WBC Oral Presentation Abstracts

Oral Presentations

Technical Session 1: Hops I

1. Influence of fermentation compounds from yeast on the quality of hop aroma. Presenter: Hitoshi Takemura, Kirin Brewery Company, Limited, Japan

Hop aroma is a very important factor that contributes to the flavor of beer; therefore, a great deal of care is taken to adjust hop aroma by controlling brewing parameters. Nevertheless, even when using the same hop variety under the same conditions (timing of addition and quantity), samples can have different aroma intensities if fermentation is performed using different brewing conditions (temperature, yeast pitching rate, etc.). Therefore, we investigated the cause of differences in aroma intensities in order to facilitate more precise control of hop aroma. We had the following two hypotheses: "the extent of conversion of hop aroma by yeast varies according to the brewing conditions" (hypothesis 1), and "hop aroma is masked by compounds that are produced by yeast during fermentation" (hypothesis 2). To test these hypotheses, samples that had different intensities of fruity aroma (lychee, citrus-like), even though the same hop variety (American Cascade and New Zealand Motueka) and hop addition conditions were used, were subjected to GC/MS analysis to determine whether there were any differences in the quantity of aroma compounds. The results indicated that the amount of linalool and the amount of beta-citronellol produced by the yeast did not significantly differ between the samples. This suggests that the validity of hypothesis 1 is low. Furthermore, as there was a difference in the amount of compounds that arise from fermentation, hypothesis 2 appears to be valid. Next, we investigated the influence of esters and alcohols on hop aroma. We observed a tendency for alcohols (e.g., 1-heptanol) to mask fruity aromas. Therefore, we brewed samples in which cold wort from the same batch was fermented (in a 20 L scale fermentor) using different fermentation conditions. Sensory evaluations revealed differences in fruity aroma between samples. Statistical analysis indicated that there was a significant negative correlation between 1-heptanol and fruity aroma. Furthermore, 1-heptanol was positively correlated with the number of yeast cells added at the start of fermentation and fermentation temperature. These results further support hypothesis 2 and suggest that it is important to control fermentation conditions. The results of this research have lead to the establishment of specific brewing conditions for more precise control of hop aroma.

Hitoshi Takemura has worked for Kirin Brewery Company Limited since receiving a master's degree in life science from Kyoto University in 2002. He worked in the Quality Assurance Department of the Tochigi brewery for three years and then entered the Laboratory for Brewing, where he conducted research on the use of hops in wort boiling. From 2008 to 2010 he worked as a guest researcher in Lehrstuhl fuer Brau- und Getraenke Technologie fuer Technische Universitaet Muenchen. Since August 2010 he has worked in the Brewing Technology Development Center.

2. Hop aroma and harvest maturity. Presenter: Daniel Sharp, Oregon State University, Corvallis, OR, USA. Co-author(s): Yanping Qian, Shaun Townsend, and Thomas Shellhammer, Oregon State University, Corvallis, OR, USA.

Hop chemical composition changes, in particular aroma development, during plant maturation are part of a rapid and dynamic process that requires a comprehensive, in-depth chemical and sensory analysis to maximize characteristics of interest to brewers. The complex aroma chemistry associated with hops in beer has been a confounding variable for practical brewers, and a deeper understanding of hop aroma development during cultivation is needed. This presentation discusses results and conclusions from a two-year study and compares these results with other studies that have examined location and harvest time and their effect on brewing quality. The effect of harvest date and location on and a variety of key chemical components of Willamette and Cascade hops were investigated for the 2010 and 2011 growing seasons. Hops were harvested at three time points within a 3-week interval (early, normal, and late), from three different farms in the Willamette Valley, Oregon, and then analyzed for moisture, hop acids content, total oil content, and essential oil composition. The response of analytes was dependent on the variety being examined, its location within the Willamette Valley, as well as the time of harvest. Hop acids did not change appreciably during plant maturation, while hop oil content increased hyperbolically to a plateau as the hops aged on the bine. Increases in oil quantity were strongly correlated ($r > 0.80$) with increases in alpha-pinene, beta-pinene, myrcene, limonene, methyl heptanoate, linalool, and eudesmol concentrations. Growing location within the Willamette Valley had a significant effect on oil concentrations for each variety at each time point, thus suggesting that individual grower practices and local environmental influence hop chemical composition.

Daniel Sharp is a master's student in the Food and Fermentation Science program at Oregon State University. His research is currently focused on hop studies being conducted in Thomas Shellhammer's lab. Daniel's primary area of study is the aroma compounds in hops and beer. Prior to joining the Food Science program at OSU, Daniel earned a B.A. degree in both Spanish and adventure leadership at the University of Oregon. After graduation he lived and worked in South America, first as a mountain guide in Venezuela and later as a brewer at the Center of the World Brewery, Ecuador's only microbrewery at the time.

3. Phenolic profiling of lager beer during aging in relation to hopping technology. Presenter: Patricia Aron, MillerCoors, Milwaukee, WI, USA. Co-author(s): Thomas Shellhammer, Oregon State University, Corvallis, OR, USA; David Ryder, MillerCoors, Milwaukee, WI, USA.

The most important class of polyphenols for consideration in beer and related products is that of the 2-phenylbenzopyrans, generally referred to as flavonoids. In contrast to the roles of other hop derived ingredients, such as the isomerized alpha-acids and their reduced products (bittering acids), the absolute value of hop derived flavonoids is not well realized. To add to the confusion, very little is understood regarding the fate of these polyphenols during the brewing and aging processes. During this experiment lager beers were produced using varying hopping regimes to investigate hop product contribution to beer polyphenol content. Finished beers were also force-aged and monitored for changes in polyphenolic profiles. Polyphenol rich extracts were produced from the beers using Sephadex LH20 resin. Finished beers varied in total polyphenols, flavanoids, and proanthyocyanidins by hopping regime.

Phloroglucinolysis was used in conjunction with RP-HPLC-ESI-MS to reveal subunit composition and proanthocyanidin mDP. Six major phloroglucinolysis products were observed; however, galloylated flavanols were not detected in any of the beers. The predominant subunits by molar ratio were (+)-catechin followed by (–)-epigallocatechin; however, beers brewed with hop solids were also high in (–)-epicatechin. The major extension subunit was (+)-catechin for all treatments. Although Sephadex extracts produced from the beers were phenolic in nature, proanthocyanidins only accounted for up to 2% of the total phenolic material. Total flavanoid and proanthocyanidin content of the beers increased initially during storage, with eventual decreases occurring after 6 weeks of storage at 30°C. Beers high in hop polyphenols did not suppress the loss of iso-alpha-acids during aging and were also assessed as the least flavor stable of the beers test by ESR T150. Conversely, the presence of hop polyphenols suppressed the formation of staling aldehydes during aging as measured by SPME-GC-MS.

Pattie Aron received a B.S. (2000) degree in biochemistry from Elmira College, Elmira, NY, and further obtained both M.S. (2007) and Ph.D. (2011) degrees in food science and technology from Oregon State University, Corvallis, OR. In February 2010 she began employment as an intern at MillerCoors as a hop chemist in applied brewing technology in the Technical Center in Milwaukee, WI. Following completion of her Ph.D. degree, Pattie continued on at MillerCoors, where she functions as a hop scientist and continues to conduct research for applied brewing applications.

4. Contributions to hop aroma in beer from the water-soluble fraction of hops. Presenter: Thomas Shellhammer, Oregon State University, Corvallis, OR, USA. Co-author(s): Daniel Sharp, Yanping Qian, and Michael Qian, Oregon State University, Corvallis, OR, USA.

Hop aroma in beer is complex. While hop oils contain over 300 different components that can contribute to beer flavor, their high volatility results in little hop oil being retained in the finished beer. Yet beers produced using a long boil do have significant hop flavor and aroma. While terpene alcohols and oxidation products (epoxides) can contribute flavor, another hypothesized source of this aroma lies in glycosidically bound aromatic compounds such as glucosides and arbinoglycosides of alcohols, monoterpene alcohols, and ketones. During fermentation, and more likely post-fermentation, yeast may hydrolyze the sugar moieties for energy and, thereby, release the volatile aglycone, thus contributing to hop aroma in beer. This study examined the impact of the water-soluble fraction from four different American hop varieties (Simcoe, Centennial, Citra, and Cascade). Samples of each variety were extracted using supercritical fluid CO_2 extraction, and the resultant extract and spent materials (along with the starting material) were dosed at (1 g/L) in hot wort to produce approximately 40 L of finished beer. Volatile analysis of beers was performed using a stir bar sorptive extraction (SBSE) with compound identification via GC-MS-FID. Key aroma compounds, alpha-pinene, beta-pinene, myrcene, limonene, linalool, caryophyllene, humulene, and terpineol were identified using selective ion monitoring mode and quantified. The linalool concentrations in the spent hopped beers were significantly higher than the other two treatments and were high enough to contribute to hop aroma. Principal component analysis revealed clustering of beers into separate groups by type of hop material (pellet, extract and spent). The sensory descriptive data identified prominent differences among the form and variety treatments. The spent hop treatments produced beers that had perceptible hop aroma. In one instance, Simcoe, the spent treatment resulted in beers that had higher aroma than extract and pellet treatments from other varieties. The intensity and nature of the hop aroma in the spent treatments was hop-variety specific, making it difficult to make a blanket statement regarding the water-soluble components of hops and their impact on hop aroma across all varieties. Nonetheless, there is sufficient evidence that in Citra and Simcoe hops the spent material contains substantial hop aroma or precursors thereof. These results point to the importance of non-oil contributions to hop aroma in some varieties.

Thomas Shellhammer is the Nor'Wester Professor of Fermentation Science in the Department of Food Science and Technology at Oregon State University, where he leads the brewing science education and research programs. His brewing research investigates hops and beer quality. He directs the brewing education component of the Fermentation Science program at OSU and teaches courses about brewing science and technology and beer and raw materials 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 a member of the Board of Examiners for the Institute of Brewing and Distilling, London, England, and the chair of the Editorial Board of the MBAA Technical Quarterly.

Technical Session 2: Analytical I

5. Brewing with barley: Comparing protease activities with the resulting proteins and peptides in beer using activity-based protein profiling and LC-MS/MS. Presenter: Lone Baekgaard, R&D, Novozymes A/S, Bagsvaerd, Denmark. Co-author(s): Renier van der Hoorn and Joji Villamor, Plant Chemetics Lab, Max Planck Institute for Plant Breeding Research, Cologne, Germany; Christian Jørgensen, Carsten Sönksen, and Niels Elvig, R&D, Novozymes A/S, Bagsvaerd, Denmark; Stefan Kreisz, Carlsberg Research Laboratory, Copenhagen V., Denmark; Hans-Peter Heldt Hansen, R&D, Novozymes A/S, Bagsvaerd, Denmark.

Today it is possible to brew beer with 100% unmalted barley using the enzyme product Ondea Pro. Although the barley beer is very similar to a malt beer in many aspects, differences could be observed when we analyzed the beers at the proteomic level. The overall content of proteins and peptides was higher in barley beer compared to malt beer. Using LC-MS/MS we have shown that the peptides mainly originated from the proline rich hordeins. This was in line with previous results, where we have seen a lower concentration of the less fermentable free amino acids, especially proline, in the barley wort. These results show that the proteolytic activities are different when you brew a barley beer with Ondea Pro compared with a pure malt beer. Ondea Pro contains a protease that works in synergy with endogenous proteases in barley (S. Aastrup [2010], Scand. Brew. Rev. 67:28-33.). Thus, to improve our understanding of protease activities in barley compared to malt, we analyzed barley extracts (with and without the Ondea Pro protease) and malt extract using technique activity-based protein profiling (ABPP). This is a new and powerful technique to be applied in brewing related research. The technique employs specific probes for different classes of proteases that bind irreversibly to the active site of the proteases, but only when the proteases are active and, thus, it is not dependent on substrates as many other protease assays are (I. Kolodziejek et al. [2010], Curr. Opin. Biotechnol. 21:225-233). Four different probes were tested under different brew-

ing relevant pH and temperature conditions for papain-like cysteine proteases (PLCPs), serine hydrolases (including serine proteases such as carboxy peptidases), proteasome (threonine proteases), and vacuolar processing enzymes (cysteine proteases). Clear differences in protease activities were observed between malt and barley under the different conditions, which likely play a role during the brewing process. For example, PLCPs were very dominant in malt, whereas no activity was seen in barley. These results support previous findings, where it has been shown that PLCPs such as EP-A and EP-B are produced during germination (S. M. Koehler et al. [1990], Plant Cell 2:769-783). However, when the Ondea Pro protease was added to the barley extract, some PLCP activity could be seen. This shows that the Ondea Pro protease surprisingly is able to activate endogenous proteases in barley and in this way work in synergy with the barley proteases.

Lone Baekgaard has a Ph.D. degree in plant physiology from Copenhagen University (2005). From 2005 to 2009, she had a post-doc position at Copenhagen University, where she worked with biochemical characterization of plant enzymes. Since April 2009, she has been working as a research scientist in the Department of Brewing and Alcoholic Beverages, R&D, Novozymes A/S, focusing on protein chemistry within brewing.

6. Monitoring flavor active epoxydecenals during beer storage at ppt levels. Presenter: Nils Rettberg, TU Berlin/VLB Berlin, Germany. Co-author(s): Konrad Neumann and Leif Garbe, TU Berlin/VLB Berlin, Germany.

Flavor active *cis*- and *trans*-4,5-epoxy-2E-decenal isomers (epoxydecenals) are important (off)-flavor compounds in wheat bread, popcorn, oils, beef meat, fruit juices, tomatoes, tea, etc. They originate from linoleic acid oxidation; therefore, their presence in wort and beer is likely. *trans*-4,5-Epoxy-2E-decenal has a very intense metallic taste and smell. Its flavor threshold is reported at 0.6 pg/L in air and 20 ng/L in water, respectively. In the literature, the aroma of *cis*-4,5-epoxy-2E-decenal is described as citrus-like, sweet, fatty, and malty. So far there are no odor and flavor thresholds published. The concentration of epoxydecenals in a foodstuff varies widely. Fresh tomatoes contain up to 600 µg *trans*-epoxydecenal per kg in fruit juices, and black tea concentrations are considerably lower. In fresh grapefruit juice 3 µg/L *trans*-epoxydecenal were traced; in black tea the sum of both isomers is in the 1 µg/L range. Even at these comparatively low concentrations, epoxydecenal isomers were identified as key odorants of these products. In fresh beer we analyzed epoxydecenal concentrations at 20 pg/L. In wort we quantified 3–4 µg/L of both isomers in sum. Determination of this ultra-trace compound requires sophisticated analytical techniques. To quantify epoxydecenals from beer, mash, and wort, we established a rapid and effective solid phase extraction (SPE) procedure. A stable isotope dilution assay (SIDA) was established for their quantification. Negative chemical ionization–selected ion monitoring mass spectrometry (NCI-SIM-MS) was used for analysis. This method increased the sensitivity/selectivity and resulted in a reliable and ultra-trace quantification. Epoxydecenals were quantified in mash and wort, as well as in fresh and aged beers. We observed an increasing epoxydecenal concentration during beer storage. The storage temperature, light, or linoleic acid addition showed remarkable effects on their concentration and their *cis*/*trans* ratio.

Nils W. Rettberg is a trained brewer and maltster from Radeberger Gruppe, Germany. In 2011, he received a diploma in biotechnology from the Berlin Institute of Technology (TUB) and started as a Ph.D. student at the TUB Chair of Bioanalytics. In addition, Nils is employed at the Research and Teaching Institute for Brewing in Berlin (VLB), Department for Special Analyses. His work includes courses for students of biotechnology and brewing science ranging from basic chemical-technical analysis to more sophisticated modern analytical techniques. As a member of Leif-Alexander Garbe's research group his scientific work focuses on brewing-relevant special analyses using mass spectrometry and stable isotope dilution assays. Initiated by his diploma thesis on "Flavor Active Epoxydecenals," he has developed a deep interest in lipid oxidation, beer staling, and trace analysis in brewing.

7. Analysis of Michigan hop varieties and easy and direct typification by paper spray ionization mass spectrometry and principal component analysis. Presenter: Andre Venter, Western Michigan University, MI, USA. Co-author(s): Kari Blain, Western Michigan University, MI, USA.

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 papers pray 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 into a mass spectrometer for analysis. In this presentation we demonstrate direct analysis of hops by leaf spray. A single bract is separated from a hop cone and analyzed directly. The entire analysis takes less than 30 sec per run, so a representative analysis can be obtained by analyzing multiple bracts from a sample. Rich spectra are obtained and the alpha- and beta-acids can be quantified relatively, 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 hops varieties by fingerprint matching or principle components analysis. Hop is a relatively new agricultural, but rapidly expanding, crop in Michigan, with around 150 acres currently under hop yards. This presentation also highlights some differences between Michigan and other U.S. hop-growing regions in the ratios of alpha- and beta-acids and other flavor compounds.

Andre Venter completed both his bachelor's and master's (cum laude) degrees at the University of Pretoria, South Africa. He received his Ph.D. degree in 2003 from the University of Pretoria, where he developed a comprehensive multidimensional supercritical fluid and gas chromatography (SFC×GC) method for petrochemical and natural product analyses. He worked with R. Graham Cooks as a post-doctoral researcher at Purdue University, West Lafayette, IN. There he investigated the fundamentals of desorption electrospray ionization mass spectrometry and other ionization methods. Since 2008 he has been an assistant professor in the Department of Chemistry at Western Michigan University, where his research in ambient desorption ionization continues. His research focuses on ambient surface analysis and ionization mechanisms involving the solvent-air interface and further development of ambient ionization and mass spectrometry technology. Applications and method development in agricultural product, food chemistry, industrial, environmental, and occupational health monitoring are being pursued.

