast propagation process by increasing wort zinc ion content

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Prior to pitching in a fermentation vessel, brewing yeast is multiplied by several scaling-up steps from a small flask culture to large propagation vessels. Usually this propagation process needs a lot of time, and moreover, the fermentation performance of the propagated yeast is often not good as repitched yeast is recovered from beer fermentation. This study attempts to solve these two problems. If brewing yeast propagation is continued after reaching stationary phase, it will cause beer quality problems such as slow attenuation speed, increasing unripe off-flavors, and high pH. Therefore, each propagation process should be stopped earlier than the late logarithmic phase, i.e., at a relatively smaller cell count, for high-quality beer production. As a result, it is necessary to repeat many scaling-up steps, and this is the reason why the total propagation process requires a lot of time and complex procedures. It is recognized that the bad fermentation performance of excessively propagated yeast originates from a lack of vital elements in the yeast cells due to exhaustion of wort nutrients. Especially, zinc content in stationary phase yeast cells was found to be remarkably lower than that in yeast at the beginning of propagation. Adequate zinc is essential for yeast metabolism, and the shortage of zinc is suggested to be the primary reason for fermentation problems due to excessively propagated yeast in wort. Considering this situation, we researched how to control wort zinc ion content. During the wort production process in the brewhouse, zinc content showed a dynamic fluctuation, such as increasing during protein rest, decreasing during maltose rest, and a remarkable loss during the wort filtration process. Studying optimum mashing conditions suggested that a longer protein rest at a lower mash concentration would be effective to increase wort zinc ion content. In this zinc-rich wort, the yeast was able to be multiplied to a cell count that was 1.5 times higher, and the yeast still had sufficient zinc content in cells even when a much higher cell count was achieved than in the case of the conventional propagation procedure. Propagated yeast in zinc-rich wort showed good fermentation performance and produced high-quality beer. Also, achieving a higher cell count in each propagation step improved the inoculation ratio to the following propagation step and reduced the number of scaling-up steps, and as a result, propagation process efficiency was much improved.

Shigehiro Yoshizaki graduated from Hokkaido University in 1997 with an M.S. degree in agricultural chemistry and joined Kirin Brewery Company Limited in the same year. He worked in the brewing section of the Kyoto, Chitose, and Okayama breweries and then returned to the Research Laboratories for Brewing Technologies. He has been engaged in the research and development of yeast and fermentation technology.

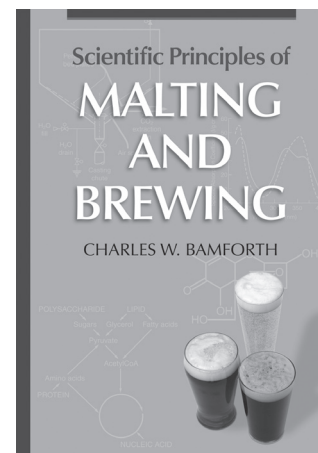
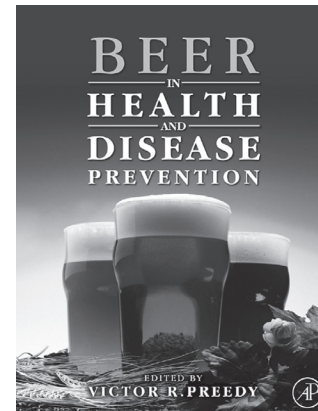
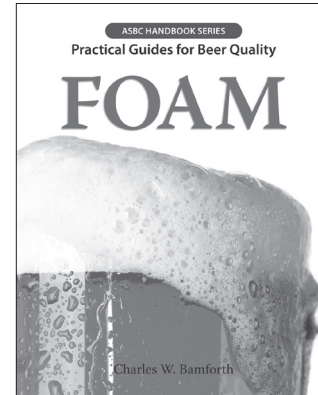
Comparative physiology and fermentation performance of Saaz and Frohberg lager yeast strains and the parental species *Saccharomyces eubayanus*

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Efficient fermentation is dependent on optimal process conditions for a given yeast strain or, alternatively, selection of a suitable yeast strain for particular process conditions. Understanding the requirements and capabilities of individual strains, therefore, is key to ensuring efficiency. In recent years, genomic studies have identified two distinct genetic groups (Saaz and Frohberg) within the *Saccharomyces pastorianus* lager yeast group and, furthermore, have identified the parental species (*S. eubayanus* × *S. cerevisiae*) of this hybrid. However, it is not known how the two hybrid groups differ physiologically, if at all. The fermentative capability of *S. eubayanus* has likewise never been studied. Here, 60 lager strains were screened to determine which hybrid group they belong to, and selected strains (3 Saaz + 3 Frohberg) were characterized to determine salient characteristics of each group. Two ale strains and the type strain of *S. eubayanus* were included for comparative purposes. Saaz strains, which are most closely related to the cryotolerant *S. eubayanus* parent, were found to be less sensitive to cold (10°C) than Frohberg strains. In 15°P all-malt wort fermentations at relatively high temperature (22°C) the Frohberg strains showed greater growth and superior fermentation performance (80% apparent attenuation in 3–4 days) compared with all other strains (including ale strains). Cropped Frohberg yeast also had the highest viability values (>93%). The fermentation performance of *S. eubayanus* was poor at 22°C, with apparent attenuation of only 33% reached after 6 days, and cell viability was reduced to 75% by the end of the fermentation. The cryotolerant Saaz lager yeasts grew relatively well during low temperature fermentations (10°C), but this did not translate to greater fermentation performance, possibly due to adaptation of these strains to lower wort gravities. Performance of *S. eubayanus* at 10°C was equal or greater to that of the Saaz strains, and growth was greatest in this strain compared with all others. Saaz strain fermentations were characterized by a relatively low production (2- to 6-fold lower than Frohberg strains) of the flavor compounds methyl butanol, ethyl acetate, and isoamyl acetate irrespective of temperature. Despite fermentation characteristics akin to those of Saaz strains, higher alcohol and ester production by *S. eubayanus* was similar to that of Frohberg yeast. It is suggested that Saaz strains may be suitable for low-temperature fermentations where beer with a clean flavor profile is desired. Fermentation of worts at or above 15°P may not be possible, however, due to the apparent sensitivity of these strains to high-gravity wort. This sensitivity may explain why the Saaz strains are used only rarely in the brewing industry at present. The ability of *S. eubayanus* to ferment wort well at low temperature (at least in comparison to Saaz strains) suggests that strains of this species have potential as new “lager yeasts,” thereby greatly increasing the genotypic and phenotypic diversity of strains available for the brewing industry.

Brian Gibson was awarded a Ph.D. degree from University College Dublin, Ireland, in 2004, where he specialized in fungal stress responses. On completion of his studies, he joined the Brewing Yeast Research group at Oxford Brookes University and later Nottingham University, UK, where his research covered a range of topics related to brewery yeast and fermentation. Since 2009 he has been employed as a senior research scientist and project manager at VTT, Finland, with responsibility for yeast physiology and fermentation research.

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THE SCIENCE OF BREWING

Comparison of 96 *Saccharomyces* isolates originating from commercial brewing environments to reveal correlations between full DNA sequence and fermentation characteristics and flavor attributes in beer

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(1) White Labs, San Diego, CA

Illumina in collaboration with White Labs Inc. has sequenced 96 closely related *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* strains used in brewing, in order to capture the biological diversity and gain insight into the differences between the strains. Comparative genomics is the study of the relationship between genome structure and function across different species or strains. The purpose of this study is to determine the phylogenetic relatedness among different *Saccharomyces* samples and compare the data to the fermentation performance and flavor characteristics of the isolates in beer production. Sequencing of the isolates was done by Illumina using HiSeq 2500 and MiSeq with different data handling tools applied. Fermentation characteristics were described on the basis of 20–80 L fermentations of brewer's wort covering a variety of beer styles true to the individual strain. All beers were analyzed by ASBC standard methods for ABV, RDF, IBU, color, and flocculation, as well as by a trained sensory panel.

Troels Prahl received a B.S. degree in biotechnology from the University of Copenhagen, Denmark, specializing in fermentation science. Troels' passion is to improve product and process quality within the brewing industry, and he has dedicated more than a decade of his working life to brewing and fermentation science and the way it is applied in the commercial brewing industry in Europe, the United Kingdom, and the United States. In addition to consulting under his own business firm, based in Copenhagen, Troels has worked closely with White Labs Inc. since 2007 on yeast R&D project management. Troels also filled the position as head brewer at Camden Town Brewery, London, UK, from 2010 to 2011. Troels is an associated teacher at the Scandinavian School of Brewing, where he lectures on brewing microbiology. In the summer of 2011 he moved back to the United States to work full time as a yeast application scientist at White Labs Inc. in San Diego, CA.

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A new strategy to improve stress resistance and fermentation performance in lager yeast

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Industrial beer brewing conditions impose a plethora of different stresses upon yeast cells, which decrease their viability and fermentation efficiency. Recent studies have indicated the correlation between strain stress resistance and fermentation performance. In this work, we proposed a novel strategy to screen strains with improved stress tolerance and fermentation performance. First, micafungin-resistant mutant strains were screened on micafungin plates. Then, these resistant mutants were isolated under high-osmolality, high-temperature, and high-ethanol content conditions. The two-round isolated strains showed faster attenuation, reduced petite cell number, and higher cell survival rate in high-gravity fermentation tests. The fermented beer had lower content of higher alcohols, higher ester content, and better foam quality, which are preferred in beer production. It was demonstrated that the mutants' cell walls contain more glucans, chitin, and mannoproteins. The increased mannoproteins reduce cell wall porosity and permeability. The glucans and chitin components contribute to cell wall mechanical strength. The strengthened cell wall structure can better protect yeast cells in stressed fermentation environments.

Qi Li received a Ph.D. degree in brewing engineering from Jiangnan University, Wuxi, China. She began working in the School of Biological Engineering in 1999. She is now in charge of the beer brewing lab and has functioned as vice president of the School of Biological Engineering since 2009. She is one of the key members of the China Beer Industry Association and China Standardization Professional Association.

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Autolysis evaluation of lager yeast using a complex parameter

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Yeast is the soul of beer fermentation, and the quality of beer significantly depends on the robustness of yeast strains. How to evaluate the process of yeast autolysis during beer fermentation has been a long-standing question. Here we established a reliable method to evaluate the autolysis process of lager yeast and tested it using several lager yeast strains. In this study, scanning electron microscopy (SEM) images and the released compounds during autolysis of four lager yeasts with different autolysis performance were recorded in order to find out the optimal evaluating parameters of yeast autolysis. Results showed that α -amino nitrogen, formaldehyde nitrogen, each molecule of protein content, and free long-chain fatty acid content had certain variations during autolysis, but there was no obvious regularity. Meanwhile, a single factor was not suitable for the evaluation of autolysis as a direct indicator. Nevertheless, the result of nucleic concentrations versus yeast mortality showed an obvious and consistent trend during autolysis. When the yeast autolysis rate increased, the ratio of nucleic concentrations versus yeast mortality gradually decreased. This indicator had the advantages of being comprehensive, highly sensitive, and less time-consuming, and the method was easy to operate. It could reflect different autolysis performance of different strains, as well as differences in the same strain at different stages of autolysis. So, the result of nucleic concentrations versus yeast mortality should be a good parameter to evaluate yeast autolysis.

Jinjing Wang received a Ph.D. degree in genetics from the Institute of Microbiology, Beijing, China. She spent 18 months at Washington State University as a visiting scholar. She began employment with Jiangnan University in 2011 as an assistant professor in the Brewing and Enzyme Technology Center of the Biological Engineering School.

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Characterization of spent brewer's yeast as a food additive

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Spent brewer's yeast represents a small but significant ($\approx 3\%$ wt/wt) residual from the brewing process. However, it often presents a waste disposal problem, particularly for smaller craft breweries, which don't produce enough spent yeast to warrant commercial arrangements for its processing as either flavoring extracts, animal feed supplements, or biogas production. In order to better understand value-added opportunities for its use, we examined spent yeast paste from a pale ale style fermentation obtained from Highland Brewing Co, Asheville, NC. Beer and residual yeast were analyzed for hop α -, β -, and isomerized- α -acids (iso- α -acids) content using HPLC-MS. Yeast paste extract showed the definitive presence of α (humulones) and β (lupulones) acids, and their amounts were ≈ 100 and $9 \mu\text{g/g}$ of wet paste, respectively (300 and $27 \mu\text{g/g}$ of dry paste). A cluster of early-eluting compounds corresponded to iso- α -acids based on UV spectra and MS fragmentation data. Total iso- α -acids were estimated at $100 \mu\text{g/g}$ of wet paste ($300 \mu\text{g/g}$ of dry paste). The beer portion obtained from centrifugation of the

yeast slurry contained $\approx 6 \mu\text{g/mL}$ of α -acids and $60 \mu\text{g/mL}$ of iso- α -acids; no β -acids were detected. Previous studies have reported spent yeast fractions containing over 10 times the concentrations of the relatively hydrophobic α - and β -acids of beer. This is the first study, however, to note appreciable levels of iso-acids in spent yeast. Given that these hop-derived metabolites have been shown to confer positive health effects, protein-rich spent yeast paste has potential food additive uses. Future work will focus on detailed elucidation of hop-derived components in spent yeast and the influence of grain bill and yeast generation on resulting compositions.

Robert "Rusty" Bryant obtained a Ph.D. degree in biochemistry from Florida State University, investigating polyketide pathways in Penicillium fungi. Following a postdoc at the University of Pittsburg, he spent seven years on the Biochemistry Faculty at the George Washington University Medical School in Washington, DC, working on the biology and chemistry of prostaglandins and related polyunsaturated fatty acid metabolites. After that, he spent 21 years in new drug discovery and natural products research at Schering-Plough Pharmaceuticals in Kenilworth, NJ. He then took a position for five years as vice president of discovery research for Redpoint Bio, a biotechnology firm in Ewing, NJ, that conducts taste and flavor discovery and development. Their particular focus is on blocking the bitter tastes of certain drugs, foods, and beverages. He and his wife moved to Asheville, NC, in 2012, where he has become interested in sustainability and brewery side streams. His company, Asheville Flavor Innovations LLC, is focused on evaluation and bitter taste mitigation of spent brewer's yeast to assess its potential as a human food supplement and new revenue source.

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Novel image cytometric method for detection of physiological and metabolic changes in *Saccharomyces cerevisiae*

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The study and monitoring of physiological and metabolic changes in *Saccharomyces cerevisiae* (*S. cerevisiae*) has been a key research area for the brewing, baking, and biofuels industries, which rely on these economically important yeasts to produce their products. Specifically for breweries, physiological and metabolic parameters such as viability, vitality, glycogen, neutral lipid, and trehalose content can be measured to better understand the status of *S. cerevisiae* during fermentation. Traditionally, these physiological and metabolic changes can be qualitatively observed using fluorescence microscopy or flow cytometry for quantitative fluorescence analysis of fluorescently labeled cellular components associated with each parameter. However, both methods pose known challenges to the end-users. Specifically, conventional fluorescent microscopes lack automation and fluorescence analysis capabilities to quantitatively analyze large numbers of cells. Although flow cytometry is suitable for quantitative analysis of tens of thousands of fluorescently labeled cells, the instruments require a considerable amount of maintenance, highly trained technicians, and the system is relatively expensive to both purchase and maintain. In this work, we demonstrate the first use of Cellometer Vision for the kinetic detection and analysis of vitality, glycogen, neutral lipid, and trehalose content of *S. cerevisiae*. This method provides an important research tool for large and small breweries to study and monitor these physiological behaviors during production, which can improve fermentation conditions to produce consistent and higher quality products.

Leo Chan currently serves as the technology R&D manager and senior scientist at Nexcelom Bioscience LLC, Lawrence, MA. His research involves the development of instrument and applications for

the Cellometer image cytometry system for detection and analysis of yeasts used in brewing and biofuel industries. He is a member of the Master Brewers Association of the Americas. He received his B.S., M.S., and Ph.D. degrees in electrical and computer engineering from the University of Illinois at Urbana-Champaign (2000–2008).

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Optimizing FAN development and β -glucan degradation and wort viscosity in North American barley varieties

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North American (NA) barley varieties typically have higher protein contents than European (EU) barley varieties. As a result, during typical malting processes the free amino nitrogen (FAN) levels are substantially higher in NA malt than EU malts. While these higher FAN levels are helpful in improving the functionality of yeast in brews with high adjunct ratios, all-malt brewers require less FAN. Additionally, excessive residual FAN in beer can potentially lead to undesirable reactions, such as Strecker degradation, that produce aldehydes that negatively impact beer freshness. Given that the majority of the FAN level observed in wort is created during the malting process, this study was designed to optimize the malting process parameters to control FAN development alongside β -glucan degradation and wort viscosity in NA malting barley varieties. Over 10 malting trials were completed with varying malting conditions and barley varieties. It was set up by sampling seven varieties to assess potential candidates' inclination to FAN development. Then, the methods were optimized around three NA varieties to determine the optimal conditions for malting. Parameters studied included steeping recipes, germination temperature and length, and kilning temperatures. Results indicated that it is possible to produce relatively low-FAN malt with an acceptable degree of malt modification from NA varieties. In the finished malts, FAN levels below 160 mg/L were achieved while keeping absolute viscosity below 1.6 Cp and β -glucans below 200 mg/L .

Katrina Christiansen received a Ph.D. degree in agricultural engineering from Iowa State University in Ames, IA, in 2011. She began working with Cargill Malt as the pilot project lead in Spiritwood, ND, that same year, supervising the innovation efforts in both the pilot malting and brewing facilities. She previously worked as a plant engineer for Abengoa Bioenergy. And way back, she completed her B.S. and M.S. degrees at the University of Nebraska-Lincoln in biosystems engineering.

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Inhibitors of foaming in specialty malts

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Although it has long since become dogma that specialty malts boost foam stability, we find that while this is true for some (e.g., black malt), quite the opposite is the case for some others. We have been investigating the nature of the foam-negative entities in some of these malts and will recommend strategies for alleviating the problem.

Justin Ang was born and raised in Chicago, IL. He received his undergraduate degree in animal science at the University of Illinois in Urbana-Champaign in 2011. While there, he researched the toxicological effects of drinking water disinfection by-products. He is now pursuing an M.S. degree in food science at UC Davis.

A laboratory-scale fermentation system and its application to developing predictable regimes for the control of volatile ester formation at production scale

TERRY W. BILVERSTONE (1), Chris A. Boulton (1), Rod White (1)
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Volatile esters, produced as a result of the metabolism of wort by yeast during fermentation, are essential contributors to the flavor and aroma of beer. Since many esters arise at concentrations close to their flavor thresholds comparatively small variations in the concentrations of individual members can have a large impact on beer organoleptic properties. An essential part of the control of the brewing process is to ensure that esters are produced in the concentration ranges characteristic for individual beer qualities. This is especially the case where a single beer is brewed at several different production sites. The metabolic pathways that are implicated in volatile ester accumulation as a result of yeast activity during fermentation and the underlying genes responsible for the synthesis of the individual enzymes and their regulation are reasonably well-characterized, albeit with several caveats. Similarly, the process factors that are known to have an impact on ester formation have been subject to intensive study over the years, although often with contradictory results. We present volatile ester data relating to a pilsner-type lager beer produced at several international breweries that demonstrate significant site-specific variability although all of the breweries ostensibly used the same yeast strain and identical wort composition and fermentation control parameters. It is assumed that the observed variability must be a consequence of differences in plant and process operation associated with each site—in particular, fermentation vessel design and management. A laboratory model fermentation system is described that has been used to study formation during fermentation of the various groups of volatile esters known to contribute to beer quality as well as the synthesis and fate of their precursors. Trials are described that have been performed with a view to elucidating the cause-and-effect relationships between process conditions and ester accumulation for this combination of yeast strain and wort composition. Preliminary results are presented that demonstrate that this model system can be used to identify production-scale regimes that when implemented will allow ester formation to be controlled in a predictable fashion and so result in improved site-to-site product matching.

Terry Bilverstone received his B.S. degree in microbiology from the University of Nottingham (United Kingdom) in 2010. Following graduation, he took a quality control position at a well-known confectionery company for one year to get a feel for industrial microbiology. During this experience, he yearned for the investigative approach of scientific research as opposed to the routine sampling of products and, thus, searched for a relevant position. In August 2012 he accepted the offer of working on a Master by Research (M.Res.) degree in brewing sciences at the University of Nottingham, under the supervision of Chris Boulton and Rod White. With regard to the work presented in this abstract, his research has also focused on methods for controlling ester formation with this particular yeast strain by altering physical fermentation parameters and wort composition.