Technical Session 3: Yeast I

8. Heterogeneous fermentation method in multi-filling cylindroconical vessels for high quality beer. Presenter: Yuichi Nakamura, Asahi Breweries, Ltd., Japan. Co-author(s): Hisao Koizumi, Asahi Breweries, Ltd., Japan.

According to the results of our previous report, the impact of the wort aeration period of multi-filling CCVs, presented at Brewing Summit 2010, we changed the ways to pitch yeast and aerate wort. In our breweries, four batches of wort are filled in a 5,000 hL cylindroconical vessel. In the process before the change, the same amount of yeast and air were pitched into the first to fourth batches of wort. As for the improved process, yeast and air were pitched into the first, second, and third worts, while neither yeast nor air was injected into the fourth wort, which was just transferred to the fermentation vessel. Though this improvement raised the sensory scores as expected, the specific gravity and the number of yeast cells of the fermenting beer were largely different from the data before improvement. Therefore, we developed new equipment capable of sampling the fermenting beer at four different heights in a fermentation tank and installed it on the 5,000 hL tank in our brewery. (Detailed specifications of the equipment are separately reported by our colleague Hisao Koizumi.) Under the previous conditions, the temperature, yeast cells, specific gravity, and amino nitrogen of the fermenting beer were the same at all four different heights. In the case of the improved method, the bottommost layer and the upper three layers of the fermenting beer were not blended, and fermentation proceeded as each layer remained independent. After 50–60 hr from initiation of fermentation, convection was generated, and the 5,000 hL of fermenting beer became homogeneous. At the time just before the fermenting beer was blended, the bottommost layer contained a rich amount of remaining amino acids and monosaccharides. Meanwhile, these amino acids and monosaccharides had already been consumed and depleted in the upper three layers. When the entire fermenting beer in the fermentation vessel was blended after 50–60 hr of fermentation, the nutrients remaining in the bottommost layer, such as amino acids and monosaccharides, were supplied to the upper layers. According to the flow cytometry results on yeast budding, the bottommost layer beer before blending contained a larger percentage of currently budding yeast cells. These results suggested that the bottommost layer beer contained many yeast cells under nutrient-rich conditions that were highly active and currently budding, and such highly active yeast cells were then diffused throughout the entire fermentation tank at approximately 50–60 hr. We therefore concluded that fermentation steadily proceeded to the end without reducing the rate, and thus the beer flavor and taste were improved and stabilized.

Yuichi Nakamura received an M.S. degree in agricultural chemistry from Tokyo University, Japan. He began employment with Asahi Breweries, Ltd. in April 1993. After working as a researcher in the laboratory, he was transferred to the brewing section of the Ibaraki brewery. He studied brewing technology at TU Muenchen-Weihenstephan in Germany for one year from 2001 through 2002 and returned to the Nagoya brewery. He has been working in the Production Technology Center, Asahi Breweries, Ltd. since 2005.

9. New insights into the mechanisms underpinning diacetyl formation and reduction in large-capacity cylindroconical fermentations. Presenter: Christopher Boulton, University of Nottingham, UK. Co-author(s): Joseph Sebastian, University of Nottingham, UK.

In previous studies we have demonstrated that when using large capacity cylindroconical vessels, where filling times may be prolonged and require several individual batches of wort, the timing of pitching and wort oxygenation can have a profound influence on subsequent fermentation performance and beer analysis. It is known that the appearance in fermenting wort of free diacetyl and its immediate precursor, alpha-acetolactate is related to the assimilation of amino nitrogen. In this regard, the extra- and intra-cellular concentrations of valine, a group B amino acid not assimilated until mid-fermentation, are significant. It would be predicted that the ordered sequence of amino acid assimilation might be perturbed where there is a long interval between pitching and the completion of wort addition. Here these possibilities are discussed, and the results of relevant trials are presented. The situation is made more complex since it has also been shown that for much, if not all, of primary and secondary fermentation, conditions within these large vessels are heterogeneous. In particular, even with relatively non-flocculent yeast strains, a large proportion of the yeast population begins to form a crop in the cone before primary fermentation has reached completion. It is accepted brewing wisdom that where it is practice to eliminate diacetyl via a warm rest period at the end of primary fermentation it is essential to ensure that sufficient suspended yeast cells are present to ensure efficient assimilation and reduction of free diacetyl to less flavor-active metabolites. Since a large proportion of the yeast has already formed a sediment in the cone during the warm diacetyl rest this brings into question how the whole of the population contributes to the removal of diacetyl in the later stages of fermentation. Here the results of trials are presented in which these aspects of fermentation performance are explored. These support the contention that the underlying mechanism that produces the visible changes in total VDK concentration throughout large-scale production fermentations is more complex than the literature would sometimes suggest. The ways in which these new insights can be applied to produce more consistent and predictable overall fermentation performance are discussed, and supporting evidence is provided.

Chris Boulton gained his first and doctorate degrees at the University of Hull. The latter for an elucidation of the biochemistry of lipid accumulation in oleaginous microorganisms. He joined the research Department of Bass Brewers in 1984, where he worked as a fermentation scientist. Over the next 25 years, working with the same company and later with Molson Coors in a number of roles, he has continued to carry out research into how the physiology of yeast is influenced by the conditions it encounters during production-scale brewing; in particular, the ways in which the genome responds to modern intensive fermentation practices, and how it can be manipulated to ameliorate the effects of applied stresses and provide consistency in performance and outcome. In 2007 he joined the Department of Brewing Science at the University of Nottingham as a teaching fellow and special professor, where he teaches and continues to pursue his interests in fermentation science.

10. "Static" Storage of a spiced beer—When is the beer mature? Presenter: Urs Wellhoener, Boston Beer Company, Boston, MA, USA. Co-author(s): Annette Fritsch, Boston Beer Company, Boston, MA, USA.

There are a variety of factors that indicate maturity based on the beer system, and the identification of a mature sample is based on the beer itself, particularly fermentation by-products, dry hopping, and spicing. Commonly, the main focus is from the analytical perspective. This includes parameters like diacetyl or acetaldehyde. However, what other factors designate the right maturation time, primarily after active fermentation is complete and the beer is in basically "static" storage? In this study, a spiced, lager beer was evaluated during storage using both chemical analysis for a wide range of fermentation by-products and sensory descriptive analysis. Similar to dry-hopping, the aroma and flavor impact of spices unfold depending on interactions with other compounds in the beer. The syn-

ergies and inhibitions among compounds change during maturation. Therefore, it was essential to evaluate how the impact of spices changes during storage. Analysis techniques including ANOVA and PCA were applied to both the chemical and sensory data to determine the optimal maturation time. In addition to identifying optimal maturation, the level of yeast carry over into static storage was explored. A moderate yeast carry-over is desired to help the beer to mature further (e.g., diacetyl) but should be kept minimal to minimize yeast autolysis, which can affect beer aroma/flavor and foam negatively. Since the beer in this study was "completely end-fermented," prior to hitting the storage tanks, the question of whether yeast should be removed completely via centrifugation at fassing was addressed as well. Through a combination of analytical techniques, we were able to identify both the impact of storage on a spiced lager beer and the effect of varying levels of yeast carry-over on the storage profile. A panel of sensory experts rated the maturity of the beer according to attributes like overall maturity, spice, diacetyl, acetaldehyde, etc.

Urs Wellhoener, the corporate manager for yeast and fermentation for the Boston Beer Company, joined the company in October 2007. His focuses are yeast management and microbiology. He is a technical graduate as a brewer and maltster (1991–1993) and received a Dipl.-Eng. degree from the Faculty of Brewing and Food Technology of the Technische Universität München-Weihenstephan (TUM) in 1999. After graduation in 1999 he was a project manager on a yeast project at Veltins Brewery, Meschede-Grevenstein (1999–2000). Between 2000 and 2007 Urs was a scientific assistant and doctorate at the Chair of Brewing Technology II at the Weihenstephan Center of Food and Life Sciences, Technische Universität München-Weihenstephan (TUM). He received his Ph.D. degree for his studies on yeast physiology during fermentation and propagation. During this time he also worked for Muellerbraeu, Pfaffenhofen/GER, as QC manager.

Technical Session 4: Hops II

11. Increasing the hop alpha-acids utilization by hop pre-isomerization and the evaluation of the bitter quality of beer. Presenter: Seiichi Takishita, Asahi Breweries, Ltd., Japan. Co-author(s): Hisato Imashuku, Asahi Breweries, Ltd., Japan; Martin Krottenthaler, Hochschule Weihenstephan-Triesdorf, Germany; Thomas Becker, Technische Universität München, Germany.

Our colleague, Hisato Imashuku, presented PIE (pre-isomerizer and evaporator) at WBC 2008. This is a system in which hops are boiled with hot water separately from the wort. By using this system, we can save energy, improve the hop alpha-acids utilization, and so on. In this study, further improvement of the alpha-acids utilization using PIE was investigated, and the bitter quality of beer was evaluated. First, optimization of hop pellet PIE treatment conditions at the laboratory scale was conducted. In consequence, boiling 60 min under pH 8.0 using KOH (at the onset of boiling) was the best condition. Secondly, some brewing trails with 70% malts and 30% syrup adapting the abovementioned PIE condition were done at a 60-L scale pilot plant. The influence of the time when PIE-boiled hops are dosed to wort/beer, the influence of aged hops, and the influence of different hop varieties were the focus of the study. Concerning the time of dosing PIE-boiled hops, there was no significant difference in alpha-acids utilization with different dosing time, during boiling, at the beginning of fermentation and at the beginning of maturation. Maximum alpha-acids utilization was approx. 67%, which was only approx. 2 and 10% lower than using isomerized kettle extract and isomerized extract, respectively, and >1.5 times higher than conventional hop dosing. There was no significant difference in the bitter quality of beer, in spite of the different dosing times for PIE-boiled hops. Concerning the influence of aged hops and different hop varieties, there was no significant difference in the rating of bitter quality between using PIE and the conventional method. Nevertheless the character of bitterness changed slightly, and we presume that it is related to the amount of non-isohumulone bittering compounds in the beer. As a result, this could make it possible to control the character of bitterness in beer.

Seiichi Takishita graduated in 1999 with a master's degree in agricultural and life sciences from the University of Tokyo, Japan. He began employment with Asahi Breweries, Ltd. in 1999 as a technical staff member in the brewing section. After he had worked at several of the breweries and Development Laboratories for alcoholic beverages, he worked as a visiting researcher at the Technology University of Munich from 2010 to 2012.

12. Hop oil analysis—The power of stable isotope dilution assays for quantification at trace levels. Presenter: Leif Garbe, TU Berlin/VLB Berlin, Germany. Co-author(s): Nils Rettberg, TU Berlin/VLB Berlin, Germany.

Hop essential oils and their oxidation products are of special interest for brewing science and quality control as well as for practical brewers. They are volatile, chemical reactive, and usually are present in very low concentrations in the final product. In hops, they are embedded in complex matrices that hinder rapid analysis and make trace analysis a challenging discipline. Various sample cleanup and target isolation strategies have been established. The more intense the sample cleanup procedures are, the more sources of analyte losses and errors have to be considered in quantification and calibration. In trace analysis, external calibration is not reasonable, and the quality of internal standard assays strongly depends on the availability and properties of the internal standard. Stable isotope dilution assay (SIDA) is a special type of an internal standard assay. In SIDA standard and analyte are isotopologues, thus they are as nearly identical as possible. Their chemical and physical properties match, and their chromatographic and mass spectrometric characteristics are very alike. This paper highlights the advantages and challenges of SIDA in trace analysis on hop oils. The important hop oil terpene hydrocarbons and terpene alcohols myrcene, linalool, terpineol, nerol, geraniol, and farnesol, as well as caryophyllene and humulene, are not commercially available as stable isotope labeled standards. Therefore, their chemical synthesis as isotopologues carrying Deuterium (hydrogen-2), oxygen-18, or carbon-13 is of crucial necessity for SIDA. We have performed lab synthesis of these terpene compounds. Using SIDA, precise and valid quantification even at low concentrations from the complex beer matrix, raw hops, and any intermediate is strongly simplified. In the presented paper, a short introduction to SIDA and SIM-MS methods are given. The major focus of the paper deals with data from hop oil analysis via conventional and SIDA methods, respectively. One disadvantage of SIDA is the necessary instrumentation—chromatography coupled to mass spectrometry. However, SIDA is also proposed as a reference method in evaluation of routine assays preformed on cost-effective non-MS equipment like GC-FID analysis.

Leif-Alexander Garbe is professor for biochemical and technical analysis at the Berlin Institute of Technology (TUB). Additionally, he chairs the Department for Special Analyses at the Research and Teaching Institute for Brewing in Berlin (VLB). Leif graduated in 1996 from TUB with a diploma in chemistry. Then he worked as a researcher and teacher at VLB and TUB. He supervised biotechnology and brewing students and performed several research projects in

brewing and life sciences. He finished his Ph.D. thesis in April 2002 on the "Metabolism of Hydroxy-Fatty Acids in Yeasts," and his habilitation thesis in 2009 on "The Biochemistry of Oxidized Lipids: Analytical Characterization of Bioactive Metabolites" at TUB. Today Leif's research interests focus on mass spectrometry, NMR, trace analysis, biotransformation, isotope dilution technique, and Maillard reaction of peptides/proteins.

13. The role of "unknown" hop proteins. Presenter: Martina Gastl, Lehrstuhl für Brau- und Getränketechnologie, Freising, Germany. Co-author(s): Christoph Neugrodda and Thomas Becker, Lehrstuhl für Brau- und Getränketechnologie, Freising, Germany.

Compared to the large quantities of malt required in beer production, the amount of hops (*Humulus lupulus*) needed is significantly smaller. This minor ingredient has a crucial impact on beer flavor and physical properties (i.e., foam stability, turbidity). In hop research, much attention has been given to the major components: hop resins (10–30%), hop oils (0.4–2%), and hop polyphenols (4–14%). Despite the recent "boom" in hop research, hitherto, the role of hop proteins remains unknown. Besides the major components present in the dry substance of *Humulus lupulus*, other valuable substances are found in hops. Depending of the variety, hop proteins constitute up to 15% (w/w) of the dry matter. Although, the influence of proteins (from barley/barley malt) on beer turbidity is indisputable, to date there is no research on the characterization of hop proteins and their impact on beer turbidity and flavor. In this research, modern analytical methods available for protein research (i.e., bioanalyzer, 2D-PAGE, off-gel-fractionation) were used to characterize hop proteins based on their molecular weight and isoelectric point (pI). Further, the hop protein compositions were monitored throughout the brewing process, that is from the raw material to the finished beer. The results show significant differences in the protein composition of different hop varieties. Furthermore, these results make it possible to estimate the impact of hop proteins relative to malt proteins on beer properties.

Martina Gastl apprenticed as a brewer and maltster from 1994 to 1996 in Klosterbrauerei Andechs, Germany. She studied brewing and beverage technology at the Technische Universität München-Weihenstephan, Germany. She graduated as an engineer in 2002. From 2002 until 2006 she completed her Ph.D. concerning the "Technological Influence on Lipid Degradation in Terms of Improvement of Beer Flavor Stability." She is currently assistant professor and head of the laboratory, as well as the raw material and beverage design research group, at the Lehrstuhl für Brau- und Getränketechnologie in Weihenstephan. Since 2008 she has been working on her post-doctoral lecture qualification. Her research interests involve characterization and interaction of flavor active taste and aroma compounds in cereal-based beverages influencing beverage harmony.

14. A study of the functionality of hop epsilon-resins as a novel brewing product. Presenter: Cynthia Almaguer, TU-München, Germany. Co-author(s): Martina Gastl, Michael Dresel, Thomas Hofmann, and Thomas Becker, TU-München, Germany.

The brewing value of hops (*Humulus lupulus*) is primarily attributed to the flavor- and bitter-active compounds found in the resins. These resins are synthesized and accumulated in the lupulin glands of female hop cones. Early work on the fractionation of hop resins, based on the solubility of resins in various organic solvents, classified them into soft resins and hard resins. Hitherto, research has primarily focused on studying the impact on beer properties of the major hop bitter acids (alpha- and beta-acids) extracted from the soft resin. Therefore, little information is available on the functionality of the hard resin and for years it has been considered of no brewing value. It has been established that the hard resin is mainly composed by oxidation products insoluble in hexane. However, to date, the brewing value of these products and their contribution to beer quality has not been determined. In this study, through the development of novel fractionation techniques, it was possible to further purify the hard resin extract. From this purification process the delta-resin and epsilon-resin were obtained, and from each resin, it was possible to further extract it to retrieve 11 fractions. It is the purpose of this work to determine which of and to what extent the fractions found in the hard resins contribute to beer quality. It is known that certain hop compounds possess antimicrobial activity. To the brewer, this is of great value since by addition of selected hop compounds, these antimicrobial properties can be exploited to enhance the microbiological stability of beer. Therefore, the minimum inhibitory concentration as well as the bitter intensity of the 11 fractions were independently determined and correlated. The fractions that proved to be active were further purified, and the obtained pure compounds or subfractions were tested for activity. As a result, inhibitory and taste active hop compounds or subfractions present in the hard resin could be identified. It was seen that the epsilon-resin was more active than the delta-resin. For this reason, the functionality of the total epsilon-resin as a brewing product was examined, and finally, the epsilon-resin contribution to the microbiological stability of beer was assessed. To achieve all this, brewing trials were conducted in which hop pellets were replaced with an epsilon-resin rich extract. In these laboratory scale experiments, it was possible to determine that independent of the addition point, the epsilon-resin contributes to the microbiological stability of beer. In the different sensory evaluations of the fresh beers, it was shown that addition of this resin had a positive impact on all taste relevant attributes. Although in terms of microbial stability the addition point had no influence, from the sensory point of view, the beer in which the resin was added upon boiling was generally preferred. As a result of this study, novel hop products that positively contribute to beer taste and stability were proposed.

In 2008, Cynthia Almaguer completed her B.S. degree in biochemical engineering at Jacobs University Bremen. She then started her graduate studies in a collaborative project between the Institute of Brewing and Beverage Technology (Thomas Becker), TUM-Weihenstephan, and the Department of Food and Nutritional Sciences (Elke Arendt), University College Cork. Her research project aims to understand and reveal the contributions of hop hard resins in beer. A significant portion of her research activities are directed toward the investigation of the taste as well as the antimicrobial properties of hops.

Technical Session 5: Malts and Grain

15. Performance of LOX-1-less malting barley—Sapporo's worldwide strategy for development of high quality malting barley varieties. Presenter: Wataru Saito, Sapporo Breweries Ltd., Japan. Co-author(s): Takehiro Hoki, Tetsuya Saito, Tomokazu Takaoka, Shinichiro Yoshida, Masayuki Shimase, Kiyoshi Takoi, Naohiko Hirota, and Makoto Kihara, Sapporo Breweries Ltd., Japan; Brian Rossnagel, Crop Development Centre, Department of Plant Sciences, University of Saskatchewan, Canada; Jason Eglinton School of Agriculture, Food and Wine, Waite Campus, University of Adelaide, Australia; Shinji Yamada, Sapporo Breweries Ltd., Japan.

Lipoxygenase (LOX) in malt is involved in the formation of *trans*-2-nonenal (T2N) which causes cardboard off-flavor in beer. There are two LOX isozymes (LOX-1 and LOX-2), with the formation of 9-hydroperoxide, a precursor to T2N, primarily catalyzed by LOX-1. The formation of trihydroxyoctadecenoic acid (THOD), which has negative effect on beer foam retention, is also catalyzed by LOX-1. Therefore LOX-1 is an enzyme affecting beer quality. Sapporo has been developing high quality malting barley varieties in Japan and with its partners in other countries. The LOX-1-less malting barley variety CDC PolarStar was developed by molecular marker assisted backcross breeding using a landrace from India with no seed LOX-1 activity as the donor parent and high malting quality Canadian malting barley variety CDC Kendall developed by the University of Saskatchewan as the recurrent parent. Applying a similar breeding strategy in Australia, a LOX-1-less variety has been developed from the joint breeding program with the University of Adelaide using the high quality Australian malting barley variety Flagship. Sapporo has conducted brewing trials comparing LOX-1-less varieties with the parents and a commercial variety to investigate the effect of the LOX-1 trait on beer quality. The results demonstrate expected positive effects on beer quality.

Wataru Saito received a master's of agriculture degree in plant breeding from Okayama University in Japan. He began working for Sapporo Breweries Ltd. in April 1985 as a barley breeder in the laboratory on the raw material. Since April 1987, he has functioned as a malting barley breeder in the Hongri Seeds Co., Ltd., Hongxinglong, Heilongjiang, China. He joined the joint breeding project on malting barley with teh University of Saskatchewan in Canada from 1997 to 2007 and since has shifted to the breeding program with the University of Adelaide in Australia.

16. Trends in the incidence of *Fusarium* and *Microdochium* species in UK malting barley: Impacts for malting and brewing quality. Presenter: David Cook, University of Nottingham, UK. Co-author(s): Linda Nielsen, University of Nottingham, UK; Simon Edwards, Harper Adams University College, UK; Rumiana Ray, University of Nottingham, UK.

In 2008, 74% of tested U.K. malting barley samples were infected with *Fusarium* spp. Reported statistics have shown that the species implicated are diversifying. While due diligence monitoring indicates that mycotoxin levels in U.K. malting barley are below the specified safety limits, the impacts of sub-acute *Fusarium* infection on the malting and brewing quality of barley are relatively unclear but have been linked variously to a number of key quality parameters such as germinative energy and capacity of the barley crop, brewing malt specifications (e.g., soluble nitrogen, wort FAN, color, and beta-glucan levels), gushing, PYF, etc. The SAFEMalt project (Strategies Against *Fusarium* Effective in MALTing Barley) is a 3-year multi-partner research initiative spanning the malting barley supply chain from barley breeder through barley grower and merchant to brewer. The project incorporates two annual surveys of U.K. spring malting barleys (2010 and 2011 harvests) and also has retrospective access to U.K. spring barley samples collected for mycotoxin screening between 2007 and 2009. In 2010, species-specific real time PCR analyses identified that the main species present across 88 samples of U.K. malting barley were *F. avenaceum*, *F. langsethiae*, *F. poae*, and *F. tricinctum*, with each species detected in 80–90% of all samples tested. Retrospective analysis of *Fusarium* species present in 2007–2009 indicated that the prevalent *Fusarium* species on U.K. spring barleys differed hugely with harvest year. For example, *F. graminearum* was identified in all samples analyzed in 2007 and 2008, but in just 9% of samples from 2010. When 2010 samples were germinated (GE 4 mL and GE 8 mL counts) there was a positive correlation between the degree of water sensitivity and the quantity of DNA of *Fusarium* and *Microdochium* spp. in each sample ($R^2 = 0.51$; $n = 24$). This correlation was more pronounced in data relating to a single barley variety ($R^2 = 0.65$; $n = 12$). A micromalting procedure was developed using 350 g batches of barley in individual steep compartments in a Micromaltings K Steep-Germinator (Custom Lab), this being necessary to investigate the impacts of the *Fusarium* and *Microdochium* spp. present without mixing them while steeping multiple samples. The effects of the presence of *Fusarium* and *Microdochium* spp. and significant malt and wort quality parameters (friability, alpha-amylase, beta-amylase, wort extract, FAN, beta-glucan, and viscosity) will be presented.

David Cook is a lecturer in brewing science at the University of Nottingham, U.K., and is course director for its innovative e-learning–based courses for brewers. He is engaged in research across the malting and brewing fields, specializing in malting science, flavor formation, stability, and perception. Other current projects focus on biorefining and the use of lignocellulosic waste for bioethanol fermentations and links between crop husbandry, barley microbiology, and the functionality of malts.

17. Studies on the kilning conditions of teff (*Eragrostis tef*) malt as alternative raw material for gluten free foods and beverages. Presenter: Mekonnen Gebremariam, Institute for Brewing and Beverage Technology, Technische Universität München, Freising, Germany. Co-author(s): Martin Zarnkow and Thomas Becker, Institute for Brewing and Beverage Technology, Technische Universität München, Freising, Germany.

Teff is a gluten free cereal with an attractive nutritional profile, making it a suitable substitute for barley, wheat, and other cereals in their food applications and foods for people with celiac disease. The demand for gluten free foods is certainly increasing. The aim of this research was to study the influence of kilning on enzyme activities and DMS level of teff variety DZ-Cr-387 and suggest a kilning condition that yields teff malt with low level of DMS with no or little damage on its enzyme activities. The teff samples were steeped for 5 hr on the first day, and 4 hr on the second day at 24°C and germinated for 4 days at 24°C in a temperature controlled chamber with 95% relative humidity. The green malts were dried using isothermal conditions at 30, 40, 50, 60, and 70°C for 40 hr with sampling at certain time intervals. To set up optimum kilning conditions, two temperature regimens were selected based on the results of the isothermal kilning and some other trial experiments: 18 hr at 30°C + 1 hr at 60°C + (3 and 5) hr at 65°C (R1), and 18 hr at 30°C + 1 hr at 60°C + (3 and 5) hr at 80°C (R2). The results from the isothermal kilning indicate that enzyme activities of teff malt decreased as the kilning time and temperature increased. At lower temperatures, there was an increase in the enzyme activities as the kilning time increased. The DMS contents decreased with an increase in temperature and time. The first kilning regimen (R1) with 3 hr curing at 65°C resulted in teff malt with alpha-amylase, beta-amylase, and limit dextrinase activities and DMS content of 68 U/g, 440 U/g, 1,072 U/kg, and 3.3 mg/kg, respectively. The same kilning regimen (R1) with 5 hr curing at 65°C yields teff malt with alpha-amylase, beta-amylase, and limit dextrinase activities and DMS content of 60 U/g, 421 U/g, 780 U/kg, and 2.5 mg/kg, respectively. Whereas the second kilning regimen (R2) with 3 hr curing at 80°C resulted in teff malt with alpha-amylase, beta-amylase, and limit dextrinase activities of 42 U/g, 406 U/g, and 736 U/kg, respectively, and DMS content of 2.15

mg/kg. The same kilning regimen (R2) with 5 hr curing at 80°C yields teff malt with alpha-amylase, beta-amylase, and limit dextrinase activities of 37 U/g, 395 U/g, and 594 U/kg, respectively, and DMS level of 1.7 mg/kg. The results in general show that the teff malt, which was kilned using the first kilning regimen with shorter curing time at 65°C, contained the highest level of amylolytic enzymes. The DMS values in all teff malts were below the threshold level (5 mg/kg) that good quality malt should contain. It can be concluded that the first kilning regimen (R1) with shorter curing time is the best kilning condition that yields a good quality teff malt. The levels of enzyme activities and DMS show that teff can be a suitable alternative raw material for production of good quality gluten free beer.

Mekonnen Melaku Gebremariam received his B.S. degree in chemistry from Debub University, Ethiopia. He began employment with the Ethiopian Ministry of Education in July 2000 as a chemistry teacher in the South Nations and Nationality People Region. He terminated his contract agreement with the Ministry of Education after four years. He next was employed as a chemist in the Federal Micro and Small Enterprises Development Authority. After 18 months with this company, he terminated the contract agreement and joined Addis Ababa University for further studies. He graduated from Addis Ababa University, Ethiopia, in 2007 with an M.S. degree (with great distinction) in food engineering. Immediately after graduation he was employed as a lecturer and researcher by Hawassa University, Ethiopia. After about two-and-a-half years of work at Hawassa University, he went to Germany for his Ph.D. studies with the support of his employer, Hawassa University. Currently he is pursuing his doctoral studies at the Technical University of Munich, Germany.

18. A comparative study of oat (*Avena sativa* L.) cultivars as brewing adjuncts. Presenter: Birgit Schnitzenbaumer, School of Food and Nutritional Sciences, National University of Ireland, University College Cork, Cork, Ireland. Co-author(s): Jean Titze and Elke Arendt, School of Food and Nutritional Sciences, National University of Ireland, University College Cork, Cork, Ireland.

The use of oats (*Avena sativa* L.) as an adjunct in brewing has not only the potential to reduce the costs of raw materials, but also to contribute to a unique beer flavor and aroma. However, the replacement of malted barley with unmalted oats can also have a negative impact on the quality and processability of mashes, worts, and beers. The aim of this study was to evaluate the mashing performance of unmalted oat cultivars used as adjuncts in brewing. For this purpose, seven husked oat cultivars (Buggy, Curly, Galaxy, Ivory, Lutz, Scorpion, Typhon) and one naked oat cultivar (NORD 07/711) were fully characterized using confocal laser scanning microscopy, lab-on-a-chip capillary electrophoresis, and standard methods specified by the Mitteleuropäische Brautechnische Analysenkommission, European Brewery Convention, or American Society of Brewing Chemists. Furthermore, the rheological behavior of mashes containing 0, 20, and 40% oats of each cultivar was monitored during mashing by applying a Physica MCR rheometer. The quality of worts obtained from laboratory-scale mashing trials has been determined, particularly with regard to their cytolytic, proteolytic, and amylolytic parameters. All analyses were carried out in triplicate. Significant differences between the studied oat cultivars with regard to their use as brewing adjuncts were revealed. It has been found that naked oats are characterized by a significant lower beta-glucan content and a higher starch content in comparison to husked oat cultivars. In addition, the replacement of 20 or 40% malted barley with naked oats resulted in a constant extract yield, whereas the use of up to 40% husked oats led to significant extract losses.

*Birgit Schnitzenbaumer successfully completed an apprenticeship as assistant tax consultant and worked in this job full-time before she studied brewing and beverage technology at the Technical University of Munich in Weihenstephan, Germany. During her studies, she completed several internships in breweries and did her master's thesis on the effect of malting on the protein profile of proso millet (*Panicum miliaceum *L.) at the School of Food and Nutritional Sciences of the University College Cork, Ireland. Birgit graduated with a Dipl.-Ing. (M.S.) in brewing and beverage technology in 2009 and started her Ph.D. project on the application of novel and industrial enzymes when brewing with unmalted cereals at the University College Cork in November 2009.*

19. Toward a DNA fingerprint to identify barley cultivars that fit specific brewers' needs. Presenter: Richard Horsley, North Dakota State University, Department of Plant Sciences, Fargo, ND, USA. Co-author(s): Magan Lewis, North Dakota State University, Department of Plant Sciences, Fargo, ND, USA; Fabio Pedraza-Garcia, Seeds 2000, Breckenridge, CO, USA; Ana Correa-Morales, North Dakota State University, Department of Plant Sciences, Fargo, ND, USA; Shiaoman Chao, USDA-ARS, Cereal Crops Research Unit, Fargo, ND, USA; Ronshuang Lin, University of Maryland, College Park, MD, USA; Paul Schwarz, North Dakota State University, Department of Plant Sciences, Fargo, ND, USA.

Brewers in the United States who use six-rowed barley (*Hordeum vulgare* L.) have historically used cultivars with similar malt quality profiles. Around the year 2000 this changed, with some brewers preferring cultivars that produce higher levels of alpha-amylase and have increased protein modification during malting, while other brewers prefer cultivars that have moderate levels of these two characters. Two cultivars that meet these differing criteria are Stander, which produces increased levels of alpha-amylase, soluble protein, and free amino nitrogen (FAN); and Robust, which produces moderate levels of these malt quality parameters. In addition, Robust and Stander differ in their resistance to preharvest sprouting (PHS), with Stander being very susceptible and Robust being moderately resistant to PHS. An interesting characteristic of Stander and Robust is that they are very closely related. This feature should make it possible for us to determine the genetic basis for the dissimilarities in the two cultivars and to use this information to design a marker assisted breeding strategy for developing cultivars that meet specific brewers' needs. The markers associated with specific quality parameters in Robust or Stander can be thought of as their "fingerprint." Geneticists call this fingerprint a haplotype. A doubled-haploid mapping population from the cross Robust × Stander was developed. A genetic map for this cross comprised of single nucleotide polymorphism (SNP), simple sequence repeat (SSR), and diversity array technology (DArT) markers was constructed. The polymorphic markers were grouped into 19 linkage groups, which were associated with six of the seven barley chromosomes. Chromosomes 2H, 4H, and 6H had relatively large portions of the chromosomes mapped, while chromosomes 1H, 3H, and 5H had many small segments mapped. Because of the specific quality parameters required for malting barley, it is not surprising that only portions of the chromosomes were mapped. Many of the segments not mapped would be regions where genes controlling malt quality are fixed in a favorable state. Additionally, the regions where a map was constructed are likely to include the specific genes that determine the quality differences observed in Robust and Stander. The map constructed was used to identify quantitative trait loci

(QTL) controlling seedling dormancy, alpha-amylase activity, soluble protein concentration, Kolbach index, FAN, wort beta-glucan, and concentrations of wort carbohydrates. QTL controlling correlated traits often mapped to similar sites. For example, QTL controlling alpha-amylase, Kolbach index, FAN, and wort color mapped to a similar region in chromosome 6H. A preliminary "fingerprint" or haplotype of markers that differentiate Robust-type from Stander-type barley cultivars will be discussed.

Richard Horsley is the barley breeder at North Dakota State University and head of the Department of Plant Sciences. Richard earned his Ph.D. and M.S. degrees in agronomy from North Dakota State University and his B.S. degree in agronomy from the University of Minnesota. The primary goal of his breeding project is to release and develop six-rowed and two-rowed malting barley varieties acceptable to barley producers in North Dakota, adjacent states, and the malting and brewing industry. Current research efforts include the determination of DNA "fingerprints" that differentiate varieties for specific brewer's needs and identification of genes for resistance to preharvest sprouting.

Technical Session 6: Quality Considerations

20. The equipment to sample the fermenting beer from four positions in the cylindroconical vessel and its practical application to flavor improvement in the brewery. Presenter: Hisao Koizumi, Asahi Breweries, Ltd., Suita Brewery, Japan. Co-author(s): Yuichi Nakamura, Asahi Breweries, Ltd., Suita Brewery, Japan.