Adapting brewery laboratory methods for a microplate reader

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The ability to increase efficiency and maximize time and labor in the brewery is of the utmost importance in an industry with exponential growth. Microplate readers are perfect instruments for analyzing a large number of samples at one time, with plates having up to 96 wells. Many popular ASBC and EBC methods require the use of spectrophotometers, which typically analyze one sample at a time. Not only do microplate readers have more wells, but they require a much smaller sample size, resulting in a decrease in reagents needed. Methods such as IBU, color, FAN, polyphenols, or any other method that requires the UV-Vis spectrum can be adopted. Additionally, multiple readings of a sample can be taken and averaged to reduce sampling error.

Kara Taylor received a B.S. degree in biology from Loyola Marymount University in Los Angeles. She began employment at White Labs in San Diego, CA, in 2009 as a yeast laboratory technician. Since 2011, she has functioned as the analytical laboratory specialist in White Lab's new analytical laboratory. She is a member of MBAA and ASBC and serves on multiple subcommittees.

An improved method for the determination of arabinoxylan content in wort utilizing high-performance anion exchange chromatographic separation with pulsed amperometric detection (HPAEC/PAD)

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Arabinoxylan and β -glucan make up the cell wall polysaccharide constituents of barley. Arabinoxylans are also known as pentosans and consist of chains of β 1-4 linked xylose units with side chains of arabinose linked via α linkages to either C-2 or C-3 of xylose (or both). Ferulic acid residues are linked through ester bonds to the C-5 position on the xylose backbone. High concentrations of arabinoxylans and/or β -glucans are problematic in brewing because they can lead to high wort viscosity, resulting in impaired liquid pumping and handling, poor wort filtration, and loss of extract yield in the brewhouse. They do this either when they are solubilized in the wort or insoluble. These compounds can also separate out of solution in the brewing process, or in the finished product, as hazes, gels, or precipitates. While analytical methods to determine β -glucan content in wort have been around for many years, and the impact of β -glucan levels in wort has been well documented in the brewing literature, much less is known about the impact of elevated levels of arabinoxylans on wort viscosity, filtration, and extract yield. That is due to the absence of a fast, easy, precise, and reliable analytical method to quantitate arabinoxylan in wort to date. Methodology exists in the literature for the determination of arabinoxylan in wort using high-performance liquid chromatography or gas chromatography; however, the sample preparation required is extensive and time-consuming, and the reproducibility is not great. A method is described in the literature for the determination of arabinoxylan content in wheat-based wort utilizing high-performance anion exchange chromatography by M. Krahl et al., which was used as the basis for the method described here. The main objective of this study was to develop an analytical method to quantitate total arabinoxylan content in malt-based wort that is quicker and easier, matching or surpassing the repeatability and reproducibility of current methodology, which is represented by the gas chromatography method. Congress mash samples of various malts were prepared and

run via both high-performance anion exchange chromatography and gas chromatography methods and the total arabinoxylan content, ruggedness, repeatability, and reproducibility were compared and contrasted.

β -Glucan test, by the Congo Red method, was also determined for each wort sample to illustrate how levels of arabinoxylan and β -glucan can vary from one malt variety to another. Total sample throughput time was found to be reduced from 3–4 days with the gas chromatography method to 1–2 days with the high-performance anion exchange chromatography method.

UnJu Kim received her B.S. degree in food science from NC State University in Raleigh, NC, in 1984. She began employment with Novozymes in 1994 in the Quality Control Department. UnJu was transferred to the Food Group in the Technical Service Department in 1999 as a research associate. She was awarded the 2005 Green Chemistry Award for her work on the inter-esterification concept using enzymes to reduce trans-fat. In 2006 UnJu joined the Novozymes Brewing group. She set up the Brewing Lab in Franklinton to support brewing customers in the Americas. UnJu has been a member of ASBC since 2010. She has participated in the ASBC Ring Analysis program since 2011. Currently, she is chair of the ASBC β -Glucan technical Subcommittee. UnJu is married to Jae Kim and has a son, Justin, and a daughter, Amanda. In her spare time, she enjoys golf and listening to books on tape. She has discovered the art and science of making beer, not to mention the love of tasting wheat beers.

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Analysis of volatile phenols in beer by EG-based stir bar sorptive extraction-gas chromatograph-mass spectrometry

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4-Ethylphenol (4-EP), 4-ethylguaiacol (4-EG), 4-vinylphenol (4-VP), and 4-vinylguaiacol (4-VG) are the main volatile phenols present in beer and other alcoholic beverages. Although they are positive aroma attributes for some types of beer, excessive concentration of these volatile phenols in beer can give the product a horsy, medicinal, smoky, clove-like off-aroma. It is important to monitor the levels of these volatile phenols during brewing processes, as well as in the final products. A stir bar sorptive extraction (SBSE)-GC-MS technique based on a polar EG/PDMS-copolymer absorption phase for volatile phenol analysis was developed and validated. The method simultaneously extracts the analytes and desorbs them into GC-MS to allow effective and reliable volatile phenol analysis in beer with minimum sample preparation. The analysis parameters, including pH, ionic strength, extraction time, alcohol concentration, and internal standard selection, as well as sample matrix on the quantification, were investigated. The linear response ranged up to 500 mg/L, with R^2 in the range 0.994 to 0.999, and relative recoveries were from 90 to 113%. A limit of quantification (LOQs) of 5 mg/L can be easily achieved, and lower LOQ can be achieved through minor method modification. The method has good reproducibility and has been successfully applied to monitor the changes in volatile phenols in beer samples during fermentation and aging.

Qin Zhou received a B.S. degree in chemistry from Wuhan University in China in 2006. She next received an M.S. degree in fermentation engineering from the China National Research Institute of Food and Fermentation Industries in 2009. After graduation, she worked as a researcher at a center for physical and chemical analysis, working on food safety. In 2010, she began working toward a Ph.D. degree in Michael Qian's flavor chemistry lab at Oregon State University in the Food Science and Technology Department. Her work focuses on development of analytical methods of flavor analysis in beer and wine, including volatile phenols, stale aldehydes, volatile sulfur compounds,

DVKs, and other volatiles like higher alcohols and esters. She presented two research papers at the World Brewing Congress 2012.

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Application of molecular dynamics simulations to explore the behavior of proteins and iso- α -acids at interfaces

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The interactions between small, discrete molecular entities and larger “biomacromolecules” in beer have a number of influences on both the production and final quality of beer. For instance the formation of trub and cold break during production and, in final beer, haze formation and beer foaming are all affected by such interactions. Here, we have focused on the dynamic situation that persists in the formation of beer foam. Using ab initio calculations and molecular dynamics simulations, we have explored the interactions of hop-derived bitter acids (represented in this study by *cis*-isochumulone) with a representative protein (LTP1) at biphasic interfaces (i.e., air-dodecane and air-vacuum interfaces) to explore the relative rates of protein adsorption to the interface and the adsorption of hop acids to the LTP1 itself. It is clear that the adsorption of the LTP1 to the interface is relatively slow and that the protein is already laden with hop acids by the time it reaches the interface. Furthermore, the LTP1 alters its geometry when adsorbing to the interface, undergoing a slight spreading. It is not clear whether this adsorption is irreversible. The primary structure was observed to be unchanged in this study. However, once at the interface, LTP1 resisted re-entering the bulk water phase, even after protracted simulation runs. The binding of the *cis*-isochumulone moieties to LTP1 did not appear to be rigorously stoichiometric. Indeed, there was a tendency for the hop acids themselves to form small clusters that also adsorbed to the interfaces studied. This is perhaps unsurprising given the presence of hydrophobic regions on the hop acids, but it would be informative to extend this study to evaluate the behavior of the iso- α -acids in bulk solution as a function of ethanol and metal cation concentrations. While these simulations cannot in isolation be considered to be definitive, they have proved to be insightful and thought-provoking, suggesting potentially useful experimental and test systems that might be applied in future research.

An organic/analytical chemist by training, Paul Hughes joined BRF in Nutfield in 1990. After nine years working on research problems in raw materials, beer quality, and analysis, Paul moved to Heineken International in the Netherlands as principal scientist, where he focused on product quality research and global product safety and integrity issues. In 2005 Paul returned to the United Kingdom and joined Heriot-Watt as professor of brewing, before assuming the role of ICB D director in 2006. Paul is currently leading strategic projects to enhance further Heriot-Watt activities at the forefront of distilling education and research, as well as leading a research program on a wide range of quality and production challenges in both the brewing and distilling sectors. These include the sensory interaction of bitterness and hoppy aroma, valorization of distillery waste, modeling whisky maturation, and sensory integration of distilled gin volatiles. Paul holds a B.S. degree and Ph.D. qualification in chemistry from the University of London, an MBA from the University of Surrey, and the IBD brewing diploma. His research has been recognized by both IBD (Cambridge Prize) and ASBC (Eric Kneen Award). He serves on the editorial boards of several journals. In his spare time, Paul runs his own publishing company, (with a primary focus to increase access to important historical brewing works), and he has a growing interest in all aspects of botanicals.

Aroma-active compounds in sour beer identified by gas chromatography/olfactometry-mass spectrometry

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Sour beer is gaining popularity among consumers. Many sour beers have a characteristic fruity, chocolate, and sour flavor partially due to secondary fermentation with *Brettanomyces* yeast. However, the flavor of sour beer is not well understood. The object of this study was to identify the aroma compounds in sour beer using gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry (GC-MS). A commercial sour beer sample was extracted with LiChrolut EN powder, and the extracted compounds were eluted with dichloromethane. The extract was fractionated into an acidic fraction and a neutral/basic fraction. Both fractions were analyzed by GC-O and GC-MS. Silica gel-based normal phase liquid chromatography was used to further separate the neutral/basic fraction to facilitate the GC-O and GC-MS analysis. The results showed that the important aroma-active compounds in sour beer were acids: acetic, isobutyric, butanoic, 3-methylbutanoic, hexanoic, octanoic, nonanoic, and decanoic acids; esters: ethyl isobutyrate, isobutyl acetate, ethyl butanoate, ethyl 3-methylbutanoate, isoamyl acetate, ethyl pentanoate, ethyl lactate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, 2-phenylethyl acetate, ethyl phenylacetate, ethyl 3-phenylpropanoate, and ethyl cinnamate; alcohols: propanol, 3-methylbutanol, 2-pentanol, benzyl alcohol, phenethyl alcohol, linalool, α -terpineol, and geraniol; and phenols: 4-ethylphenol, guaiacol, 4-vinylguaiacol, and 4-ethylguaiacol. In addition, γ -nonalactone, vanillin, acetophenone, and β -damascenone were identified to be important in the sour beer sample. It is proposed that differences in the concentrations of these aroma-active compounds are responsible for the unique flavor among sour beers.