In our breweries, large vessels with a capacity of several thousand hectoliters are used to brew beer. For reasons of cost-effectiveness, sanitary conditions, and appropriate cleaning, only one sampling device is usually installed on the large vessels, and no assurance is given that the sampled liquid taken from the device represents the whole beer in the large vessel. Therefore, we developed equipment to directly sample the beer in the large vessel from several positions at different heights. This paper reports on the application of the equipment to the 5,000 hL fermentation vessel, which is filled with four batches of wort prepared as 1,250 hL each for fermentation. Four perfluoro alkoxyl alkane resin tubes were extended from the vessel bottom to the intermediate heights between the liquid levels, where four batches of 1,250 hL wort reached, respectively, and the fermenting beer was sampled from each layer. The bottommost layer (3.8 m above the vessel bottom) was designated sample #1, the 9.2 m high position was sample #2, the 12.5 m was sample #3, and the topmost layer (15.8 m high) was sample #4, and the fermenting beer and yeast were analyzed. We used the equipment to compare the uniform pitching method, where the same amount of yeast and air were pitched into the first to fourth batches of wort, and the early pitching method, where the same amount of yeast and air were pitched into the first to third batches of wort, while neither yeast nor air was injected into the fourth batch of wort. Under the uniform pitching condition, the temperature, number of yeast cells, specific gravity, and amino nitrogen level during fermentation were almost the same at all of the four positions in the fermentation vessel. However, in the case of the early pitching method, the bottommost layer and the upper three layers were not blended, and fermentation started as each layer was kept independent. After around 50–60 hr from initiation of fermentation, convection was generated, and the 5,000 hL of fermenting beer became uniform. According to the sensory tests, the beer brewed by the early pitching method had a higher rating with smaller variations. The sampling equipment we developed for this enabled us to sample fermenting beer and yeast from an arbitrary height at any time and evaluate the distribution and uniformity of the fermenting beer components, temperature, and yeast cells, which was applicable to improvement of beer flavor in practical brewing processes.

Hisao Koizumi received an M.S. degree in biotechnology from Hiroshima University, Japan. He began employment with Asahi Breweries, Ltd., in April 2001. After working as a researcher in the laboratory, he was transferred to the brewing section of the Suita brewery.

21. Primary gushing: The explosive love story between CO_2 and hydrophobin. Presenter: Christina Schönberger, Barth-Haas Group, Barth Innovations, Joh. Barth und Sohn, Germany. Co-author(s): Sylvie Deckers, KU Leuven, Department M^2S, Malt & Beer Sciences and LFoRCe, Belgium; Jean Titze, National University of Ireland, University College Cork, School of Food and Nutritional Sciences, Ireland; Vladimir Ilberg, Hochschule Weihenstephan-Triesdorf, Fakultät Gartenbau und Lebensmitteltechnologie, Germany; Guy Derdelinckx, KU Leuven, Department M^2S, Malt & Beer Sciences and LFoRCe, Belgium.

During the last decade a lot of research was performed on an undesirable and unexplainable phenomenon called gushing, which is characterized by a spontaneous and wild liquid expulsion of carbonated beverages that occurs immediately after opening the bottle without any shaking. Gushing is a tremendous problem for breweries as it is unpredictable and can cause severe delivery and image problems. This work reviews all relevant findings in brewing science throughout the last 20 years and explains the gushing phenomenon based on the facts that are known today. While secondary gushing is due to technical and technological problems, primary gushing is related to the use of raw materials contaminated by a filamentous fungi, a producer of a human safe and amphiphilic protein called hydrophobin. By forming a solid condensed pellicle (i.e., crystalline layer) around gaseous CO_2 Class II hydrophobins are responsible for the presence of gaseous nanobubbles insulated from the liquid and pressurized at 4 bars in a bottled beverage. These nanobubbles explode upon opening due to the drop in pressure. This explosion provides the energy required for nucleation, which results in the fast escape of dissolved CO_2 and gushing. Knowing the interaction of hydrophobins and CO_2, possible solutions for gushing have to be looked for in any material that may interfere with the formation of stabilized nanobubbles. In this regard various hop components seem to be of interest. As gushing is a worldwide problem, it seems important to make a review of this phenomenon and which possible hop characteristics, as a typical ingredient in beer, could offer a solution.

Christina Schönberger studied brewing and beverage technology at the Technische Universität München-Weihenstephan, Germany (1995–1999), graduating as an engineer in 1999. After working as a brewing intern in 2000 at Suntory, Japan, she pursued doctoral thesis work at the Chair of Brewing Technology I on "Sensory and Analytical Characterisation of Non-volatile Taste Compounds in Bottom Fermented Beers," with which she graduated summa cum laude in December 2003. For her doctoral thesis she received the Dr. Nienaber Award in 2005. After working for the German Brewers Association for one year as a consultant for technical and governmental issues, she joined the Barth Haas Group in 2005 as manager of technical sales. Within this role she is also responsible for the guidance of research projects and authors hop related professional articles. Christina currently holds the role of International Director on the ASBC Board of Directors.

22. Mid-infrared sensors: Testing in-progress product quality at critical process control points (CPCP) in the brewing and packaging processes. Presenter: Robert O'Leary, VitalSensors Technologies LLC, USA.

Many in-line or on-line instruments use physical properties of fluids to determine concentrations of desired ingredients. These traditional measurements include temperature, pressure, sound velocity, and density. Mid-infrared is a technique that can directly measure product properties, including sugar, ethanol, and CO_2, by looking at molecular absorption. Mid-infrared technology is currently being used at critical process control points (CPCP) in brewing and packaging processes. Current installations include "direct" measurement of sugar in wort, ethanol in low alcohol beer, ethanol in high gravity dilutions, ethanol and CO_2 in flavored alcoholic beverages (alcopops), phase transition, and beer in the finishing and release to packaging lines. Mid-infrared's principal advantages are temperature immunity, process line hydraulic immunity, and no requirement for product flow at the point of measurement. Mid-IR is an instantaneous in-process measurement as opposed to slipstream or membrane based (inferred) measurement. Mid-IR directly measures fermentation parameters, including fermentable sugars, ethanol, and CO_2; Mid-IR does not need to convert from physical properties (including density) to determine the concentration of desired measured analytes. This eliminates the need for reoccurring "product dial-in" that other measurement techniques require. The miniaturization of the VS-3000 beer monitor allows for one analyzer to measure product ethanol, CO_2, and sugar using one compensation contained within the analyzer. The sensor process interface is sapphire, 316L stainless steel, and virgin PEEK; the sensor is mounted directly in the CIP stream for maximum sterility and sanitization. The solid-state construction and 100,000 hr mean time to failure of the VS-3000 mid-infrared beer monitor decreases maintenance and is the most cost-effective measurement technique.

Robert O'Leary is currently the chief technology officer and a founder of VitalSensors Technologies LLC. He is the inventor of the Mid-Infrared ATR beer monitor. Bob's background includes 20 years at PerkinElmer, where he designed custom sensors and optical benches for spectroscopy, thermal imaging, and medical devices. He was president and CEO of Optical Coating Corporation, where he developed custom infrared optical filters for nondispersive mid-infrared instruments. Bob lives in Newton, MA, with his wife and three sons.

23. 35 years of malting and brewing—Experience with improvements in quality characteristics of raw materials and changes in technologies in maltery and brewhouse. Presenter: Udo Kattein, Technische Universität München (retired), Germany. Co-author(s): Sebastian Kappler, Technische Universität München, Germany.

In the last few decades a profound change in processes both in malteries and brewhouses could be observed. Striking success in breeding has provided new barley varieties that allow shorter germination times with lower moisture contents. Along with easier handling in malteries the quality characteristics of the finished malts could be improved. This exerted immense influence on the following treatments in the brewhouse. The processing steps of milling, mashing, lautering, and even wort boiling were affected. With regard to hops a lot of new varieties were launched by breeders. Higher contents of alpha-acids were achieved, a widespread diversity of hop products could be established in the market, and new possibilities for storage and especially exact dosage of alpha-acids opened up. Along with the improvements regarding the quality of raw materials an amazing change in the construction details of brewhouses took place. This was induced by the energy crisis and the demand for shorter production times. The most significant changes could be observed in the lautering and wort boiling equipment. Along with remarkable progress in monitoring devices and automation engineering, the output of brewhouses could be raised from 6 up to 12 or even 14 brews a day with the use of lauter tuns. This paper is an experience report about 35 years of leadership in the research and educational brewery of Technische Universität München. Udo Kattein was charged with the commercial production of malt and beer starting in 1975 and was able to observe the evolution described, along with revising a lot of new installations both in the maltery and brewhouse.

Udo Kattein received a diploma engineer degree from the Technische Universität München-Weihenstephan in 1972; afterward he performed an economic study at the University of Munich, finishing a diploma merchandiser degree in 1976. At this time he started work on his doctoral thesis and employment at TU München. He was in charge of the technical leadership of the research and educational brewery at Weihenstephan. He served as head brewer and was responsible for production of commercially sold malts and top-fermented beers. In addition to these tasks he was involved in the development of new beer types and training students. In 1984 he received a Ph.D. degree in engineering sciences, with a thesis on investigations of sulfur compounds in malt, wort, and beer. Since 2002 he has been responsible for the construction of the new malting and brewing facilities of the research brewery, which began in 2005. In autumn 2010 he retired and occasionally acts as a consultant.

Technical Session 7: Sustainability

24. High rate anaerobic digester systems for brewery wastewater treatment and electricity generation: Engineering design factors and cost benefit analysis. Presenter: Manaf Farhan, EMG International, Inc., Media, PA, USA. Co-author(s): James Kuhr, The F.X. Matt Brewing Company, Utica, NY, USA; Yassar Farhan, EMG International, Inc., Media, PA, USA.

A brewery can incur significant costs associated with wastewater treatment and disposal. Increasing financial and regulatory pressures can drive brewery management to consider investment in a wastewater treatment system that will reduce annual operating costs. Anaerobic fluidized bed digester (AFBD) technology with electricity generation and waste heat recovery offers breweries an innovative high rate wastewater treatment system that can provide cost and operations and maintenance (O&M) savings. However, onsite wastewater treatment systems require a significant upfront capital investment and proper long-term operation and maintenance. In today's volatile and highly competitive business environment, brewery management cannot afford to commit to large capital expenditures for such systems without a detailed technical and economic feasibility evaluation. This paper discusses technical and economic evaluation criteria required for successful implementation of a high-rate anaerobic digester system for breweries. Technical evaluation criteria discussed in this paper include selection of digester technology; evaluation of regulatory and compliance requirements; analysis and determination of brewery wastewater generation rates (average and design flow rate measurement and calculation, hourly, daily, and seasonal variability, and growth projections); required wastewater analyses (chemical oxygen demand, 5-day biochemical oxy-

gen demand, total and volatile solids, nitrogen and phosphorous levels, and pH and temperature trends and variability); high-rate digester system layout and space requirements; ability to handle overloading and shock loading conditions; recovery from operational upsets; and system automation and reliability. Cost benefit analysis elements discussed include evaluation of wastewater disposal costs; brewery energy usage; digester system capital costs; long-term operation and maintenance costs (labor, materials, and consumables); expected sewer savings; energy savings/income; renewable energy credits (RECs); and available grant funding. This paper uses a detailed technical and economic feasibility evaluation completed for a high-rate anaerobic digester system at the F.X. Matt Brewing Company in Utica, NY, as a case study. Based on the results of this evaluation, the Matt Brewing Company is currently installing an AFBD system for wastewater treatment, electricity generation, and waste heat recovery.

Manaf H. Farhan is the president and CEO of EMG International based in Media, PA. He holds a B.S. degree in civil engineering from the University of Notre Dame, a master's degree in environmental engineering from Columbia University, and master's and Ph.D. degrees in systems engineering from the University of Pennsylvania. He is a licensed professional engineer. His doctoral research focused on design and optimization of various anaerobic digester processes to maximize process efficiency and biogas production. He has authored several peer-reviewed articles on anaerobic digestion and has served as an adjunct professor in the Department of Electrical and Systems Engineering at the University of Pennsylvania. He has over 20 years of experience providing a wide range of environmental engineering design and consulting services to private industry and governmental clients. His professional experience includes design and construction of digester systems for wastewater treatment and biogas and electricity generation for food and beverage facilities and for dairy farms; technical evaluation, process modifications, and operational support for various full-scale anaerobic digester installations; development and testing of bench- and pilot-scale wastewater treatment systems; pollution prevention and wastewater minimization audits; and biogas collection, clean up, and utilization.

25. Malt manufacture: Being practically sustainable. Presenter: Nigel Davies, Muntons, UK.

It is untenable to simply say your factory is sustainable these days—you must prove in very practical ways that you are serious about making changes right along the supply chain. This paper looks at how a detailed analysis of its carbon footprint has lead the malting company Muntons to challenge the supply chain to adopt major changes in practice from farming to consumer and to make environmental assessment of carbon simple with easily recognizable actions. The benefits of being "green" and environmentally aware are shown to be financially viable and to develop competitive advantage. Opportunities to develop new products that are cost-effective and environmentally preferred are explained, along with the ability to work right across the supply chain to encourage and lead environmental excellence.

Nigel Davies is manufacturing and technical director of Muntons plc, a U.K.-based malting company and the largest producer of malted ingredients in the world. He has led Muntons to achieve numerous environmental accolades and developed a farming footprint calculator specifically for growers of malting barley. After earning his doctorate he lectured in biological sciences at London University before joining Brewing Research International (BRi), where he specialized in cereal physiology and pioneered the use of freezing-stage electron microscopy to study many different foods, becoming manager for confidential international malting and brewing projects. He is also experienced in flavor analysis of malts, beers, and wines and regularly acts as an expert witness in cases where food safety of cereals is at issue.

26. Brewery wastewater recycling: A case study. Presenter: Michael Eumann, EUWA Water Treatment Plants, Gaertringen, Germany.

Pushed forward by ambitious sustainability targets wastewater recycling in breweries is getting more and more ready for implementation. These plants usually include germ-safe filtration, like micro- or even better ultrafiltration, followed by reverse osmosis treatment for desalination. Nevertheless, due to the raw water composition and compared to normal water treatment, some very specific points, especially regarding the pretreatment of the reverse osmosis, have to be adhered to. This presentation provides deep insight into the topic, starting from the basics elaborated in pilot trials, coping with the design and planning phase of the whole project, and finally resulting in the commissioning of a large-scale plant sized for 100 m^3/hr. Different points of use for recovered water are discussed in detail, considering technical, technological, and ethical aspects. Special focus is put on the impact on the residual wastewater, which may emerge to be the limiting factor. The impact on the overall water balance is shown, and the new limits for the overall water consumption figures are presented. Operation data and first-hand operation experience, as well as business figures, are given.

Michael Eumann is the owner of EUWA Water Treatment Plants, Germany, which he joined in 1987. EUWA has one subsidiary in Singapore and customers in more than 100 countries on 5 continents. Michael is a well-known specialist in water treatment technology and holds numerous patents.

Technical Session 8: Sensory

27. Impact of fermentable and non-fermentable sugars on oxidative processes during brewing, SO_2 formation, palate fullness, and flavor stability. Presenter: Thomas Kunz, Technische Universität Berlin, Department of Biotechnology, Chair of Brewing Sciences, Berlin, Germany. Co-author(s): Torsten Seewald, Niklas Brandt, Christof Reinhardt, and Frank-Jürgen Methner, Technische Universität Berlin, Department of Biotechnology, Chair of Brewing Sciences, Berlin, Germany.

The aim of this study was to investigate the influences of fermentable and commonly used unfermentable sugars, usually added during wort boiling to increase the beer's palate fullness, on oxidative processes during wort boiling, the SO_2 formation, palate fullness, flavor, and formation of specific aging compounds. The first results demonstrate that the increase of osmotic pressure by addition of non-fermentable sugars is responsible for higher SO_2 formation during fermentation. Addition up to 2% prior to fermentation leads to a better palate fullness and higher concentration of antioxidant substances like SO_2 without notable influence on flavor or sweetness directly after filling. Otherwise, a previous investigation showed that sugars behave differently at low pH than the generally known behavior described by Fehling, resulting in a sugar type specific influence on oxidative processes during brewing and beer storage. The newly developed "Chapon" method (MBAA 2011) demonstrates that at low pH (4.2), the strongest reducing potential results from isomaltulose followed by fructose, Vitalose®, and maltotriose. The low reduction potential of the so called "reducing sugar" glucose at low pH can be explained by the inhibited formation of the open-chain aldehyde structure. In contrast, fructose possesses a higher ability to generate the open-chain-structure, resulting in stronger reducing proper-

ties. In this context the increasing reducing potential of the "non-reducing sugar" sucrose results from the acid hydrolyzed formation of invert sugar. Additional investigations at higher temperatures (90°C) and pH (5.2) provide evidence about the behavior of fermentable and unfermentable sugars during wort boiling. As a result the strong reducing potential of maltotriose in comparison to maltose is remarkable. In addition, the partial unexpected reduction potentials of sugars in the pH range of wort and beer have a direct influence on oxidative processes. In correlation to the measured reduction potential, the sugars show the same effect on oxidative processes during wort boiling as specific intermediate Maillard reaction products with reductone/endiol structure, resulting in an acceleration of radical generation (EPR spectroscopy) by the Fenton-/Haber-Weiss reaction mechanism and the formation of specific aging compounds (GC-MS oxygen indicator: 3-/2-methylbutanal). This phenomenon is demonstrably caused by the rapid reduction of Fe^{3+} to Fe^{2+} in direct dependence with the reduction properties, resulting in a higher activation of oxygen and stronger radical generation. In consideration of the reduction properties, the sugar profile of the wort should be characterized by a low content of fructose and maltotriose. In the case of non-fermentable sugars addition during wort boiling is disadvantageous. Our recommendation is direct addition before fermentation to avoid the negative effects on radical generation and to use the positive effect on SO_2 formation during fermentation. Additional experiments provide information on the influences of the types of unfermentable sugar used on oxidative processes, aging compounds, and taste during storage.