Michael C. Qian is a flavor chemist at Oregon State University. He received his B.S. degree in chemistry from Wuhan University of China, his M.S. degree from the University of Illinois at Urbana-Champaign, and his Ph.D. degree from the University of Minnesota under the guidance of Gary Reineccius. Michael's research interests at Oregon State University have covered aroma/flavor chemical/biochemical generation in dairy products, small fruits (blackberry, raspberry, and strawberry), wine and wine grapes, beer, and hops. He has published more than 50 peer-reviewed original research papers and 12 book chapters in the field of flavor chemistry and analytical chemistry. He is a co-editor of four books, Flavor Chemistry of Wine and Other Alcoholic Beverages, Volatile Sulfur Compounds in Food, Flavor and Health Benefits of Small Fruits, and Micro/Nano-encapsulation of Active Food Components, published by the American Chemical Society and is a frequent speaker at national and international meetings. Before he came to academia, Michael spent 10 years in industry as a research scientist. He is a former chair of the American Chemical Society-AGFD Flavor Chemistry Sub-Division and is currently serving as chair-elect of the American Chemical Society-Agricultural and Food Chemistry Division.

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Automated quantification of budding *Saccharomyces cerevisiae* using a novel image cytometry method

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Measurements of concentration, viability, and budding percentages of *Saccharomyces cerevisiae* are performed on a routine basis in the biofuel and brewing industries. Generation of these parameters is of great importance in a manufacturing setting, where they can aid in

the estimation of product quality, quantity, and fermentation time of the manufacturing process. Specifically, budding percentages can be used to estimate the reproduction rate of yeast populations, which directly correlates with metabolism of polysaccharides and bioethanol production, and can be monitored to maximize production of bioethanol during fermentation. The traditional method involves manual counting using a hemacytometer, but this is time-consuming and prone to human error. In this study, we developed a novel automated method for the quantification of yeast budding percentages using Cellometer image cytometry. The automated method utilizes a dual-fluorescent nucleic acid dye to specifically stain live cells for imaging analysis of unique morphological characteristics of budding yeast. In addition, cell cycle analysis is performed as an alternative method for budding analysis. We were able to show comparable yeast budding percentages between manual and automated counting, as well as cell cycle analysis. The automated image cytometry method is used to analyze and characterize corn mash samples directly from fermenters during standard fermentation. Since concentration, viability, and budding percentages can be obtained simultaneously, the automated method can be integrated into the fermentation quality assurance protocol, which may improve the quality and efficiency of the bioethanol production process.

Leo Chan currently serves as the technology R&D manager and senior scientist at Nexcelom Bioscience LLC, Lawrence, MA. His research involves the development of instrument and applications for the Cellometer image cytometry system for detection and analysis of yeasts used in brewing and biofuel industries. He is a member of the Master Brewers Association of the Americas. He received his B.S., M.S., and Ph.D. degrees in electrical and computer engineering from the University of Illinois at Urbana-Champaign (2000–2008).

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Beer reference sample correlation between free amino nitrogen (FAN) and NOPA (nitrogen by OPA)

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In the fermentation process to produce beer yeast, *Saccharomyces* normally converts sugars to ethanol and carbon dioxide. The yeast synthesizes proteins required for healthy growth from amino acids, which may be created by the yeast from ammonia or by removal of the amino group from other α -amino acids. The α -amino acids available to the yeast in the fermentation are known, therefore, as free amino nitrogen (FAN). The ninhydrin method measures ammonia content in addition to FAN and so measures total assimilable nitrogen. In the beer industry EBC (European Brewery Convention), MEBAK, and ASBC methods of analysis describe FAN measurement using a ninhydrin-based assay. In this paper we show the correlation between the BAPS beer samples measured according to the EBC FAN protocol and the Thermo Scientific Gallery system NOPA (α -amino nitrogen by OPA) method. The rapid 2-reagent method was developed for a multipurpose discrete analyzer. The method is easily adapted to a manual spectrophotometer as well. The use of blank buffer eliminates possible sample color interference. Total analysis time for 6 samples and 60 requests was approximately 45 min. A method performance study was done by Thermo Scientific Gallery and Arena discrete analyzers at a wavelength of 340 nm. An additional side wavelength of 700 or 750 nm can also be used. Method linearity was determined between 20 and 300 mg/L with aqueous glycine standard solutions. In addition to the BAPS samples, 16 commercial beer samples and 2 wort samples were analyzed. Values used as a reference were assigned by the ninhydrin method. Method comparison for more than 20 samples showed good correlation between the two methods ($R^2 > 0.99$). Beer and wort samples tested showed excellent repeatability, with

typical variation being 2% or less. Furthermore, we see that no additional ammonia measurements are needed.

Liisa Otama received a B.S. degree in analytical chemistry from the University of Helsinki. She began employment with Thermo Fisher Scientific in 2008 in the Processing Engineering Department. From there she moved into the Research and Development Department, where she worked as an R&D scientist responsible for the development of beer analysis applications, like β -glucan and NOPA, as well other food and water chemistry tests for discrete analyzers. Since June 2012 she has functioned as an application specialist and is the primary contact for supporting industrial customers. In addition her responsibilities also include handling industrial product feedback and inquiries, supporting customer training, and identifying new customer needs.

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Beer tastes and quality evaluation using e-tongue

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In this study, body and “richness” of taste of beer were studied using a taste sensing system, i.e., e-tongue. In our previous report at the ASBC annual meeting in 1999, we reported bitterness evaluation of beers using our newly developed bitter sensor on the basis of bitter aftertaste derived from iso- α -acids found in beer, corresponding to human sensory quality. The purpose of this study is to evaluate, qualitatively, body and richness of tastes of beer using our taste sensing system. Effective R&D and strict quality control of a broad range of food and beverage products require objective taste evaluation. The taste sensing system using artificial lipid/polymer membranes has been developed based on concepts of global selectivity and high correlation with human sensory score. These sensors respond to similar basic tastes in a similar manner to human taste reception with high correlations to sensory score. Using these unique properties, these sensors can quantify the basic tastes of saltiness, sourness, bitterness, umami, astringency, and richness without multivariate analysis or artificial neural networks. In the present experiment, large varieties of beers were measured using the taste sensor. The result suggested that savory aftertaste would be possible to assess the body and richness of taste of beers. As reported at the ASBC annual meeting in 1999, bitter aftertaste and how quickly it diminishes were significantly useful information in order to characterize beer taste quality. From the results of these studies, the taste sensing system would provide an effective method to optimize production methods, ensure quality control, and assess shelf life in the beer industry.

Masaaki Habara received his B.E. and M.E. degrees, both in electrical engineering, from Kanazawa Institute of Technology in 1995 and 1998, respectively, and a D.Eng. degree from Kyushu University in 2002. He worked as a visiting associate professor in the Graduate School of Kyushu University until 2011. He joined Intelligent Sensor Technology, Inc. as a research and development engineer in 2011.

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Carbon utilization and key fermentation performance indicators during very high-gravity (VHG) brewing of lager and ale type beers

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Very high-gravity (VHG) fermentations are increasingly attractive within the brewing industry as a means of energy-saving and to optimize process efficiency. However, under high sugar conditions, yeast cells are subjected to osmotic pressure, low water activity, and potentially toxic ethanol levels, as well as other stress factors, that can inhibit yeast growth and affect fermentation performance. Such effects include

longer fermentation time, altered flavor characteristics, and decreased foam stability. In order to eliminate or reduce the detrimental effects caused by these extreme conditions, brewing yeasts must respond to this environment by shunting carbon into different metabolic end-products, which may assist in the protection of cells but may also impact final ethanol yield. In this study, the key fermentation performance indicators ethanol and carbon dioxide were quantified against anti-stress molecules such as trehalose and glycerol, as well as intracellular glycogen and cell biomass. This was conducted in a series of lab-scale fermentations with 13, 18, and 24°P wort using both lager and ale brewing strains. In conjunction, the effects of VHG brewing on osmotic stress during fermentation were also investigated. Here, the effect of wort gravity on yeast physiology and key fermentation performance indicators will be shown. In particular the effect of VHG brewing on carbon end-products and potential trade-offs with ethanol will be explored. In addition, the contribution of ethanol and glycerol to osmolality and the potential effects of this on yeast viability will also be investigated. Our results demonstrate that many aspects of yeast physiology and fermentation performance are affected during VHG fermentations; however, the results suggest that lager and ale brewing strains exhibit different responses to VHG environmental stresses. It is anticipated that the data presented here will provide a greater understanding of the response of yeast to VHG conditions, potentially leading to process optimization in the future.

Shiwen Zhuang received an M.Eng. degree in food science from Tianjin University (China) in 2010. During his studies at Tianjin University, Shiwen explored several yeast isolates that originated from a variety of food materials, including molecular identification and flavor formation of wild yeast strains, as well as construction of genetically engineered strains. He is currently working toward a Ph.D. degree at the University of Nottingham (United Kingdom), focusing on microbial physiology and fermentation performance under high-gravity fermentation. In addition to research on beer and biofuel, he is also interested in fermented food products such as wine, soy sauce, and Chinese liquor. He is an associate judge for the International Wine and Spirit Competition (IWSC). Aside from work Shiwen enjoys calligraphy, swimming, and squash, as well as playing saxophone.