After qualifying as a certified technician in preservation engineering (1991–1993), Thomas Kunz completed his basic studies in chemistry at the University of Applied Sciences, Isny (1994–1995), and his basic studies in food chemistry at Wuppertal University (1995–1998), before studying food technology at the University of Applied Sciences, Trier (1998–2002). After graduating, he worked as a chartered engineer in the area of ESR spectroscopy at the Institute of Bio Physics at Saarland University (2002–2004). Since January 2005, he has been employed as a Ph.D. student at the Research Institute of Brewing Sciences, Berlin Institute of Technology (Technische Universität Berlin). His main research focus lies in analyzing radical reaction mechanisms in beer and other beverages using ESR spectroscopy.

28. Going the last mile: Better draft beer presentation. Presenter: Michael Lewis, UC Davis Extension, Davis, CA, USA.

Wine is always served in a stemmed glass with a tulip-shaped head. I have never seen milk, iced tea, cola, or (God forbid!) water served in a wine glass. Why then, in pubs all across this country, do I see beers served in the all-purpose, any beverage you want, straight-side pint glass? This glass is surpassingly ugly and does not support the product in any way, but instead demeans it. In this glass, beers that brewers have struggled to make superbly well are sold to consumers as a common commodity, as something ordinary, plain, not deserving of special presentation. "Commoditization" is the opposite of "premiumization," and premium is the message craft brewers (or any brewer for that matter) should be sending to consumers. During recent travels, I realized the United States is miles behind the rest of the world in premium presentation of draft beers. Although we have perhaps the world's most innovative and creative brewing industry and excellent products, we fatally miss out on the last essential step—splendid presentation. We do not go the last mile. This paper is a plea to change that approach.

Professor Emeritus Michael Lewis taught the program in brewing science at the University of California at Davis for more than 30 years before retiring in 1995, and many former students now hold distinguished positions in the American brewing industry, large and small. Michael has been recognized by the university with the Distinguished Teaching Award and by the industry with the MBAA Award of Merit and Life Membership. Michael remains active in the brewing industry; he is the academic director of brewing programs in the University of California Extension, where the Master Brewers Program, which prepares students for the Institute of Brewing and Distilling examinations, is the flagship educational offering.

29. Influence of maltodextrins on palate fullness of beer. Presenter: Heinrich Rübsam, Institute of Brewing and Beverage Technology, TUM-Weihenstephan, Freising, Germany. Co-author(s): Martina Gastl, Institute of Brewing and Beverage Technology, TUM-Weihenstephan, Freising, Germany; Martin Krottenthaler, Hochschule Weihenstephan-Triesdorf, Freising, Germany; Thomas Becker, Institute of Brewing and Beverage Technology, TUM-Weihenstephan, Germany.

The content of high and low molecular weight maltodextrins, as well as their structural properties in beer, may influence the palate fullness of this beverage. Therefore, the possible association between sensory impressions and structural analysis of maltodextrins was investigated. To achieve this, a series of tasting sessions using different beer and maltodextrin samples was performed in parallel with various measurements of different structural parameters using field-flow fractionation techniques. For these purposes a selection of different maltodextrins products (2–100 kDa) was required. These maltodextrins were classified, using different dextrose equivalents (DE). First, the taste threshold of each maltodextrin in beer was determined. To do this, the different maltodextrins were independently added in increasing concentrations in a pilsner beer, and all samples were tasted. Seven samples (control and 0.25, 0.5, 1, 2, 4, and 8% maltodextrin in beer) were presented to the panelist, and these were evaluated for the intensity of the palate fullness. Further, in the same tasting, the threshold concentration of each maltodextrin at which the flavor was not pleasant for the degustation panel was also determined. To validate the previously determined threshold values and to compare the characteristics of each type of maltodextrin in the beer sample, a series of taste sessions was conducted. In these sessions, 4% of maltodextrin was added to the corresponding beer sample. Furthermore, the influence of maltodextrin to palate fullness was determined by the application of defined matrices (maltodextrin-beer and maltodextrin-water). Finally, a number of different commercial pilsner beers (same wort extract and alcohol content) was selected and tested for sensory palate fullness. All sensory tests were conducted 10 times to obtain trustworthy results. Finally, the structural analysis of the tasting matrices was carried out by means of a field-flow fractionation-refractive index-light scattering measurement system. The system was calibrated with different standards (pullulans, beta-glucan, and proteins) at a molecular weight range of 6–800 kDa, and the corresponding reproducibility was examined. All pilsner beers used for the tasting sessions were analyzed. The correlation between the analytical results and the sensory impressions was determined by the comparison of the molecular weight distributions of the pilsner beers with the degree of intensity of the palate fullness of each beer. From the tested beers, it was found that the molecular weight distribution ranged from 2 to 15 kDa. By correlation of the analytical data and the results obtained from the tasting sessions, it is possible to conclude that the beers with the higher palate fullness intensity corresponded to the beers with molecular weight distributions from 6 to 15 kDa, while the beers with molecular weight

distributions from 2 to 15 kDa were considered by the panelists to have lower palate fullness.

Heinrich Rübsam completed his Dipl.-Eng. degree in chemical engineering at the Metropolitan University, Caracas, Venezuela, in July 2004. He was then employed by Polar Brewery Company at the research center. He participated in different research projects: synthesis of alpha-dicarbonyl compounds by application of HPLC, GCMS, and mass spectrometry and quantification of alpha-dicarbonyl compounds and Strecker aldehyde during the aging process of beer (2004–2006). The results of this work were published in the Journal of Agricultural Food Chemistry (56(11), pp 4134-4144, 2008). In 2007, he did an internship at the Paulaner Brewery in Munich, Germany. He worked in the Production, Bottling, and Quality Assurance departments. Additionally, he participated in an independent project for the taste stability determination of beer. Later, he studied brewing and beverage technology at the Technische Universität München (TUM) in Germany and obtained his M.S. degree in 2009. His work, in cooperation with the Paulaner Brewery on optimizing brewhouse technology with reference to dimethyl sulfide, was published in Brauwelt International in 2010. Currently he is pursuing his Ph.D. at the Institute of Brewing and Beverage Technology, TUM-Weihenstephan, Germany. His research focus is on the characterization of the degradation of starch during the mashing process, as well as the contribution of the content of high and low molecular weight maltodextrins and their structural properties to palate fullness of beer.

30. Sensory evaluation of Belgian and U.S. red/brown sour beers. Presenter: Jeff Clawson, Oregon State University, Corvallis, OR, USA. Co-author(s): Victor Algazzali, Yanping Qian, Michael Qian, and Thomas Shellhammer, Oregon State University, Corvallis, OR, USA.

Sour ales from the Northern region of Belgium are unique in their sensorial aspects, with varying degrees of sourness and aromatic qualities. These "Flemish" red or brown ales are traditionally aged in oak barrels for up to 2 years, although some are aged in stainless steel. Some are blended with younger beer before packaging. Sour beers brewed in a similar style are being produced by some U.S. craft brewers. To date, there has been little research comparing and contrasting the Belgian beers with those produced in the United States. This research focused on examining this style of beer from the two respective countries using instrumental and sensory approaches. Six commercial sour beers from the Flanders region of Belgium were chosen based on their popularity and availability, while seven U.S. beers were chosen based on their similarity to the Flemish sour beer style with regards to aging, color, and sour character. Instrumental analyses included measures of acidity (pH, TA, and organic acid profiles), volatile aromas (ethyl and lactate esters), and markers of *Brettanomyces* activity (4-ethylphenol and 4-ethylguiacol). Descriptive sensory analysis was performed using a panel of 13 trained beer tasters and a ballot of 22 attributes. The U.S. beers were significantly higher and more variable in OG, ABV, and acidity than their Belgian counterparts but were similar in visual appearance and color. The U.S. beers were perceived as being more bitter, salty, sour, and astringent, with greater *Brettanomyces* character than the Belgian beers, while the Belgian beers tended to be sweeter and possessed greater coca/coffee notes. There was less differentiation between the two countries for descriptive terms such as dark fruit, cherry, fruity, caramel, tobacco/black tea, and sweaty/cheesey. Instrumental results of esters and *Brettanomyces* markers mirrored these similarities. These results reveal that sour beers originating from the United States and Belgium have many similarities, while at the same time expressing their own uniqueness.

Jeff Clawson received both his M.S. and B.S. degrees from Oregon State University in Corvallis, OR. In 1993 he began employment with the Food Science and Technology Department at OSU as a faculty research assistant working with Mina McDaniel. He conducted both descriptive and commercial sensory panels involving beer and food products. Since 2001 he has been professional faculty, managing both the food processing plant and research brewery, supporting the research program of Thomas Shellhammer. He has been an active member at the local level of the Institute of Food Technologists, ASBC, and MBAA.

Technical Session 9: Analytical II

31. Recent discoveries in beer foam. Presenter: Karl Siebert, Department of Food Science, Cornell University, Geneva, NY, USA.

A statistical experiment design (central composite face centered) was used to select combinations of conditions in a foam model system in which the levels of protein (ovalbumin), iso-alpha-acid, ethanol, and pH were varied. The results were used to construct a response surface model; this provided insight into some of the contradictions in the foam literature. Intermediate ethanol levels led to the best foam, with poorer foam at higher and lower ethanol contents. Increasing pH led to poorer foam. For predictions of a model system to be useful, it must behave like the modeled phenomenon (in this case beer foam). Ethanol was added to commercial non-alcoholic beer; the effect on foam behavior was similar to the model system. When a commercial lager was adjusted in pH, however, the foam increased with increasing pH, opposite to the model system. Dimethyl formamide, a hydrogen bond acceptor; dioxane, a non-polar but water-miscible solvent; and NaCl solution were each added to the model system and to beer. Salt greatly reduced foam in the model system, suggesting the bonding between ovalbumin and iso-alpha-acid is mainly ionic. DMF caused by far the largest reduction in the foam of the commercial beer, indicating a hydrogen bonding mechanism. These results show the mechanisms are different in this foam model system and beer. The barley proteins that have most often been associated with beer foam are lipid transfer protein 1 (LTP1) and proteins Z4 and Z7. Ovalbumin has considerable similarity to proteins Z4 and Z7 in the proportions of amino acids of different types and in the charge on the molecule at various pHs but is quite different from LTP1. The results suggest possible greater involvement of LTP1 than the other two proteins in beer foam.

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, Karl joined Cornell University as professor of biochemistry in the Department of Food Science and Technology. He served five years as department chair and now has a predominantly research commitment. Karl is active as a consultant in beverage technology and chemometrics. He has twice received MBAA Presidential Award for papers he presented, and he and his colleague, Penny Lynn, received the ASBC Eric Kneen Memorial Award (for the best paper published in JASBC in the prior year) three times. Karl was made an honorary professor of the Moscow (Russia) State Academy of Food Processing in 1996, and in 1999 he received the ASBC Award of Distinction. He received the MBAA Award of Merit in 2011. He is currently a member of the JASBC Editorial Board and the ASBC Foundation. Karl's research interests involve foam and haze in beverages, perception of astringency and other flavors, the application of chemometric methods in food science, and assessment of microbiological risk.

32. The measurement of carbon dioxide in packaged beer: A critical review. Presenter: Donald Hutchinson, Anheuser-Busch InBev, St. Louis, MO, USA.

The concentration of carbon dioxide (CO_2) levels in packaged beer is a critical parameter that defines the quality of a bottle, can, or keg of beer both from sensory and processing standpoints. CO_2 content below a brewer's specification can lead to customer complaints of flat beer. Conversely, CO_2 content above specification can cause problems with the package and packaging process. At the present time, the basis of all methods used to determine the CO_2 content of packaged beer is the pressure produced by the CO_2 at a given package temperature. The CO_2 content can then be derived from these parameters based on the solubility of CO_2 in beer at those conditions under the context of Henry's law. Over the years, the principal brewing industry associations, such as ASBC, EBC, and Brewers Association, as well as various CO_2 instrument manufacturers, have each developed and subscribed to different mathematical expressions to carry out these calculations. Unfortunately, each algorithm has its own set of foibles that lead to a situation where an identical pressure and temperature measurement will result in a significantly different CO_2 concentration. This paper reviews the behavior of CO_2 in packaged beer, critically examines each of the primary calculations used to determine the CO_2 content, assesses the pros and cons of each, and offers recommended changes to bring the methods more in line with each other.

Don Hutchinson received his B.S. degree in chemistry from Miami University and Ph.D. degree in analytical chemistry from Northern Illinois University. He joined Anheuser-Busch in 1988 as a senior group leader in analytical chemistry in the Corporate Research and Development Department. In August 2011, he assumed the role as manager, packaging and material science, with the Anheuser-Busch InBev Brewery Technical Center in St. Louis and is the corporate subject matter expert for package gas analysis.

33. Carbon dioxide solubility in wort and beer. Presenter: Alex Speers, Dalhousie University, Halifax, NS, Canada. Coauthor(s): Andrew MacIntosh, Dalhousie University, Halifax, NS, Canada.

Carbon dioxide (CO_2) is a key component of beer; however, the amount of CO_2 within beer is dramatically affected by temperature and pressure. The CO_2 level in a beer is dependent on CO_2 solubility, which in turn is affected by temperature, containing pressure, and beer composition. There is a substantial gap in the literature as to how substances in wort and beer affect CO_2 solubility. In fact, the origin of various pressure-temperature solubility charts contained in ASBC's *Methods of Analysis* or MBAA's *Beer Packaging: A Manual for the Brewing and Beverage Industries* are largely unknown and poorly referenced. This is especially problematic as there are discrepancies between the most commonly used charts, and explanations for these differences are not readily apparent. This presentation details the findings of an exhaustive literature search through electronic and pre-electronic cited literature. The methods used to create these charts will be discussed, including the assumptions reported by the original authors. ASBC and MBAA solubility charts and those generated by simple formulas will be compared to each other and to the van 't Hoff equation, which describes how gas solubility is affected by temperature. The influence of other variables unaccounted for in the aforementioned solubility charts will be reported. Specifically, the effect of ethanol (0–8.3 g/100 g) and solids (0–13 g/100 g) on Henry's constant in water, model worts, and beers will be presented. Reports concerning CO_2 solubility made outside of the brewing literature and their applicability will be noted. Finally, measurements in our laboratory concerning the time at which freshly fermenting wort reaches CO_2 saturation will be compared to predictions based on measured sugar, alcohol, pressure, and temperature levels.

Alex Speers is a professor in the Food Science program at Dalhousie University, Halifax, NS, Canada. Born in Creston, BC, he gained B.S. (Agr.), M.S., and Ph.D. degrees in food science at UBC. At "Dal" he instructs students in brewing science, quality assurance, and product development. 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) beta-glucan and arabinoxylan polymers. He has organized and/or presented brewing workshops in Australia, China, America, and Canada. Alex also organized the International Brewers Symposium: Yeast Flocculation, Vitality, & Viability in Boston, MA, in 2009 sponsored by MBAA. Alex has spent sabbaticals at CUB/Fosters in Melbourne and the Columbia Brewing Company in Creston. He is a past chair of Editorial Board of the MBAA Technical Quarterly. Alex belongs to several professional societies and is a member of the editorial boards of Food Research International, JASBC, JIB, and the TQ. He has published or presented more than 150 papers and edited and was recently named a Fellow of the Institute of Brewing and Distilling and awarded the W.J. Eva Award by the Canadian Institute of Food Science and Technology.

Technical Session 10: Microbiology I

34. Investigation into the antibacterial activity of mesoporous zirconium phosphate against beer-spoilage bacteria. Presenter: Guangtian Zhou, School of Food and Bioengineering, Shandong Institute of Light Industry, Jinan, China. Coauthor(s): Xinxia Ge, School of Food and Bioengineering, Shandong Institute of Light Industry, Jinan, China; Wen He and Xiaoyong Du, Key Laboratory of Glass and Functional Ceramics, Shandong Polytechnic University, Jinan, China; Xiaolei Dong, School of Food and Bioengineering, Shandong Institute of Light Industry, Jinan, China

Hygiene is a major concern of the brewing industry. The aim of this study was to examine the antimicrobial effect of mesoporous zirconium phosphate (M-Zrp), which is an inorganic nanometer material with an aperture of 2~50 nm that could be used as an effective antibacterial agent against beer-spoilage bacteria. Template synthesis of M-Zrp is an important method. A biological template has a lot of advantages in terms of nontoxic and environmental protection. M-Zrp's were produced by natural fresh yeast through bio-mimetic synthesis, which conforms to the green chemical concept advocated at the present. The antibacterial activity of M-Zrp from the yeast bio- template was investigated by the inhibition zone test. The impact of particle size, concentration, and action time on antibacterial behavior was examined. The results showed that M-Zrp has bacteriostatic activity against *Saccharomyces diastaticus*, *Candida albicans*, *Pediococcus damnosus*, and *Escherichia coli*. But it had no effect on *Lactobacillus brevis* and *Pectinatus cerevisiiphilus*. The mean diameters of the inhibition zone were 11.67, 12.37, 8.64, and 14.36 mm, respectively. Antibacterial activity increased as the concentration of M-Zrp increased and particle size decreased. Within a certain range, the effect was enhanced with the prolonging of action time. The minimal inhibitory concentrations (MIC) were found by spectroscopic method to be 10 mg/L for *S. diastaticus*, *C. albicans*, and *E. coli*, and 25 mg/L for *P. damnosus*. When M-Zrp was exposed to bacteria for 24 hr, the average sterilization rate can reach 96.78% at MIC of 12.5 mg/L and up to 98.54% after 36 hr. The morphology of *S. diastaticus* before and after treatment with M-Zrp was observed by means of polarization micro-

scope. It was observed that the presence of M-Zrp damages the membrane of the bacterium at first, and further, penetrates the cell wall and interacts with internal components, resulting in leakage of intracellular contents and eventually the death of bacterial cells. Our findings suggest that M-Zrp exhibits effective antimicrobial properties and could be exploited for the application of antibacterial coatings or additives for the food and beverage industries.

Guangtian Zhou received his B.S. degree in bioengineering from Shandong Institute of Light Industry, Jinan, China, in 1982. He was then employed with the Jinan Beer Group as a brewer. Guangtian studied in Doemens Akademie, Munich, Germany, from August 1987 until November 1988. After graduation, he became chief brewer of the Jinan Beer Group. Since July 1994, Guangtian has functioned as professor, tutor of M.S. degree students, and the director of the China-Germany Beer Technology Center in the School of Food and Bioengineering, Shandong Institute of Light Industry, teaching and researching beer production. At present, he serves as an editor of China Brewing and a council member of Shandong Society for Microbiology.

35. *Pediococcus claussenii* genetic expression during growth in beer assessed by transcriptome sequencing (RNA-seq). Presenter: Vanessa Pittet, University of Saskatchewan, Saskatoon, SK, Canada. Co-author(s): Trevor Phister, University of Nottingham, Nottingham, UK; Barry Ziola, University of Saskatchewan, Saskatoon, SK, Canada.

The beer environment is very inhospitable for microbes as it typically contains high levels of CO_2, hops, and ethanol and has low pH, oxygen, and available nutrients. As such, most microbial growth is inhibited in beer. However, specific organisms have adapted to overcome the stresses found in beer and, therefore, can grow in and spoil the product. The most common beer-spoilage bacteria are lactobacilli and pediococci, and their presence in a brewery can have a major economic impact due to product spoilage. To elucidate the mechanisms that these organisms use to grow in a beer environment, we sequenced the genome of the brewery isolate *Pediococcus claussenii* ATCC BAA-344T. We found that this organism has the means for genetic diversity, particularly via the eight plasmids that are present. To gain a better understanding of the role that various genes play, we performed whole transcriptome sequencing (RNA-seq) of *P. claussenii* during growth in beer and a non-beer medium (MRS). RNA was extracted during mid-logarithmic growth from both MRS broth and a Canadian-brewed beer (5% [v/v] ethanol, pH 4.2, and roughly 11 BU). Transcriptome sequencing was done via illumina technology, and bioinformatic analyses were performed to determine gene expression in both growth conditions. We found that the majority of genes are expressed in both environments, indicating that most of the coding capacity of this isolate is used (i.e., at least basally expressed). Roughly half of the genes did not show differential expression in either medium. However, of those showing significant differential expression, half of the genes had increased expression during growth in beer, while half showed decreased expression. Several operons were very highly expressed during growth in beer but not during growth in MRS, suggesting that these genes play a major role in the ability of *P. claussenii* to grow in beer. As expected, the previously described hop-resistance gene *horA* had increased expression during growth in beer. Interestingly, a number of the genes with increased expression in beer played a role in nutrient acquisition and hop resistance, leading to the conclusion that the other stress factors found in beer (e.g., ethanol) may not play a large role in determining if growth of the isolate will be inhibited in beer or not. This is most likely due to the intrinsic resistance that most lactic acid bacteria have to a range of stressors, including ethanol and low pH. This study provides a better understanding of the genetic mechanisms that bacteria can use to overcome the inhospitable environment of beer. This information can be used as the basis for further studies into potential targets for detection of beer-spoilage bacteria in a brewery setting.

Vanessa Pittet graduated from the University of Saskatchewan in 2008 with a double honors B.S. degree in microbiology and immunology and in biochemistry. She then started a master's program under the supervision of Barry Ziola in the area of brewing microbiology at the University of Saskatchewan. She converted from an M.S. program to a Ph.D. program in May 2010 and is looking to finish her Ph.D. degree in September 2012. Her Ph.D. work uses genomic and bioinformatic approaches to study beer-spoilage bacteria.

61. Impact of *Fusarium culmorum* infection on barley malt protein fractions, brewing process, and beer quality. Presenter: Pedro Oliveira, University College Cork, Cork, Ireland.

Malt infected with *Fusarium culmorum* entering into the brewing supply chain can have a major impact on the processability and quality of beer. High *F. culmorum* infection levels in barley grains result in substantial malt loss, changes in enzymatic activity, kernel ultrastructure deterioration, and DON accumulation. In this study, the protein fractions and protease activity from the resulting infected barley and malt were first characterized. Protein Osborn fractions were extracted and electrophoresed, while the four protease groups were analyzed via specific inhibitors. The results showed significant and relevant differences. Second, in vitro *F. culmorum* infected malt was used to produce lager beer in a pilot scale facility. The impact of the *Fusarium* infected malt on a wide range of brewing parameters was measured. It was found that the wort containing infected malt (IW) had a lower pH, higher FAN, higher beta-glucan, and 46% increase in purging rate than the control wort containing uninfected malt (CW). IW caused premature yeast flocculation (PYF), although final extract and attenuation degree were not significantly affected by *Fusarium* contamination. The final beer quality was fully characterized. The beer produced with infected malt (IB) was compared to the control beer produced with uninfected malt (CB). The IB amino acid profile was considerably different from the CB, while sugar and organic acid profiles were comparable. Flavor characterization of IB revealed a higher concentration of esters, fusel alcohols, fatty acids, ketones, and dimethylsufide (DMS). Acetaldehyde was particularly higher for the IB compared to the CB (98 and 7 mg/L, respectively). Another notable difference for IB compared to the CB was the greater proportion of Strecker aldehydes and Maillard products contributing to an increased beer staling character. Final IB had a 67% darker color with a trend to higher foam stability. The mycotoxin deoxynivalenol (DON) was measured in the malt as well as in the final beer. It was found that 78% of accumulated DON present in the raw material was transferred to the final beer.

Pedro Oliveira studied food science and engineering at the Instituto Superior de Agronomia, Technical University of Lisbon. Pedro was awarded a mobilization scholarship, and for one year he studied at the University College Cork, where he completed his final graduation project on NPD and beverages sciences. During his master's degree studies , Pedro performed research in the field of beverages and fermented processes focusing on "New Fermented Beverages Using Immobilized Yeast." Pedro performed an internship in the Manufacturing Support Department at Nestlé PTC, Konolfingen, Switzerland, and gained practical training in the Quality Department at Les Mousquetaires Group on Sensory Analysis. In 2010, Pedro was awarded a

postgraduate scholarship by the Irish Research Council for Science, Engineering & Technology and he joined Elke Arendt's research team at the University College Cork for his Ph.D. Project. His research focus is 1) the impact of infections from Fusarium species on malt quality, with emphasis on mycotoxin production and kernel ultrastructure; and 2) the characterization and identification of antifungal compounds from lactic acid bacteria and their application in malting and brewing. Pedro is a peer support leader and lecturer in the UCC Food Science and Technology course. He is also a member of ASBC and IBD.

Technical Session 11: Brewhouse Operations

37. The influence of nitrogen compounds on beer characteristics. Presenter: Taichi Maruhashi, Suntory Liquors Limited, Osaka, Japan. Co-author(s): Tetsuya Arita, Yutaka Yamaguchi, Yoshinori Hida, and Kaneo Oka, Suntory Liquors Limited, Osaka, Japan.

The nitrogen compounds in mash contribute not only to beer taste, but also to the brewing process, nitrogen source for yeast, and beer filterability. For this reason, control of protein modification in malting and mashing is very important, and we must consider optimizing mashing procedures depending on malt quality and vice versa. Because it is both easy and economical, it has become popular to use malts with relatively high protein modification. However, this may cause low fullness or an unpleasant aftertaste if the mashing method is not carefully considered. I reported at MBAA in 2010 that relatively low protein-modified malt and low mashing-in temperature led to better fullness and a bitter quality. Decoction beer has better fullness and bitter quality than infusion beer when relatively low protein-modified malt and a high mashing-in temperature were used. In order to improve the quality of decoction beer further, the influence of the mashing-in temperature and the heating process in the kettle mash was investigated using laboratory scale mashing apparatus (50 g of malt). Because it is also known that the protease activity of low-modified malt is improved by a low mashing-in temperature of 35°C, we first investigated the effect of mashing-in temperature in the kettle mash on extraction and degradation of nitrogen compounds. A low mashing-in temperature of 35°C produced more nitrogen compounds in the mash than a temperature of 45°C. The appropriate heating rate and rest temperature of the kettle mash produced larger quantities of nitrogen compounds than were produced without a rest process in the same length of time. We have described the suitable single decoction mashing procedure for better extraction and degradation of nitrogen compounds as determined by laboratory scale mashing experiments, and its influence on beer quality was evaluated on a 100-L pilot brew scale.

Taichi Maruhashi graduated with an M.S. degree from the Tokyo University of Science in 2001. After joining Suntory, he worked for six years in the position of second brewmaster at the Suntory Tonegawa brewery in Gumma, Japan. He then went to Technische Universität München, Weihenstephan, in Germany as an international student and studied there for three years. He currently works in beer development.

39. Brewing intensification—Successes and failures. Presenter: Graham Stewart, GGStewart Associates, UK. Co-author(s): James P. Murray, Better Lines Co. Ltd., Galashiels, Scotland.

Brewing, similar to most manufacturing industries, has two overlapping primary objectives, namely to brew quality beers in the most efficient and cost-effective manner. Many brewing production stages have evolved together into a more efficient and rapid process, and most stages have resulted in cost savings. Brewing has a long and proud manufacturing tradition, and a major part of this tradition is focused on "a slow cold process." This prolonged processing time largely focuses (but not entirely) upon maturation because "a slow process" usually (but not always) produces consistent palatable beer, but at a cost! Inflation (including labor and management, new materials, utilities, equipment, and real-estate costs) and diverse taxation initiatives have necessitated that brewing companies rigorously examine their overhead (fixed and variable). Research in all relevant areas of the technical aspects of the process has enabled development of more efficient procedures for brewing beer with consistency, drinkability, quality, and stability. Although much of this research and development in process efficiency has been successful, some relevant initiatives require further attention. The positives and negatives of these research and development initiatives will be considered.

Graham Stewart is Emeritus Professor in Brewing and Distilling at Heriot-Watt University and Special Professor in Bioethanol Fermentation at Nottingham University. He was director and professor of the International Centre for Brewing and Distilling, Heriot-Watt University, from 1994 to 2007. He received his B.S. degree (with honors) in microbiology and biochemistry from the University of Wales, Cardiff, and Ph.D. and D.S. degrees from Bath University. He was lecturer in biochemistry in the School of Pharmacy at Portsmouth University from 1967 until 1969. From 1969 to 1994 he held a number of technical positions with Labatt's in Canada and from 1986 to 1994 was its brewing technical director. He was the president of the Institute of Brewing and Distilling in 1999 and 2000. He is a member of the American Society of Brewing Chemists (ASBC) and the Master Brewers Association of the Americas (MBAA). He holds fellowships in IBD, the Institute of Biology, and the American Academy of Microbiology. He has over 250 publications to his name. Since retiring he has established a consulting company—GGStewart Associates. He was awarded the IBD Horace Brown Medal in 2009, the ASBC Award of Distinction (Excellence) in 2008, the MBAA Presidential Award in 1983 and 1998, the MBAA Award of Merit in 2009, and the Society of Industrial Microbiology Charles Thom Award in 1988.

40. Optimized conditions for pre-treatment of hops in the brewhouse to maximize utilization rate without a decrease in beer quality. Presenter: Sebastian Kappler, Technische Universität München, Institute for Brewing and Beverage Technology, Germany. Co-author(s): Martin Zarnkow and Thomas Becker, Technische Universität München, Institute for Brewing and Beverage Technology, Germany.

Iso-alpha-acids are the main contributors to bitterness perception in beer. In the brewing process, however, only about 30% of the alpha-acids present in hops are isomerized and transferred into the finished beer. To reduce costs for hopping of beer by an increase in utilization rate, several solutions have been suggested over the past few years. Mostly either the use of pre-isomerized hop products or a pre-treatment of hops prior to dosage has been described. Formerly presented results showed a highly significant decrease in bitterness quality and aging stability with the increase in utilization rate achieved by isomerization of hops prior to dosage. Probably due to a high amount of degradation products of alpha-, beta-, and iso-alpha-acids, a harsh bitterness was created. Also, aging stability was reduced due to polymerization reactions of hop and malt polyphenols during thermal treatment of hop pellets together with common lauter wort. It was obvious that further studies to increase the quality of the resulting beer while using pre-isomerization technology was necessary. In this work the influence of various technologies and varying parameters for treatment of hop products was evaluated. Pilot-scale trials, as well as industrial-scale trials, were done to evaluate the influence of various technologies on sensorial and analytical attrib-

utes, as well as behavior during aging of beer. Particular attention was paid to the bitterness profiles of fresh and forced-aged beers. All brews were analyzed in comparison to common brewed beers. This paper presents solutions to increase utilization rate while maintaining the quality of bitterness as well as aging stability. Pre-treatment of hops can be enhanced by optimizing the time and temperature of treatment while reactions are minimized, for example, by optimizing the composition of the medium. The influence of the use of different catalysts and lauter fractions, as well as variations in pH value, is shown. Finally, the influence of variations in point of dosage to the boiling wort is exposed. Suitable approaches toward an improved yield of bitter acids together with an acceptable bitter quality are shown!

Sebastian Kappler received a Dipl.-Ing. degree in brewing and beverage technology from Technische Universität München in 2008. He began his employment with the Augustiner-Wagner Brewery in Munich as an apprentice to a brewer and maltster in 2000. After becoming an assistant he started his studies on brewing science at the Technische Universität München. Since May 2008 he has been working as a scientific employee at the Institute for Brewing and Beverage Technology in Weihenstephan. The topic for his doctoral thesis is the evaluation of the factors affecting the yield of isohumulones during preparation of wort.

Technical Session 12: Engineering

41. Future brewery concepts and upcoming streams. Presenter: Roland Folz, VLB-Berlin, Berlin, Germany.

Studies to investigate the right strategic behavior for breweries to invest into the future under the premises of performance and technological behavior and their quality impact have been carried out. Trials were performed in pilot scale as well as in industrial applications. Results for different approaches that can be considered as cutting-edge technology will be presented, and different concepts of how brewing can change will be discussed. On the organizational level this includes the application of lean manufacturing and the calculation of overall process times compared to value added time in the different production steps, while at the same time being able to keep a necessary flexibility over the supply chain. The planning of future green fields will include technology that is not yet fully proven; presented small scale results can provide direction. Sustainability considerations will include alternative energy usage and reduction of solid waste. Results will be presented for new technology across production, including continuous production segments, changes in filtration and stabilization, packaging in alternative formats, and process control management. A holistic view will be created of current innovations, as well as possibilities that can have an impact on future brewing in order to help brand owners decide on new possibilities based on independent technological results.

Roland Folz apprenticed as a brewer and maltster at the Beck's Brewery in Bremen, Germany. After working another year for the Beck's Brewery, he started his studies in Berlin and received a diploma engineer degree in brewing technology from the Technical University, Berlin. After graduation, he was head of the Technical Department/Production at the Preussen Pils Brewery in Pritzwalk, Germany, for two years. In October 2006, he started at VLB-Berlin as a global consultant for brewing and beverage technology, working in the Engineering and Packaging Department as the specialist for the Filling Department, where he performed his Ph.D. research in beer in PET. With regard to his consulting practice, he is involved in teaching and research projects and the internationalization of VLB. Since autumn 2008, Roland has been head of the Department of Brewing & Beverage Technology and Applications at VLB-Berlin. This department includes the education and teaching programs of VLB, as well as the research activities regarding technological topics, global consulting, analytics, and services. Since 2012, the microbiological laboratory and research programs of VLB are part of this department. Since 2010, Roland has also headed the Labotec GmbH, which is a subsidiary of VLB and is working with laboratory equipment, supplying turnkey laboratories on a global scale.

43. Passivation of austenitic stainless steels for the purpose of manufacturing and handling beer. Presenter: Harvey Claussen, The Zythos Project LLC, Portland, OR, USA.

Creating and maintaining a suitable non-reactive surface for brewery vessels requires an understanding of the nature of austenitic stainless steels. The selection of the appropriate chemical and electrochemical system for passivation is critical. All of this can be brought into perspective for the brewer. An example is the composition of the surface during passivation of molybdenum containing austenitic stainless steels such as Avesta AB with 3.6 a/o Mo and Sandvik AB with 1.7 a/o Mo in 0.1*M* HCl + 0.4*M* NaCl. Low levels of chlorides can seriously alter the nature of the seemingly inert coating. The alloys exposed to certain electrolytes under polarization can develop varying corrosion potentials, as well as active and passive potentials. Electrochemical polarization can vary depending on the initial voltaic potential, as well as the rate of an increasing potential. Ni and Fe cations can be present to varying degrees in the oxide. An enrichment of Ni by 10 a/o may also develop on the surface of the metallic phase. At the passive region, the film formed on the alloy can be chromium oxide. Bringing all of the passivation technology together can be daunting, so the presentation focuses on a simplified approach to the understanding of passivation technology.

Harvey Claussen received a B.S. degree in chemical engineering from the University of Washington in Seattle. He has been involved with the design, construction, and operation of fine chemical, agricultural, and food facilities since 1961. He first entered the brewing world in 1984, siting and designing microbreweries. In addition, Harvey enjoys home brewing when time permits. He and his associates founded The Zythos Project LLC, a beer think tank, in 2011.