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Construction and application of sensory evaluation system of hop aroma using standardized hop boiling method

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Hop imparts a characteristic flavor to beer. Hops have been evaluated by sensory testing of hop cones to estimate their aroma characteristics; however, most of the volatile essential oils in hops are evaporated significantly during the boiling process after hop dosing, and the composition of the essential oils will be changed from their original composition according to chemical and physical properties such as polarity and the boiling point of each volatile. In this study we introduced a new sensory evaluation system for hop aroma using a standardized hop boiling method in which the hops were dosed according to the α -acids level of 0.5 g/L to distilled water in glass flasks and boiled for 5 min at 105°C using autoclave equipment. Quantitative descriptive analysis (QDA) was adopted in the sensory evaluation and nine words were set to cover the aroma properties. The method was demonstrated using three different categories of hop cones, aroma hop (Saazer), high-alpha hop (Magnum), and flavor hops (Furano Beauty and Citra), harvested in different crop years. The result of subsequent principal component analysis (PCA) suggested that the method is a promising way to characterize and distinguish the aroma properties of different categories of hops. Using the aroma evaluation system,

the major hop varieties grown in Europe, the United States, and Japan were evaluated and characterized using PCA plots. Consequently the new sensory evaluation system enables us 1) to evaluate hop aroma characteristics at identical bitterness levels, even though the α -acids levels were different among hop varieties; and 2) to evaluate the aroma characteristics assumed to be closer to those after the boiling process than raw hops from the point of view of the composition of volatile aroma components and, hence, closer to beer brewing. The system can be applied to the selection or screening of breeding lines in hop breeding programs.

Koichiro Koie received a master's degree in agriculture from Kyoto University. He began employment with Sapporo Breweries in 1999 as a hop breeder/researcher in the Bio-engineering Laboratories. He bred 'Little Star', 'Furano Special', 'Furano Royal Green', and 'Furano Beauty'. He also dealt with research on the genetic marker construction of the bitter-acids components in the hop cone. Since 2010, he has moved to Frontier Laboratories of Value Creation and conducted research on the flavor of beer and other beverages. In April 2012 he moved to the Bioresources Research and Development Department, where he is working as a hop breeder/researcher.

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Determining the premature yeast flocculation potential of malt by using the miniature fermentation assay with synthetic wort and a malt washing technique

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Premature yeast flocculation (PYF) has proven to be troublesome for the malting and brewing industries. It causes production difficulties and quality issues characterized by poor attenuation and low yeast cell counts post-fermentation. These issues result in variability in fermentation and flavor profiles. For this reason it is critical for brewers to assess their malts for PYF potential. To test for PYF potential in malt, the industries rely on a variety of fermentation assays to indicate if a sample has PYF potential. This study used a modified miniature fermentation assay to determine if malt displays PYF potential. Instead of using traditional wort preparation methods, a wash technique was used to extract the PYF factor for use with synthetic wort. This modification to the assay eliminated the need for a controlled mashing device and reduced the number of fine adjustments of the wort pre-fermentation to reach target start values. Control malt known to display no PYF attributes and PYF-inducing malts were used in the study. To further our understanding of the PYF factor the "PYF solution" extracted using the wash technique was subjected to various conditions pre-fermentation. The PYF solution was mixed with the synthetic wort with varying ratios from 0 to 100% of the PYF solution (mixed with distilled water) to find the threshold at which PYF characteristics were displayed. The wort was treated with a combination of pre-fermentation boiling (60, 90, or 120 min), chilling at 5°C, and freezing at -30°C to further our understanding of the how these conditions affect the PYF factor. The trials were conducted using a 15 mL fermentation with a consistent temperature and pitch rate (21°C, 1.5×10^7 cells/mL). The change in absorbance and Plato was monitored throughout the fermentations. Control malt had no significant ($P > 0.05$) difference in absorbance and Plato measurements from PYF-inducing malt when the synthetic wort had <70% PYF solution. Synthetic wort containing >70% PYF solution had significant ($P < 0.05$) differences from the control malt for both absorbance and Plato measurements. Boiling treatments alone did not produce significant ($P > 0.05$) differences in the trials. Boiling followed by chilling yielded the same results and did not have significant changes ($P > 0.05$) in fermentation characteristics. While most of the pre-fermentation treatments did not create differences in fermentation characteristics, boiling wort for >60 min and freezing before fermentation caused the >70% PYF solution to

display the same fermentation characteristics as the control ($P > 0.05$). These findings show that the PYF-inducing factor may be susceptible to further processing. This experiment established two further findings. First, that the factor causing PYF must meet a threshold before it affects fermentation. Second, that mashing the suspected grain may not be absolutely crucial for testing PYF potential. Further testing of known PYF-inducing malts is needed to determine if this washing technique works for all PYF-inducing malts.

Joshua Adler received a B.S. degree in biology from Dalhousie University in Halifax, NS, Canada. While pursuing his degree he became very interested in food science and was the first Dalhousie student to obtain a minor in the discipline. His undergraduate thesis focused on problems encountered in wheat beer production, which he presented at the 2011 ASBC Annual Meeting. Josh is continuing his brewing research as an M.S. candidate at "Dal" where his research is focused on using various techniques to detect the premature yeast flocculation potential of malt, which he presented at the WBC 2012. In 2011, Josh became the quality manager at Propeller Brewery. At Propeller Josh strives to routinely produce beers of the highest quality, as well as create innovative and useful brewing research. When outside the laboratory, Joshua can usually be found training at the boxing club, at the movie theater, or enjoying a pint with his friends. One of his life's ambitions is to visit as many of the worlds' brewing and distilling regions as possible.

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Development and validation of a physical stability forcing test

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A forcing test was developed to produce chill haze that was similar to that at the end of shelf life. Beers were punished at 50°C for various lengths of time, up to 6 weeks, and their chill haze was measured. Samples were also stored at room temperature for up to 1 year to encompass the beer's shelf life. End-of-shelf-life chill haze was subsequently compared with earlier results from the 50°C storage study. The appropriate length of punishment was determined that would give results similar to the end-of-shelf-life chill haze. Once the length of punishment was determined, the new forcing test was validated by running a number of additional samples and comparing results with end-of-shelf-life chill haze.

Stephen L. McCarthy is a senior chemist in the Brewery Technical Center Department of Anheuser-Busch InBev. He received a B.S. degree in chemistry at the University of Missouri-St. Louis in 1976 and was employed as a senior technologist at Smith-Kline Clinical Laboratories from 1977 to 1984. In 1984, he joined Anheuser-Busch as a chemist in the Analytical Services group. He currently works in the Brewing Science group. His duties include work on chillproofing, beer haze, atomic spectroscopy, and HPLC. His work on behalf of ASBC includes chairing the technical subcommittees on Iron in Beer by Ferrozine Method and also Fermentable Carbohydrates by HPLC, as well as presentations at four annual meetings.

Evaluating the impact of sample composition on color in specialty malts

ELIZABETH A. ROBERTS (1)

(1) Briess Malt and Ingredients Co.

The different methods used in malt drying produce a variety of colors and flavors. Specialty malts are produced to contribute a wide variety of colors and flavors to many styles of beer and food products. Color is often a key component of the desired attributes and is a key release criterion for malt quality. Kilned, caramel, and dark roasted malts are all made from the same type of grain, but the differences in their manufacture lead to a wide spectrum of Maillard reactions that form color and flavor compounds essential to their identity. It has been shown that kilning and roasting produce different proportions of low molecular weight and high molecular weight color compounds. (Adriaenssens, 2004) The different amounts of these compounds in specialty malts lead to different color profiles. In order to better understand the variability in color of malts produced with different methods, four malt samples were taken and divided into the whole-kernel portion (on 5/64) and the thin and chaff portion (thru 5/64). Each component was analyzed in repeat analysis to determine the contribution of the chaff and thin portion to the color of the combined sample. The following malt types were used as samples for analysis: Bonlander Munich, Caramel 20°L, 2 Row Caramel 120°L, and Black Malt. In addition to determining the chaff and thin contributions, the internal variability of each test was determined. To achieve this, large samples of Caramel 20°L, Caramel 120°L, and Black Malt were taken and run repeatedly by lab technicians to quantify internal variability. This study showed that kilned, caramel, and roasted malts all have different color contributions from the chaff and thin portions of a sample. The chaff and thin portions of Bonlander had the smallest percentage of overall color contribution. Black malt had the highest thru percentage and also the largest thin color contribution. Black malt had the most significant negative color contribution. The data obtained in this study will be useful in determining variance in the analytical color of malt samples, in particular dark roasted products. This information can be used in identifying variance among samples, as well as to predict the effect of malt cleaning and aspiration on color.

Elizabeth Roberts received a B.S. degree in both food science and biochemistry from the University of Wisconsin-Madison. Prior to joining Briess, Elizabeth worked as a quality control supervisor for the J.M. Smucker Company. She gained experience in both malting production and quality analysis while working for Anheuser-Busch and the USDA Cereal Crops Research Unit in Madison, WI. She joined Briess Malt and Ingredients in 2009 and is currently a quality assurance chemist in the Technical Services Department at Briess, where she is involved in malt quality and research and development.

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Evaluation of international bittering unit calculations based on measurements of bitterness units via spectrophotometry and iso- α -acid concentrations via HPLC

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(1) Appalachian State University, Boone, NC

While it is believed that hop β -acids, once oxidized to hulupones, impart a minimal overall bitterness, it is the α -acids, via isomerization to iso- α -acids during the boiling of wort with hops, that are the most important bittering constituents of hops. To determine the bitterness in a beer, the bitterness units (BUs) can be measured spectrophotometrically or the iso- α -acids can be quantified via HPLC analysis. However, most small- to medium-sized breweries do not have the laboratory facilities to measure BUs or iso- α -acids in their beers and determine overall

bitterness. Rather, they rely on calculated estimates of the quantity of α -acids that isomerize and dissolve into the beer, the international bittering units (IBUs). There are several equations that are used for calculating IBUs, the most common of which are those posed by Garetz, Rager, and Tinseth. The Rager method is the oldest and generally results in the highest IBU values. The Garetz formula takes into account more factors and typically results in the lowest overall IBUs, largely because short boil times are estimated to provide no utilization in the equation. The Tinseth method is considered by many to be the most accurate, with IBU values that fall somewhere between the Rager and Garetz methods. However, no laboratory study has been conducted to determine which method is the most accurate and under what conditions, until now. Multiple small batches (20–80 L) of beer were brewed, varying different factors each time, including boil time, starting gravity, hop variety, hop addition (time and amount), hop type (pellet versus whole leaf), and yeast variety. The α -acid concentrations of hops used in the study were measured using HPLC, and the IBUs were calculated using the three formulas based on the measured concentrations. The BUs of the finished beers were determined by isooctane extraction followed by spectrophotometric absorbance at 275 nm. The iso- α -acid content of the finished beers was also measured using HPLC. For the most part, BUs and iso- α -acid concentrations correlated well, although the measured iso- α -acid concentrations of beers made with hops that were locally grown in North Carolina were lower than expected. Interestingly, the measured BUs for most of the beers analyzed matched the Garetz calculation of IBUs most closely. This was particularly true for the beers brewed with North Carolina-grown hops, in which isomerization of the α -acids appeared to be limited. The reason for the apparent limited isomerization of the α -acids in North Carolina-grown hops will be explored further, and additional conditions will be tested to assess which IBU calculation is most accurate and under what conditions.