Technical Session 13: Hops III

44. Development of SNP-based identification method of hop varieties. Presenter: Hiromasa Yamauchi, Suntory Business Expert Ltd., Kawasaki, Japan. Co-author(s): Yuri Mukouzaka, Susumu Furukubo, Kazuhiko Nakashima, and Takayuki Taniguchi, Suntory Business Expert Ltd., Kawasaki, Japan; Masami Harada, Suntory Holdings Ltd., Tokyo, Japan.

Hop is one of the key raw materials affecting beer quality, and the correct identification of hop varieties is very important. Generally, hop varieties are identified by differences in cone structures, sensory analysis, and the content of substances such as alpha-acids and essential oils. However, these methods have limitations because the content of the substances in hop can be variable depending on cultivation conditions and pelletized hop cannot be identified by observation of external appearance. Several DNA analysis techniques have been developed for the identification of hop varieties, e.g., SSR method, RAPD method, RFLP method, AFLP method, etc., which generally utilize the polymorphism of PCR-amplified products or restriction enzyme-digested fragments of hop DNA. These methods are generally complicated and have limitations to detection of mixing of other varieties. Analysis of SNPs (single nucleotide polymorphisms) in genome DNA can be a powerful tool for the identification of varieties. However, in order

to obtain sufficient SNP positions for the identification of many varieties, large amounts of DNA sequences should be needed. In recent years, high throughput DNA sequencing technology has been developed using a so-called "next generation sequencer." Using this technique, we tried to develop SNP-based identification method for hop varieties. Large amounts of DNA sequence data in several European hop varieties were obtained using the next generation sequencer. By comparing DNA sequences between the varieties, several SNP-rich DNA regions in hop genome were selected as candidates for identification markers. DNA sequences of these regions in other European hop varieties were also determined using the traditional Sanger method, and it was evaluated whether these regions could be DNA markers for the identification of all varieties tested. As a result, 14 hop varieties could be identified by using four SNP-rich DNA regions. Moreover, it was studied whether a mixture of two varieties could be correctly evaluated by this method. A hop pellet sample of one variety was mixed with that of another variety at various ratios (0, 5, 10, 50, and 100%), and their DNA was extracted to sequence the DNA marker regions. By observing the electropherogram of SNP positions, it was suggested that the mixture of with the other variety at a 5% level could be detected. A quantitative determination method of mixture rate can be expected using DNA techniques, such as quantitative real-time PCR, etc. Because this method utilizes the DNA sequence itself, it could be a simple and reproducible tool for the identification of hope varieties.

Hiromasa Yamauchi received his doctor of agriculture degree from the University of Tokyo in 1991. In 1978, he began employment with Suntory Ltd. as a researcher in the Institute for Alcoholic Beverages, and later, in the Institute for Fundamental Research. He conducted research on bacteria, yeast, fungi, and plant genetics and biochemistry. In 1996, he attended the 62nd ASBC Annual Meeting and made a presentation on "Rapid Methods of Detecting Beer Spoilage Yeasts by Using Polymerase Chain Reaction." Since April 2001, he has served in the Quality Assurance Division, in which he has developed several identification techniques for plant and living organisms using DNA analysis.

45. Growing hops is stressful! Presenter: Douglas Walsh, Washington State University, Prosser, WA, USA.

Today's beer consumer knows that hops are a key ingredient in beer. An increasing population of connoisseurs has gained an appreciation for hops' essential role in creating the distinctive flavors that characterize specialty brews. Yet, few consumers are aware that producing hops is stressful. Hop growers face the stress of uncertain market demand, shifting price structure, consolidation of key customers, cancellation of contracts, increasing input costs for labor and fuel, and environmental regulation, along with the often stressful challenge of growing this unique specialty crop. Hop plants are subject to stress, as well, from a variety of biotic and abiotic factors. Biotic stress comes from pests and diseases, while abiotic stress comes from bright sunshine, high temperatures, wind, and dust that are typical of summer conditions in the inland Pacific Northwestern United States, in addition to water availability. To assist growers in understanding and overcoming stress factors, a transdisciplinary team sought and received USDA Specialty Crop Research Initiative (SCRI) Coordinated Agricultural Project (CAP) funding to study the plant stresses resulting from spider mites, aphids, downy mildew, powdery mildew, and varying levels of deficit irrigation with respect to impacts on hop quality and quantity and also on the subsequent quality of the beer brewed with hops subjected to controlled amounts of various stresses. The team includes entomologists, plant pathologists, weed scientists, irrigation specialists, economists, a sociologist, a sensory scientist, and an outreach specialist, with researchers from Washington State University, Oregon State University, the University of Idaho, and the USDA Agricultural Research Service. The impacts of the various stresses have been measured quantitatively on yield (kg/ha) and on the levels of alpha- and beta-acids (determined by high-performance liquid chromatography) and qualitatively in controlled laboratory sensory (taste) analysis. In general, results thus far indicate that aphid feeding had no impact on alpha- and beta-acids. Spider mite feeding reduced alpha- and beta-acids, and powdery and downy mildews increased alpha- and beta-acid levels. Deficit irrigation (water stress) decreased yield and tended to decrease alpha- and beta-acids. The interaction of mite spider mite feeding and deficit irrigation did not have a significant effect on alpha- and beta-acids. Single-hop ales were brewed within each hop stress type, with the amount of hops adjusted to compensate for the variability in alpha-acids content. These brews were evaluated by sensory panels at the School of Food Science at Washington State University. Flavor panels rated brews that sustained mite and aphid feeding or infection with downy mildew as inferior to brews made with undamaged hops. Flavor panels preferred brews made with powdery mildew damaged hops. At the submission of this abstract the brews made from deficit irrigated hops had yet to be evaluated by the sensory panel. The results of these beer studies will be described in greater detail in the presentation.

Douglas B. Walsh is the integrated pest management coordinator for Washington State, a professor in WSU's Department of Entomology, and the research director of the Environmental and Agricultural Entomology Laboratory at the Irrigated Agriculture Research and Extension Center in Prosser, WA. He functions as the overall coordinator and director of the SCRI-CAP project and directs the activities relating to arthropod management. Douglas works closely with and has research supported by the Washington Hop Commission and the Hop Research Council.

46. Development of new hops varieties in the Czech Republic and new opportunities in brewing. Presenter: Jiri Smetana, ARIX Co., Zatec, Czech Republic.

Until 1994, only a single hop variety was cultivated in the Czech Republic—the world-renowned fine aroma hop Saaz. It is a traditional Czech variety and has been exported to breweries in Europe and overseas since the 19th century. Over the years many premium brands have used this variety. Significant changes in brewing technology in the 1990s, e.g., installation of cylindroconical tanks, HGB systems, reduction of the bitterness of beer, etc., encouraged Czech hop growers to develop new varieties. In 1994, the first new varieties Sladek and Bor were developed. Sladek proved to be suitable for second hopping, giving the beer additional bitterness and an aroma similar to Saaz hops. In 1996 a further variety, Premiant, was developed. This variety is primarily used as a second hop gift for "mainstream beers." Later, Czech hop growers focused on two broad directions. 1) Development of Czech high alpha-acid varieties that would also be suitable for base hopping in the form of pellets or an extract. This led to the development and registration of Agnus (2001), Rubin (2007), and Vital (2009). 2) Development of an aroma variety that would provide a specific feel to the resulting beer, significantly distinguishing it from other products. This led initially to the development of Harmonie (2004), which has a balanced profile of bitterness due to the ideal 1/1 ratio of alpha- and beta-bitter acids. Another variety, Kazbek (2009), was developed by the unique

breeding of Saaz and wild hops from the Caucasian mountains. The resulting variety is very durable, stable under changing climatic conditions, and gives a distinguished earthy aroma to beer. In tastings it has also been described as "herbal," "natural," or "wild." Recently, partially due to economic reasons, research has focused on finding "more economical variants" of the traditional Saaz and Sladek varieties. At the end of 2010, the Saaz Late variety, a second generation of the traditional Saaz variety with a higher alpha-acids content and similar aroma profile, and the Bohemie variety, a second generation of Sladek with an alpha-acids content of about 6% and mild, pleasant aroma with typical Saaz character, were developed and registered.

Jiri Smetana graduated from the Faculty of Brewing at the Institute of Chemical Technology of Prague in 1994. He has worked in the hop industry as a technician, purchasing manager, and sales manager of reality trading and, later, Saaz hop products. In 2004 he co-founded the hop company ARIX, and is a partner there today. He also manages the largest hop farm in Europe as the chair of the board of PP Servis.

Technical Session 14: Yeast II

47. Effects of non-sugar nutrient concentrations on fermentation and beer flavor. Presenter: Takeshi Kawakubo, Kirin Brewery Company, Japan. Co-author(s): Kentaro Iwasaki, Yuichiro Mese, Nobuyuki Hayashi, and Hiroyuki Yoshimoto, Kirin Brewery Company, Japan.

In 2011, low-malt beer comprised approximately 20% of Japan's alcohol market. Compared to normal beer wort, low-malt beer wort contains less non-sugar nutrients, such as amino acids, minerals, and vitamins. It has been reported that yeast metabolism is negatively affected by insufficient non-sugar nutrients, thus reducing fermentability and the production of fermentation by-products. To make high quality beer, it is important to control the balance of sugar and non-sugar nutrition concentrations. However, the relationship between the concentration of non-sugar nutrients and yeast metabolism remains unclear. The purpose of this study was to elucidate the relationship between the concentration of non-sugar nutrients, especially vitamins and minerals, and yeast metabolism and their effects on fermentability and beer quality. To investigate the effects of low concentrations of non-sugar nutrients on fermentation and beer flavor, we performed fermentation tests using a synthetic medium or a low-malt beer wort with adjusted concentrations of several minerals and vitamins. As a result, insufficient fermentation and increased pyruvate production above the threshold limit were observed in the fermentation with a low concentration of some minerals and vitamins. This increase in the pyruvate concentration resulted in an unbalanced, acidic flavor. Moreover, this tendency was enhanced with increased repitching. In these experiments, potassium and thiamine concentrations were found to have important roles in yeast fermentation performance. To monitor the physiological state of yeast cropped from this fermentation test, we estimated intracellular metabolite concentrations using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS). The CE-TOFMS analysis revealed that the organic acid metabolism of this yeast differed from that of normal yeast cropped from all-malt beer fermentation. It is assumed that this difference in yeast metabolism is the cause of the insufficient fermentation and increased pyruvate production. These data suggest that such nutrients as potassium and thiamine have important effects on fermentability and beer flavor, especially during the production of low-malt beer.

Takeshi Kawakubo graduated in 2009 from the Department of Agriculture at Kyoto University in Japan. He began his career in yeast technology development in the Brewing Technology Development Centre at Kirin Brewery Company, Limited.

48. Bottle conditioning of beer: Strategies to improve yeast refermentation performance. Presenter: Tinne Dekoninck, Catholic University of Leuven, Heverlee, Belgium. Co-author(s): Filip Delvaux and Freddy Delvaux, Catholic University of Leuven, Heverlee, Belgium.

Beer refermentation, i.e., bottle conditioning, is a frequently used technique among breweries in Belgium, the United Kingdom, and the United States. To achieve a secondary fermentation in the bottle, mature beer is inoculated with yeast and fermentable extract, whereupon it is refermented in preferably less than two weeks. Bottle conditioning results in fully saturated beer with an enriched flavor perception and prolonged flavor stability. Since export and consumption of bottle conditioned beers still increases, it is of major economic importance that constant product quality can be assured. Although beer refermentation seems uncomplicated, the process faces important pitfalls presumably because of yeast stress. Indeed, beer is far from an excellent fermentation medium since it differs from an ideal wort medium in its alcohol and carbon dioxide content and low nutrient availability. To improve the process of bottle conditioning, several perspectives can be considered. In a first experiment, the refermentability of several Belgian beers (both lager and ale types) was investigated to reveal the impact of beer related parameters on refermentation. A striking finding was a strong influence of initial beer alcohol levels on refermentation performance, especially when a less ethanol tolerant yeast strain was used. To improve the refermentation performance of different yeast strains, a promising strategy could, therefore, be the adaptation of yeast to alcohol, prior to beer inoculation. In a second experiment, yeast was propagated both in a dynamic and static way, with variable extract and alcohol levels. Throughout propagation and refermentation, important yeast physiological parameters were monitored, such as viability, glycogen and trehalose content, fatty acid and ergosterol levels, as well as the expression of (stress related) genes. These analyses revealed physiological differences between statically and dynamically propagated yeast, as well as between alcohol conditioned and reference yeast populations. Our findings indicate that the use of appropriate conditioning of yeast provides promising opportunities to increase yeast refermentation performance during bottle conditioning of beer.

Tinne Dekoninck graduated in 2008 as a bio-engineer in chemistry (food technology) from the Catholic University of Leuven. For her M.S. thesis, she joined the Centre for Malting and Brewing Science to study the feasibility of high cell-density brewery fermentations. After graduation, she obtained a grant from the Institute for the Promotion of Innovation Through Science and Technology in Flanders (IWT) and started a Ph.D. program at the Centre for Malting and Brewing Science, under the supervision of Freddy Delvaux. Her research focuses on the impact of yeast physiology on bottle conditioning of beer.

49. Genetic roots of lager-brewing yeast: *Saccharomyces eubayanus* and the Patagonian hypothesis. Presenter: Diego Libkind, INIBIOMA, Bariloche, Argentina.

The lager-brewing yeast (*Saccharomyces pastorianus*) is a domesticated microbe arising through the hybridization between *S. cerevisiae* (ale yeast) and a cryotolerant *Saccharomyces* relative as a result of cold brewing practices that Bavarian brewers made famous in the 15th century. Despite being avail-

able as pure culture since the late 1800s, its hybrid genetic nature was only discovered a few decades ago. So far, all the industrial or wild strains isolated of cold adapted *Saccharomyces* have been discarded as progenitor candidates due to considerable genetic dissimilarities or due to hybrid condition. Recently, a worldwide survey yielded a novel yeast dubbed *S. eubayanus* from Patagonian native forests of Argentina that was shown to be the closest known match (99.5%) to the non-ale portion of lager yeasts and, thus, its putative progenitor. Identifying the wild genetic stock of the cryotolerant side of *S. pastorianus* allowed resolution of the hitherto confusing taxonomy of the most relevant brewing yeast and the understanding of key events that led to the domestication of lager yeast. For example, specific genetic changes related to sugar and sulfite metabolism were detected in lager yeasts when compared to *S. eubayanus*. Furthermore, the available information relevant to the discussion on how and when such a half European and half Patagonian yeast hybrid might have been originated will be addressed in this presentation.

Diego Libkind, licenciate in biological sciences (2001); Ph.D. degree, in Biochemistry, Comahue's National University, Bariloche, Argentina (2006); Tucuman National University, Argentina. Researcher of the Argentinean National Council on Science and Technology Research (CONICET); teaching assistant in genetics, Comahue National University. Dedicated to the investigation of yeast biodiversity and biotechnology with special focus on Patagonian natural habitats and with over 50 publications on the field. Member of the ASBC Craft Brewers Committee and of the International Commission on Yeasts.

Technical Session 15: Cleaning and Packaging

50. Keg cleaning and root cause analysis. Presenters: Jeffrey Hutchison, Ecolab, St. Paul, MN, USA; Kenny Gunderman, Summit Brewing Company, St. Paul, MN, USA.

Cleaning kegs is similar to the CIP of a bright beer tank, only faster. Kegs are purged, cleaned, rinsed, sterilized, and filled at simultaneous stations, each cycle typically lasting less than one minute. To ensure beer quality in the keg, it is critical to be able to verify that the keg cleaning program is functional and consistent. Additionally, the keg cleaning operation must be cost-effective. This includes chemical selection, chemical concentration control, chemical monitoring, and proper programming for the cleaning and rinse functions. Excessive chemical use, including caustic loss, may be due to mechanical or programming issues. This paper will focus on troubleshooting the performance of a keg line and keg cleaning operations. It will discuss some real world examples of issues that can be encountered with keg cleaning and the steps that were used to identify the root cause of the issues. Corrective actions will be reviewed with corresponding field results.

Jeffrey Hutchison received degrees in both chemistry and mathematics from St. Olaf College in Northfield, MN. He began his career with Ecolab in 2007, working in the Food and Beverage Division. Since 2010 Jeff has worked as a senior chemist in the Global Lubricant Development group within Ecolab's Food and Beverage Division. Jeff has been able to work both at the lab bench and in field applications, developing and deploying global conveyor lubricant solutions for the food and beverage packaging industries. His work has led to one patent and several patent applications. Jeff has been a member of MBAA since 2011 and enjoys skiing, traveling, and performing music in his free time.

Hailing from rural Iowa, Kenny Gunderman received degrees in both mathematics and philosophy from Macalester College in St. Paul, MN. He has worked at Summit Brewing Company since 1995 as a bottler, racker, brewer, and now packaging manager. When time allows, Kenny enjoys spending time with his wife and two young daughters, as well as pickin' on an old banjo and playing the odd round of golf here and there.

51. Conveyor lubricant for stainless steel chains that saves water. Presenter: Chad Thompson, Ecolab, USA. Co-author(s): Don Rich, New Belgium Brewing, USA.