Brett Taubman has been a faculty member of the A.R. Smith Department of Chemistry at Appalachian State University (ASU) since 2007 and is engaged in instruction and academic research within the chemistry and fermentation sciences. He has B.S. degrees in both finance and chemistry from the Pennsylvania State University and Montana State University, respectively, and a Ph.D. degree in analytical and environmental chemistry from the University of Maryland. Brett has successfully developed a pilot instructional brewing facility (Ivory Tower Brewery [ITB]) on the ASU campus and currently serves as president and head brewer of ITB, co-director of the Fermentation Sciences Program, and president of the High Country Beer Fest, an annual fundraiser for the program that showcases regionally crafted fermented foods and beverages.

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From after-the-fact air to instantaneous oxygen: Lessons learned to date from implementing chemiluminescent dissolved oxygen monitoring technology

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For the sake of flavor stability, it is imperative to minimize exposure of beer to oxygen, particularly during packaging operations. In order to improve our oxygen management capabilities, chemiluminescent oxygen sensors were commissioned for monitoring dissolved oxygen (DO). These sensors were installed in order to measure the DO in beer entering the bottle filler, and in addition, an apparatus was constructed to measure DO in crowned bottles. Individual fill heads quickly emerged as a significant factor. In stark contrast to our previous headspace test, real-time DO measurements have allowed improvements in efficiency of fill head maintenance. Additional improvements have been made to optimize

bottling run startups and identify and eliminate other oxygen ingress points on the bottling line. We will also discuss our approach in training tasters to recognize oxidation flavors and correlation of sensory panel results and analytical DO values.

Lauren Torres earned a B.A. degree in chemistry in 2010 and entered the brewing industry straight out of college. She quickly developed a passion for brewing and has quickly become a key member of the quality team at Bell's Brewery. Her ever-growing responsibilities span all aspects of the quality program, from analytical method development to microbiology and sensory analysis. Her colleagues at Bell's expect she will continue to thrive as a brewing chemist and will have a long and brilliant career in brewing quality.

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Hop flavor database

MARK ZUNKEL (1), Christina Schönberger (1), Martina Gastl (2), Dana Sedin (3)

(1) Joh. Barth & Sohn, Nuremberg, Germany; (2) Technical University of Munich, Freising, Germany; (3) New Belgium Brewing Company, Fort Collins, CO

The purpose of this work was to identify chemical compounds in hops that contribute to the flavor and aroma of beer and to create a database for professionals to use in sensory research. Each identified molecule in the database is shown with synonyms, flavor descriptors, typical concentration, formation, compound classification, structure, and molecular weight, when found. In addition, the authors identified thresholds determined in beer and flavor units. Separate categories are listed for each of these components. This preliminary work contains many important molecules that describe hop flavors contributing to beer. The significance for the brewing chemist is to be able to search from a large database of molecules to help find sources of flavors and aromas derived from hops.

Mark Zunkel is working on an M.S. degree from the Technical University of Munich – Weihenstephan, Chair of Brewing and Beverage Technology. He has been a member of ASBC for five years and recently received an ASBC Technical Committee grant to complete work on this hop flavor database. After completing a B.S. degree, also at Weihenstephan, and interning in breweries in the United States and Germany, he started working as a technical manager for Joh. Barth and Sohn based in Nuremberg, Germany.

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Hydrophobic behavior of gaseous carbonic acid is responsible for primary gushing of beer: Essential consequences on curative methods

ZAHRA SHOKRIBOUSJEIN (1), An Philippaerts (2), Sylvie Deckers (1), David Riveros Galan (1), Vladimir Ilberg (3), Jean Titze (4), Kurt Gebruers (5), Guy Derdelinckx (1)

(1) KULeuven-FBIW-M²S-MbS-LIBR, Heverlee, Belgium; (2) KULeuven-FBIW-COK, Heverlee, Belgium; (3) Hochschule Weihenstephan-Triesdorf, Germany; (4) University College of Cork, Cork, Ireland; (5) KULeuven-FBIW-M²S-CLMT-LFoRCe, Heverlee, Belgium

Gushing is vigorous overfoaming of carbonated beverages upon bottle opening. Primary gushing in beer originates from a group of proteins called hydrophobins that are secreted by filamentous fungi, which contaminate CO₂ gaseous molecules during carbonation and form nanobubbles. Gushing occurs when nanobubbles grow and explode as the result of the pressure drop when the bottle is opened. Research to prevent gushing within chilling and beer bottling was carried out without any success. Indeed, from a physicochemical point of view, the

properties of gaseous CO₂ interactions with hydrophobins do not allow such an approach. It is known that dry-hopping positively influences primary gushing. The influence of hop oil extract on primary gushing showed a complete suppressing effect in sparkling water, a decreasing effect in wort, and no influence on gushing-positive beer. Therefore, it is hypothesized that hop compounds may prevent primary gushing if added at the right point in the production process. This shows the importance of the critical point of addition of this product in the brewing process. GC/MS analysis shows that commercially available lypophilic hop extract comprises fatty acids (SFA and UFA) and waxes. SFA and UFA behave in a different manner regarding gushing. In contrast to SFA, *cis*-form UFA do not induce gushing. Waxes provide sufficient hydrophobic structures to interact with gaseous CO₂ molecules and induce gushing. It is hypothesized that hop antifoam molecules interact with hydrophobins and this interaction neutralizes the hydrophobic patch of hydrophobins, preventing full interaction with CO₂ and consequently reducing gushing.

Zahra Shokribousjein was born in Tehran, Iran, in 1978. She started her education in food science and technology at the University of Tehran and continued her M.S. degree work at the Isfahan University of Technology, Iran. She worked on the relationship between the structure of gum tragacanth and its physicochemical characteristic for her M.S. thesis. After graduation, she joined to a research group at Tarbiat Modaress University (Iran) and started work on water pollutants in northern Iran. In 2010 she applied for a Ph.D. position at KULeuven (Belgium) and started her Ph.D. research on the topic of "Interaction of the Hydrophobic Patch of Class II Hydrophobins with Hydrophobic/Hydrophilic Interfaces as a Basis for Curing Primary Gushing of Beer."

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Improving accuracy of pitching yeast using an Aber yeast monitor with flocculant yeast strains

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(1) MillerCoors, Irwindale, CA

Accurate and repeatable yeast slurry pitching is a crucial factor in achieving a consistent fermentation and flavor profile. Unfortunately the concentrations of yeast slurries are difficult to quantify because the traditional methods of laboratory cell counts or spun solids are prone to error, as well as being labor-intensive. The Aber yeast monitor is an ideal solution to these issues because it counts the yeast just before pitching. It also does not count dead cells, making it superior to other methods of in-line cell counting, such as turbidity meters. Initially our breweries were using this meter to take a "snapshot" of the yeast slurry as it was being pitched. These data were then passed through the PLC to calculate a total yeast mass for pitching. This "snapshot" method was not successful because with some yeast strains the yeast brinks were not homogenous. To deal with the lack of uniformity in these yeast brinks, two modifications were made to the pitch method: 1) the yeast mixing system in the brinks was upgraded with new mixing blades; and 2) the data produced by the Aber yeast monitor were integrated by multiplying the yeast concentration by the volume as determined by a mass-flow meter. Only by these means could a consistent and repeatable yeast concentration be achieved. The program now pre-calculates the amount of yeast required and pitches the total number of cells needed for that batch size. The percentage pitched for each fermenter is displayed for the operator to see in real time during the pitching process. This system has resulted in a 50% improvement in the accuracy and reproducibility of the pitching system. Such methods should be considered in planning and executing yeast automation systems to improve product quality.

Aaron Taubman received a B.S. degree in biochemistry from the University of Iowa, Iowa City, IA, in 1996 and then worked for several years in a genetics lab as a research assistant at the University of Iowa. After taking the short course at the Seibel Institute in Chicago, IL, he

decided he would rather be making beer. In 2001, he became part owner and head brewer at Millstream Brewing Co. in Amana, IA. He relocated to California in 2007 and is now the brewing quality specialist for MillerCoors in Irwindale, CA.

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Malt color: Its effects on yeast propagation and fermentation

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There have been suggestions that darker malt variations have an adverse effect on yeast propagation. Do the darker malt variations promote lower yeast yield than lighter malt variations and what are the effects on fermentation? This study contains the propagation and fermentation data that provide the answers to these theories. The study addresses fermentation issues such as slow attenuation, off-flavor compounds, etc. The data presented in the study were collected from multiple propagation cycles using light and dark malt variations, fermentation trials, and analytical test results.

Stephanie McKinley joined the White Labs team in 2011. She is currently working in the microbiology lab, where she performs QA testing, yeast production, and special projects. She home brews as a hobby.

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Malt protein analysis using amino acid tagging technology

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(1) Briess Malt and Ingredients Co., Chilton, WI

Traditional methods of protein analysis of malt such as Kjeldahl and combustion are based on the concept of relating total nitrogen content in a sample to the total protein content. However, not all nitrogen contained in a sample is necessarily present as an amino acid. This leads to potential overestimation of protein in a sample due to total nitrogen quantification. An alternative method of protein analysis that uses amino acid tagging technology was evaluated to determine its effectiveness as a method of malt analysis. CEM has developed this patent-pending technology, which is already being used in the dairy and meat industries. It uses a patent-pending iTAG colored tagging reagent that binds to specific amino acids in proteins. The amount of iTAG that binds is measured and then quantified as a total protein value. The CEM Sprint protein analyzer was used to determine the protein values of a variety of malt samples. Comparative analysis was done using the amino acid tagging technology and Kjeldahl analysis. A variety of malt samples were tested, including 2-row and 6-row varieties that had been malted and kilned in different ways to produce different malt products. Each sample was tested for repeatability and reproducibility on the CEM Sprint protein analyzer. Results of analysis with the CEM Sprint protein analyzer had good repeatability for each malt sample that was tested. However, there were inconsistencies between certain specialty malt proteins values via Kjeldahl compared with the CEM Sprint protein analyzer. These differences may have been due to the malt being exposed to higher temperatures during kilning, which may have affected the structure of one of the amino acids to which the iTAG solution binds. Overall, this method appears to show promise for malt protein analysis for certain types of malts, but not as a universal method.

Elizabeth Roberts received a B.S. degree in both food science and biochemistry from the University of Wisconsin-Madison. Prior to joining Briess, Elizabeth worked as a quality control supervisor for the J.M. Smucker Company. She gained experience in both malting production and quality analysis while working for Anheuser-Busch and the USDA Cereal Crops Research Unit in Madison, WI. She joined Briess Malt and Ingredients in 2009 and is currently a quality assurance chemist in the Technical Services Department at Briess, where she is involved in malt quality and research and development.

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Oxidative stress during very high-gravity lager brewing fermentation

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(1) University of Nottingham, Nottingham, UK; (2) SAB Miller

For many years the brewing industry has used high-gravity (HG) brewing as a means of increasing productivity without incurring additional plant costs. Typical HG fermentations, in the order of 15–17.5°P and producing an average yield of 6–7% ABV, are generally implemented on a worldwide basis. For continuing financial gains to be afforded, very high-gravity (VHG) fermentations, above 20°P are an increasingly attractive proposition. However, the potential use of VHG wort creates a number of issues across the brewing process: from wort production through to fermentation, as well as in matching final product specifications. From the perspective of the brewing yeast culture, the environment of VHG wort during fermentation is considerably different than that of standard gravity. Although previous studies have been performed to determine nutrition- and environment-related stresses associated with VHG brewing, there are additional responses that need to be understood. One example is the relationship between the extra demands placed on yeast metabolism and the generation of compounds that can be harmful to cells. In particular, during yeast metabolism, reactive oxygen species (ROS) are produced that can lead to oxidative stress. ROS are normal by-products of cellular activity, and although they are more frequently associated with respirative metabolism that occurs immediately after pitching, they have been shown to be produced throughout fermentation. Yeast possesses mechanisms to prevent damage by ROS, such as the free-radical scavengers superoxide dismutase (SOD) and catalase (CAT). However, when an imbalance occurs between SOD and the production of ROS, damage to nucleic acids, proteins, and lipids, as well as other cellular components, can occur. Furthermore, in the presence of elevated levels of ROS, yeast stress response systems are promoted, glycolytic enzyme expression can be reduced, and the ability of cells to produce ethanol may be compromised. Here we investigate the relationship between VHG lager fermentations, ROS generation, and cellular redox potential. In particular we focus on the generation of compounds that act as biomarkers for oxidative stress, as well as determining the antioxidant response of cells. It is anticipated that the data generated and presented here will provide insight into some of the challenges faced by yeast in fermenting worts with particularly high sugar concentrations.

Since graduating in 2005 with a B.S. in biology, Alexander Mott has enjoyed a variety of research-based positions. In 2007 Alex joined the Antimicrobial Research Group at the University of Birmingham to investigate the mechanisms of action and resistance to antibiotics, as well as exploring the role of antibiotic resistant pathogenic bacteria. In 2008 he joined the University of Nottingham, where he worked as a research technician in the Centre for Genetics and Genomics to study genome evolution, speciation, and natural variation in Saccharomyces yeasts. In 2011 Alex undertook an M.Res. degree on the analysis of the phenotypic characteristics of active dried yeast for brewing, and he is currently working toward a Ph.D. degree, looking at strategies for increasing ethanol yields from brewery fermentations.

Quantitative analysis of α - and β -hop acids by direct analysis paper spray ionization mass spectrometry

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(1) WMU, Kalamazoo, MI

Paper spray ionization is a novel method of mass spectrometric analysis that allows for rapid, easy, and accurate direct chemical analysis of plant materials and extracts. Paper spray is an ambient ionization method related to desorption electrospray ionization (DESI) and direct analysis in real time (DART). With paper spray a small isosceles triangle made from paper is used directly as the ion source. An extension of this technique (known as leaf spray) uses plant material directly. A small drop of aqueous solvent, typically 5–20 μL , is spotted onto a leaf to which 3–5 kV is applied by alligator clip. Ions are then produced from compounds in the leaf or on the surface of the leaf when these are soluble in the spray solvent. These ions are sampled in a mass spectrometer for analysis. In this presentation we demonstrate direct analysis of hops by paper spray and leaf spray. A single bract is separated from a hop cone and analyzed directly. The entire analysis takes less than 30 sec per run and, so, a representative analysis can be obtained by analyzing multiple bracts from a sample. Rich spectra are obtained and the α - and β -acids can be quantified relatively, and so, for example, cohumulone ratios can be calculated. In addition various classes of lipids and polyphenolic compounds are also observed, allowing for accurate typification of hop varieties by fingerprint matching or principal component analysis.

Gregg Hasman, Jr. graduated from Michigan Technological University in 2011 with a B.S. degree in general chemistry. While at Michigan Tech, he was the president of the American Chemical Society–Michigan Tech Student Chapter for his final two years of undergraduate studies and taught chemistry lab sections that included studio chemistry, quantitative analysis, and physical chemistry. Gregg is currently studying for his Ph.D. degree under Andre Venter at Western Michigan University.

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Quantitative analysis of total purine content using the HPLC-UV method in beer, low-malt beer, and third-category beer: 2012 BCOJ Collaborative Work

BCOJ Analysis Committee (1), TAKUYA HASHIMOTO (2), Takako Handa (3), Yoichi Kakudo (2), Muneyoshi Kanai (4), Kiyoko Kaneko (5), Chihiro Kenjo (6), Masaaki Nakahara (7), Wataru Nakamura (8), Atsushi Ohuchi (3), Takayuki Watanabe (9)
(1) Brewery Convention of Japan, Chuo-ku, Tokyo, Japan; (2) Suntory Liquors, Ltd., Shimamoto-cho, Mishima-gun, Japan; (3) Asahi Breweries, Ltd., Moriya-shi, Japan; (4) National Research Institute of Brewing, Higashihiroshima-shi, Japan; (5) Faculty of Pharmaceutical Sciences, Teikyo University, Itabashi-ku, Tokyo, Japan; (6) Shimadzu Co., Nakagyo-ku, Japan; (7) Kirin Group Office Co. Ltd., Tsurumi-ku, Yokohama-shi, Japan; (8) Orion Breweries, Ltd., Nago-shi, Japan; (9) Sapporo Breweries, Ltd., Yaizu-shi, Japan

Although beer contains a fairly small amount of purine, consumption of large amounts of alcoholic beverages, particularly beer, is associated with an increasing risk of gout. The BCOJ subcommittee was charged with evaluating the high-performance liquid chromatography (HPLC)-UV method for quantification of total purine content in beer, low-malt beer, and third-category beer. The collaborative work was carried out by 10 collaborators. Collaborators were provided with eight sample pairs consisting of low-purine content beer (≈ 20 – 30 mg/L, A/B, C/D, and E/F), moderate-purine content beer (≈ 40 – 90 mg/L, G/H, I/J, and K/L), and high-purine content beer (≈ 100 – 140 mg/L, M/N and O/P). Each sample was degassed and hydrolyzed by 70% perchloric acid.

The hydrolyzed sample solution was neutralized with 8.0 mol KOH/L, followed by centrifugation. The supernatant of the solution was filtered and injected into the HPLC system for analysis of adenine, guanine, hypoxanthine, and xanthine. The total purine content was calculated using the absolute calibration method. HPLC was performed under the following conditions: instrument, HPLC-UV system without regard to manufacturer; column, Shodex Asahi Pak GF-310 HQ (7.5 mm i.d. and 300 mm length) or GS-320 HQ (7.5 mm i.d. and 300 mm length); mobile phase, 150 mM sodium phosphate buffer (titrating 150 mM sodium dihydrogenphosphate aqueous solution to pH 2.5 with phosphoric acid); flow rate, 0.6 mL/min; column temperature, 35°C; detector wavelength, 260 nm; and injection volume, 20 μL . Measurement of adenine, guanine, hypoxanthine, and xanthine was performed in duplicate. The subcommittee recommended that each collaborator should check any peak that was separated completely in the pretest. If the peak was not separated completely, the appropriate pH of mobile phase from 2.3 to 2.8 needs to be selected. Results from 10 collaborators who performed the HPLC-UV method were received for the eight sample pairs (A/B, C/D, E/F, G/H, I/J, K/L, M/N, and O/P). The statistical summary of results is shown as follows: RSD_r ranged from 0.8 to 4.6%; r^{95} ranged from 1.7 to 7.6 mg/L; RSD_r ranged from 11.6 to 16.8%; R^{95} ranged from 8.0 to 49.5 mg/L, respectively, and were judged acceptable. It was concluded that the HPLC-UV method is capable of determining total purine content in beer, low-malt beer, and third-category beer containing 20–140 mg of total purine/L. The subcommittee recommends that the HPLC-UV method be adopted for inclusion in the *Methods of Analysis of the BCOJ*.

Takuya Hashimoto graduated with an M.Eng. degree from Osaka University in 2009. Since joining Suntory, he has worked for three years in the Beer Development Department of Suntory Liquors Limited.

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Research on the relationship between malting barley fungi and premature yeast flocculation

YAN LIN (1)
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Previous research showed that premature yeast flocculation (PYF) is associated with malt husk and can be induced by fungal infection. Fungal extracts and fungal xylanase had an impact on PYF. In our study, eight kinds of fungi from malting barley and malt were separated and identified by PCR-DGGE and sequencing of amplification analysis. Malt husk was treated with purified and activated fungi for PYF testing to determine PYF sensitive and non-sensitive fungi. At the same time, xylanase in fungal extracts also was analyzed to determine the xylanase activation range for PYF-positive malts. PYF sensitive and non-sensitive fungi were pitched on malts at the beginning of germination during micro-malting to quantify fungi level for PYF-positive malt. Lab brewing tests were carried out to investigate incomplete yeast utilization of fermentable sugars in wort and insufficient yeast numbers for beer maturation in PYF-positive malts.

Yan Lin obtained a D.Eng. degree in molecular biology from China Ocean University, Life and Technology Department, School of Pharmacy (2006); an M.Agric. degree from Shenyang Agricultural University, Life Fundamental Department, School of Plant Physiology and Biochemistry (1994); and a B.S. degree from Liaoning Normal University, Biological Department (1990). After obtaining a master's degree in 1994, Yan trained in analysis using ABI and Agilent instruments, computer applications, malting and brewing technology processes, quality, and research management at Tsingtao Brewery and other training centers. In 2004, Yan trained in molecular marker technology for yeast and microbes, malting barley and hops, food safety, lab management, and yeast and microbe management, as well as other fields, at BRI. In 2006, Yan trained in malting barley QA at an Anheuser-Busch brewery.

In 2007, Yan trained in hop production and quality control at Yakima Haas and in Australian malting barley QC at CBH Grain. In 2008, Yan trained in Canadian malting barley QA at CWB (Canadian Wheat Board) Cigi, and CMBTC (Canadian Malting Barley Technical Centre). Yan has received the Second National Prize for Progress in Science and Technology from the Chinese government; Second Provincial Prize for Progress in Science and Technology from the Shandong government; and First Municipal Prize for Progress in Science and Technology for the innovative research project "Beer Flavor Chromatographic Technology Development and Application," from the Qingdao government.

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Sowing dates and malting barley quality in Guanajuato, Mexico

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In Mexico, malting barley is produced under rain-fed conditions during summer and irrigated conditions during winter. Under irrigated conditions it is cultivated in the Mexican "El Bajío" region. The largest malting barley-producing state in this area (>90%) is Guanajuato, which produces 260,000 tons a year. One of the problems observed in recent decades in Guanajuato, aside from water availability, are the maximum and minimum temperatures required, which define a very short period for growing malting barley. Because of the difficult production conditions, the national barley program of INIFAP (National Institute for Forestry, Agriculture and Livestock Research) has generated short-cycle barley genotypes (90–120 days) that allow some adaptation. In the past, the recommended planting date for malting barley in El Bajío was established considering grain yield and was set from December 1 to December 26, but malting quality was not considered. The aim of this study was to evaluate the effect of five planting dates on malting barley grown in Guanajuato. Four malting barley genotypes (six-rowed) were evaluated in two growing seasons (winter 2010/2011 and winter 2011/2012), and planting dates were November 15 (F1), November 30 (F2), December 15 (F3), December 30 (F4), and January 15 (F5). Genotypes were advanced lines M173, M10542, and RV9850 and the most planted malting barley variety in El Bajío, Esperanza; all were grown in Guanajuato, Mexico. Planting dates affected both agronomic and malt quality traits. Maturity decreased as the dates progressed (122 to 101 days), which was related to minimum temperatures observed during November and December (0–10°C) and maximum temperatures observed in February (25–35°C). Grain yield was higher in F2 (6,686 kg ha⁻¹) and F3 (7,326 kg ha⁻¹), while the lowest grain yield was observed in F5 (4,629 kg ha⁻¹). The most sensitive genotypes to date changes were Esperanza and M10542, with a 40% reduction of grain yield in F5 compared with F3. Grain protein was lower in F3 (12.6%) and higher in F5 (>13.6%); as a result, diastatic power in malt was lower in F3 (114°ASBC) and greater in samples with high protein contents (166°ASBC), which was expected because of the known β -amylase and hordein correlation. The same behavior was observed with malt extract. Considering these results, the best planting date for malting barley in Guanajuato was between December 1 and December 15, but it will be necessary to determine the days leading up to December 15 to ensure the best grain yield and quality are attained. This represents a big challenge for the farmers of Guanajuato, who would have to irrigate 60,000 ha of malting barley in less than 2 weeks.

Ramón Huerta received food engineering and M.S. degrees from Universidad Autónoma Chapingo, México State, México. He began employment at Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) in 2008. He is a researcher in the lab of malting barley quality and collaborates with INIFAP's national barley breeding program. This program, since 1957, has collaborated with the brewing

industry (Grupo Modelo and Cervecería Cuauhtemoc-Moctezuma) and has delivered all malting barley varieties grown in Mexico.

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The impact of pro-oxidative storage conditions on the aroma profile of Hallertauer Mittelfruh hops

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The prolonged exposure of noble hops to pro-oxidative conditions during storage results in the formation of new flavor compounds. Brewers hypothesize that these oxidation products can potentially create positive sensory attributes in the aroma profile of these hops and beers made with them. This study focused on measuring changes in aromatic compounds in Hallertauer Mittelfruh (HMF) after exposure to pro-oxidative conditions. Control HMF hops were stored at -20°C under inert (N₂) atmosphere, whereas oxidized hops were stored under O₂ atmosphere at 37°C and measured at two time points, 7 and 14 days. All samples were stored in glass vials with septum-lined screw-top lids. Solid-phase micro-extraction (SPME) and gas chromatography-mass spectrometry (GC-MS) were used to determine the relative change in concentration of aroma compounds in the headspace of stored HMF samples. Gas chromatography-olfactometry (GC-O) was used to obtain descriptive data and a subjective assessment of the aroma profile of HMF samples. GC-MS results indicated that oxidized HMF contained an increased terpenoid fraction, as well as the formation of new oxygenated compounds not found in the control. GC-O evaluations revealed differences in aromas and corresponding eluting peaks between the oxidized and control HMF samples. In some cases aromas were observed with no corresponding peaks, suggesting the presence of an aroma compound with a low sensory threshold.

Victor Algazzali is a master's student in Thomas Shellhammer's lab in the Department of Food Science and Technology at Oregon State University. His research encompasses sensory science, hops, and beer. He has researched the bitterness quality of hop acids, sensory changes in sour beer production, and aromatic qualities of raw hops. Prior to joining the food science program at OSU, Victor earned a B.S. degree in food science at the University of California, Davis. During his time at UC Davis Victor worked in a sensory science lab, assisting with consumer studies and complementary data analyses. Victor spends his free time playing soccer, camping, and brewing beer.

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The loss of hop bittering compounds from various hop sources during the brewing process

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During production and over time iso- α -acids precipitate, oxidize, and degrade. The purpose of this study was to investigate the extent to which individual types of iso- α -acids are lost and whether the mode of hopping (pellets or extracts) impacts the losses observed during production. Five beers were brewed, in duplicate, and samples at various stages were collected and extracted for analysis via HPLC-diode array detector. The *trans/cis* ratio observed in the liquid did not match that observed in the loss, and ultimately, it appeared that precipitation/adsorption played a minimal role in the loss of these compounds. The best retention of iso- α -acids was observed with the use of isomerized kettle extract, and overall during production the loss of *cis*-iso- α -acids was greatest for all brews. Minimizing foaming using suppressants had no effect on the retention of iso- α -acids.

Aaron Golston received his B.S. degree in chemistry from the University of Washington in Seattle and, after a year helping establish Fremont Brewing in Seattle, returned to graduate school at UC Davis, where he obtained an M.S. degree in agricultural and environmental chemistry. Aaron worked as a quality analyst at the Anheuser-Busch brewery in Fairfield, CA, during graduate school and, upon completion of his studies, began work at MillerCoors in Irwindale, CA, as an analytical specialist in the product quality laboratory.

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The purification and properties of ascorbic acid oxidase from malted barley

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Barley and the pale malts derived from it contain a range of oxidase enzymes that catalyze the oxidation of substrate with molecular oxygen. Among these are polyphenol oxidase, lipoxygenase, and thiol oxidase. A hitherto unreported enzyme is ascorbate oxidase, which we have now identified in malted grain. The enzyme has been purified and its properties investigated. The importance of the enzyme in mashing has been illustrated in mashes to which exogenous ascorbic acid has been added. The addition of ascorbic acid results, among other things, in a decrease in color and soluble polyphenol in the resultant wort. We have investigated the role of ascorbic acid oxidase in preferentially scavenging oxygen in mashes and, as a consequence, the merits of ascorbic acid additions in sweet-wort production to counter potentially deleterious impacts of oxygen at this stage.

Makoto Kanauchi graduated from the Tokyo University of Agriculture in Tokyo, Japan, in 1996 and received a Ph.D. degree in bioregulation control from that university in 1999. He was a postdoc in Charlie Bamforth's laboratory in the Department of Food Science and Technology, University of California at Davis (1999–2003). Subsequently, he was employed at the Institute of Food Science in Fuji Oil Co. Ltd. in Moriya, Ibaraki, Japan, as a researcher (2003–2005). Since 2005, he has been employed at the Department of Food Management, Miyagi University. He has also been a lecturer on enzymology and alcoholic beverages (mainly spirits and wine) at the Tokyo University of Agriculture since 2005.

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The role of flavoring agents in carbonation rate for yeast carbonated non-alcoholic soda: A general chemistry experiment with interesting problems

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The use of yeast to naturally carbonate soda can serve as an excellent capstone experience for a year-long, college-level general chemistry lab experience. A broad set of topics can be integrated into the experiment and discussion, including gas laws, kinetics, catalysis, the role of temperature in promoting reactions, biochemical reaction pathways, aerobic and anaerobic metabolism, simple organic structure

and nomenclature, and others. It has the added benefit of being very interesting and engaging for students without bringing the difficulties associated with combining alcohol and underage students. A repeatable, reliable, safe procedure has been developed using Lallemand Nottingham ale dry yeast, Gnome Beverages soda extract, commercial table sugar, and bottled water. The procedure provides individual samples for students to test and uses materials that make it inexpensive and easy for them to repeat on their own. Under identical conditions, flavoring agents such as root beer and vanilla cream had dramatically different carbonation rates compared with results obtained using spicy ginger beer or autumn red birch beer flavorings. To better understand the promotion or inhibition of these flavoring agents and their active components, several other flavoring compounds and additives have been investigated. Results show similar effects for certain organic structures that may be important in inhibiting aerobic fermentation and carbon dioxide production.

Mark Thomson is currently an associate professor of chemistry in the Department of Physical Science at Ferris State University in Big Rapids, MI, where he teaches courses in general chemistry, biochemistry, and science education. He received his B.S. degree (1987) from the University of Utah in chemistry and his Ph.D. degree (1995) from Colorado State University in inorganic chemistry.



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