Conveyor lubricants designed to save lubricant dilution water have become known as "dry lubricants." Over the last 10 years the industry has seen the successful development of dry lubricants for plastic conveyor chains, and the use of these dry lubricants has grown substantially in that application. Stainless steel conveyors, however, present unique challenges for dry lubricant application, including persistence to chain material, cleanliness, microbiological growth, and cost-effectiveness. This presentation will discuss the development process of dry lubricants for steel and how these hurdles are addressed. It will discuss the laboratory and field testing, data collection, test method development, and new analytical methods. The presentation will also include how one brewery analyzed the dry lubricant offerings in the marketplace and the decision-making process they went through when starting a new product. It will conclude with results (water savings, drier floors, foam level, performance) from an installation at New Belgium Brewing in Fort Collins, Colorado.

Chad Thompson has over 19 years of experience in brewing, and in 2007 joined the Brewery group in the Food & Beverage Division of Ecolab, Inc. His responsibilities include the development and commercialization of conveyor lubrication products. He has been brewing for 14 years and has been with Ecolab for 7 years. During his time at Ecolab he has contributed to numerous business segments within the corporation. Chad is a contributing member to the Master Brewers Association of the Americas (MBAA) and received an Honorable Mention for Best Paper in 2009. He received a degree from Michigan State University in packaging engineering and has been granted three patents for his work.

52. Utilizing ozone: Energy savings in automated CIP sanitization. Presenter: Lars Larson, Trumer Brauerei, Berkeley, CA, USA. Co-author(s): Darren Moser, Trumer Brauerei, Berkeley, CA, USA.

Trumer Brauerei recently installed a system to generate ozone for sanitizing product lines during CIP. Ozone has long been recognized as an effective sanitizer due to its extremely high oxidative power. Typically when used as a sanitizer it is used for surfaces and not in CIP systems. The primary impetus behind the installation was the energy savings provided by the sanitizing method. Trumer was conducting hot water sanitizing on its wort line. With the new method, ozone is created on demand and injected into cold/ambient water circulating through the line. Ozone degrades rapidly at higher temperatures and stays effective longer the colder the water, therefore cold sanitization is desired and most effective. The energy and cost savings are due to the reduction in natural gas consumption. Cold sanitizing would also be possible with chemicals; by using ozone there are the additional benefits of generating only the amount of sanitizer needed on demand, and the reduction of chemical usage, which is beneficial both from handling and cost perspectives. The cost of the installation was subsidized in part through grants from PG&E (the natural gas provider) and from the city of Berkeley, which had funds available through a federal program called Money for Energy Efficiency. This novel approach required an installation with a high degree of precision control of the ozone levels generated, as well as a high level of process automation, so the operator would not be required to spend much time monitoring the process. The system was installed in one area of the brewery and

once proven will be utilized in additional areas, increasing the savings benefit. This presentation will discuss the cost/benefit calculations, design elements of the system, commissioning issues, operation, and results.

Lars Larson studied brewing science at the Technical University of Berlin, Germany, and received a Diplom-Braumeister degree. He has worked in breweries ranging in size from small brewpub to microbrewery to regional, as well as large national, on three continents. Since 2004 he has been master brewer at the Trumer Brauerei in Berkeley, CA.

53. A novel air ingress test method. Presenter: Eric Samp, MillerCoors, Golden, CO, USA. Co-author(s): Eric Maskwa, MillerCoors, Milwaukee, WI, USA; Chaz Benedict, Hach Company, Loveland, CO, USA; Kendal Nichols, MillerCoors, Eden, NC, USA.

Protecting beer from the deleterious effects of oxygen is critical for ensuring flavor stability in packaged beer, thus brewers strive to not only control oxygen pickup during beer processing but also oxygen entrained during packaging operations. However, for non-hermetically sealed containers such as bottles, it is known that oxygen will ingress into the headspace over time. Knowledge of how much oxygen permeates across the closure could provide brewers with the ability to detect problems with crowner operations or identify other issues associated with closure applications, yet today methods are scarce or limited for breweries to utilize. Laboratory based methods do exist but are limited in that evaluations cannot be done on production scale samples; therefore, brewers cannot troubleshoot issues on their own crowners/cappers or evaluate alternatives to cap/crown modifications with their own fillers under normal operating conditions. We have developed a novel technique that can be employed on fillers under normal operating conditions. The test method can be carried out with any package oxygen analyzer and provides relative results to the amount of oxygen that permeates into the container. This paper will discuss the method and review some case studies illustrating the impact of process changes on air ingress results. The case studies will include 1) evaluation of oxygen scavenging liners, 2) optimization of top-load force on a closure system, and 3) evaluation of variation between closure elements on a bottle filler. The results from these studies agree with the anticipated outcomes, thus proving the method produces results that are meaningful for breweries.

Eric Samp is a quality engineer/senior statistician for MillerCoors working in the Corporate Quality Organization. He holds a Ph.D. degree in applied statistics from the University of Northern Colorado and an M.S. degree in brewing and distilling from Heriot Watt University. Eric is a CQE and CQM from the American Society for Quality and a diploma brewer from the IBD. He is also a certified Six Sigma Master Black Belt and serves on the MBAA Technical Quarterly and JIB editorial review boards. Eric is also a recipient of the ASBC Eric Kneen Memorial Award (2001 and 2011).

Technical Session 16: Yeast III

54. Observation of flocculation protein during propagation of brewing yeasts. Presenter: Kei Asada, Sapporo Breweries Ltd., Yaizu, Japan. Co-author(s): Ryouichi Fukuda and Akinori Ohta, University of Tokyo, Tokyo, Japan; Masahide Sato and Tatsuro Shigyo, Sapporo Breweries Ltd., Yaizu, Japan.

The brewer's yeast genome encodes a "Flo" flocculin family responsible for flocculation. Controlled floc formation or flocculation at the end of fermentation is of great importance in the brewing industry since it is a cost-effective and environmentally friendly technique for separating yeast cells from the final beer. Yeast flocculation is a very complex process that depends on the expression of specific flocculation genes such as *FLO1*, *FLO5*, *FLO8*, *FLO11*, and Lg-*FLO1*. Among these genes, Lg-*FLO1* is the most effective gene for brewing beer because Lg-Flo1 protein recognizes not only mannose but also glucose and maltose, and it contributes to flocculation at the end of fermentation. For this reason, brewers want to understand the behavior of Lg-Flo1 protein during fermentation. In this study, we report the localization of Lg-Flo1 protein during the propagation of genetically modified bottom fermenting yeasts (*Saccharomyces pastorianus* W34/70). We used *E. coli* as a host for DNA cloning, and we subcloned the upper region of the Lg-*FLO1* promoter, Lg-*FLO1* promoter domain, and Lg-*FLO1* N terminal domain. Subsequently, we ligated the upper region of the Lg-*FLO1* promoter, drug resistance gene, Lg-*FLO1* promoter domain, *EGFP* gene, and Lg-*FLO1* N terminal domain in that order. We transformed this fragment into wild-type bottom fermenting yeast and screened the cells by drug resistance and obtained recombinant strain (*EGFP*-Lg-*FLO1*). We verified that this recombination occurred correctly in the specific location using the colony PCR method. Finally, we observed the localization of the protein with a fluorescence microscope. As a result, we found that the EGfp-Lg-Flo1 protein was localized in the cell wall.

Kei Asada received a master's degree from the Graduate School of Biostudies, Kyoto University, Japan. He began employment with Sapporo Breweries Ltd. in 2008 as a microbiologist in the Frontier Laboratory of Value Creation. From April 2011 to March 2012, he studied the flocculation of bottom fermenting yeast as a researcher in the Department of Biotechnology, University of Tokyo.

55. The effect on fermentation by-products of the amino acids in wort. Presenter: Takuya Hashimoto, Suntory Liquors Limited, Osaka, Japan. Co-author(s): Taichi Maruhashi, Yutaka Yamaguchi, Yoshinori Hida, and Kaneo Oka, Suntory Liquors Limited, Osaka, Japan.

Amino acids in the wort, measured as free amino nitrogen (FAN), are extracted during the mashing process and are essential nutrients for sufficient fermentation performance. Too low a level of amino acids causes incomplete fermentation due to insufficient nutrition of the yeast, but too high a level remaining in the finished beer may negatively affect sensory variables such as foam quality. Therefore, for good fermentation, it is necessary to ensure the appropriate quantity of amino acids in the wort. In this study, we investigated the influence of fermentation conditions that differed in the amount of amino acids in the wort with most variables held constant. We made the wort at 12.0°P with 115, 160, and 230 mg/L of FAN and fermented. This showed that the more FAN in the wort, the more isoamyl acetate was produced. We analyzed the relationship between the quantity of this ester and the amount of the amino acids valine and leucine, which are biosynthetic precursors of isoamyl acetate. As expected, in the wort with low FAN content, valine and leucine were depleted during fermentation, inhibiting the formation of isoamyl acetate. The uptake of amino acids involved in yeast metabolism during fermentation is also connected with the generation of sulfur compounds, so the quantity of FAN also correlated with the quantities of hydrogen sulfide (H_2S) and sulfur dioxide (SO_2). Thus, the FAN utilized by the yeast during fermentation was confirmed to be an important factor determining the quality of the beer. In conclusion, in addition to being an essential yeast nutrient for fermentation, FAN in the wort is involved in the biosynthesis of a number of fermentation by-products. In particular, we found that the quantity of isoamyl acetate can be controlled.

This suggests that the adjustment of FAN is a factor that can control the flavor of the beer.

Takuya Hashimoto graduated with a master's of engineering degree from Osaka University in 2009. He has worked for three years in the Beer Development Department of Suntory Liquors Limited.

56. Standardized fermentation parameter for probiotic and non-probiotic lactic acid bacteria in barley malt wort. Presenter: Martin Zarnkow, TU München, Germany. Co-author(s): Thomas Becker, TU München, Germany.

The goal of this study was to find a normative parameter for the growth of lactic acid bacteria in barley malt wort, probiotic or not. Four bacteria (*Lactobacillus brevis*, *L. casei*, *L. perolens*, and *Leuconostoc lactis*) and five well-established probiotic bacteria of the food industry (*L. rhamnosus*, *L. acidophilus*, *L. casei*, *Streptococcus thermophilus*, and *Bifidobacterium lactis*) were selected. Out of three different initial cell counts, three different pH values, and three different incubation temperatures, the most appropriate parameter set to the growth should be found. With this set, a general statement should be made, which can be accepted for a multitude of probiotic lactic acid bacteria in barley malt wort as substrate. The pH value, absorbance at 600 nm, and percentage amount of lactate were measured daily for this experiment. At the first and last day of this test run, the concentration of the extract and the alcohol concentration were determined. The face centered design of response surface methodology and the analysis resulted from the software Stat Ease Design Expert. The absolute growth, difference in absorbance between the end and beginning of the experiment, pH value, and amount of lactate at the end of the experiment were analyzed. In four out of five tested strains, the pH value of the substrate had the highest impact on growth. A pH value of 5.6 caused maximum growth behavior of these strains, although the amplitude was quite different. The determined growth ranged between a doubled and a 25-fold growth. In four out of five strains, the percentage amount of lactate was dependent on the pH value of the substrate as well. The highest amount of lactate was produced between a pH value of 4.8 and 5.6. The averaged amount of lactate was between 0.07 and 0.38%. The decline in pH was conspicuous for all of the strains. Predominately, a pH value below 4.0 was reached at the end of the experiment. The consumption of the extract was very different. Between 0.21 to 6.12% of the extract was fermented by the end. Because of the differing results, a normative procedure for these lactic acid bacteria is not easy to determine. Thus, a compromise has to be made that is close to the optimum but that cannot be the optimum for all probiotic and non-probiotic strains.

Martin Zarnkow apprenticed as a brewer and maltster from 1989 to 1991 at a small brewery in Frankonia. Finished a Diplom-Ingenieur (FH) degree, option brewing technology, in 1996 at TU München, Weihenstephan. Worked as a brewmaster for one year in a medium-sized brewery in Germany. Since 1997 Martin has been head of the research group for brewing and beverage technologies and microbiology at the Lehrstuhl für Brau-und Getränketechnologie (Institute for Beer and Beverage Technology) at TU München in Weihenstephan. Finished his external Ph.D. research in 2010 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."

57. Mechanism of suppression of pyruvate and acetolactate formation by use of yeast of modified mitochondrial transportation system. Presenter: Hiroshi Kitagaki, National Saga University, Japan.

Residual pyruvate and acetolactate during alcoholic fermentation leads to synthesis of off-flavor diacetyl. Therefore, suppression of these substances during alcoholic fermentation is desirable. In order to circumvent this problem, we came up with the idea of modifying the mitochondrial transportation system, fortifying the transportation of pyruvate from the cytosol to the mitochondria, and decreasing the amount of pyruvate and acetolactate. To accomplish this, we isolated mutants of *sake* yeast resistant to ethyl alpha-*trans*-cyanocinnamate, an inhibitor of mitochondrial pyruvate transport. The brewery yeast of *sake*, the Japanese traditional rice wine, was used as the parent strain. This strain indeed exhibited a decreased amount of pyruvate and acetolactate during *sake* brewing on a factory scale. This was the first success of development of a brewery yeast that produces a decreased amount of pyruvate and acetolactate without deteriorated fermentation ability. However, although we supposed that pyruvate transportation from the cytosol to the mitochondria during alcoholic fermentation lowered the pyruvate content, its mechanism had not been elucidated. Therefore, we constructed *sake* yeasts that overexpress various mitochondrial transporters and investigated the resistance of the strains to ethyl alpha-*trans*-cyanocinnamate. As a result, the strain overexpressing the mitochondrial ATP/ADP translocator gene *AAC1*, exhibited resistance to ethyl alpha-*trans*-cyanocinnamate. This strain also exhibited a low pyruvate-producing ability during *sake* brewing. These results suggest that transportation of ATP from the cytosol to the mitochondria enhances pyruvate turnover within mitochondria during alcoholic fermentation. It can also be inferred that since an electron transport system using molecular oxygen does not occur during alcoholic fermentation, ATP within mitochondria is depleted, and mitochondria need to import ATP from the cytosol, where ATP is synthesize through glycolysis. This research is the first to propose a role of ATP transport from the cytosol to the mitochondria to modify fermentation characteristics and suggest a novel strategy for developing brewery yeasts that produce decreased amounts of pyruvate and acetolactate.

Hiroshi Kitagaki received a Ph.D. degree from the University of Tokyo. He began employment as a brewing analyst at the National Taxation Bureau in 1995. He moved to the National Research Institute of Brewing as a researcher in 2001. He worked as a visiting researcher at the Medical University of South Carolina from 2005 to 2006. He is now an associate professor at the National Saga University. He is the president of the Symbiotic Microbial Fermentation Engineering Forum and has been selected as an associate member of the Science Council of Japan and a program officer of the Ministry of Education, Culture, Sports, Science and Technology, Japan. He has received the Young Scientists' Award in the Commendation of Science and Technology from the Minister of Education, Culture, Sports, Science and Technology, Japan; The Foundation of Agricultural Sciences of Japan; and The Society for Biotechnology, Japan.

Technical Session 17: Mashing

58. About the influence of different mashing methods on the beer quality of classical beer styles. Presenter: Jens Voigt, Technische Universität München, Weihenstephan, Germany. Co-author(s): Andreas Richter and Thomas Kraus-Weyermann, Weyermann Specialty Malts, Bamberg, Germany.

The goal of this work was to show the effects of mashing procedures on the process performance and resulting quality of beers. Three different, classic beer styles (German pilsner, American pale ale, and Bavarian dunkel) were brewed in a 250 L pilot scale. Each beer style was produced in three variants using different mashing regimes: single step infusion, multi-step infusion, and decoction The recipes for the malt bill, fermentation procedure, and storage parameters were identical.

Each of the three trial beers were compared in terms of analytical and sensory parameters to explore the influence of mashing regime. While the differences in sensory analyses were not too large, the paper shows that choosing simpler mashing procedures still resulted in very acceptable qualities and characteristic sensory values. The paper reports that not only the originally preferred methods of brewing led to high quality products, but alternative mashing regimes also provided good options, especially if the brewing process equipment only allowed limited possibilities.

Jens Voigt received a diploma engineer (M.S.) degree in brewing and beverage technology from TU München-Weihenstephan, Germany, in 1985. He started his career with A. Steinecker GmbH, Freising, as a technical engineer in brewhouse, fermentation, and filtration equipment. He held sales, product, and manager positions with Steinecker until 1995. From 1988 until 1992 he worked on his doctorate in the brewing technology of beer foam from Weihenstephan (Prof. Dr. Narziß). In 1996 he joined Doemens Brewing School in Munich, Germany, as managing director. In late 1997 he joined Heinrich Huppmann GmbH, Kitzingen, Germany, as key account manager for brewery equipment and was managing director of brewmaxx, a supplier of software solutions for the brewing industry. Since early 2004 he has been a research associate with Karl Sommer (mechanical engineering and process technology) at the WZW (Wissenschaftszentrum Weihenstephan [Center of Life Science, Technische Universität München-Weihenstephan]) working on brewing and beverage process technology issues. He is a member of MBAA and IBD, the editorial board of the Journal of the Institute of Brewing and MBAA Technical Quarterly. He is a publicly certified expert in brewing and beverage technology.

59. Mashing without primary energy—The path to an autarchic brewery. Presenter: Peter Gattermeyer, Krones AG, Freising, Germany.

Brewing beer is an exceptionally energy-intensive process, and the brewhouse accounts for about a third of the energy used, made available as heat in the form of steam or high-pressure hot water and in the form of electricity for driving pumps and motors. Increasing priority is being accorded to downsizing the operating costs involved by significantly reducing the consumption of energy and media. In view of the lengthy lifetimes now being achieved by plant components, continually rising energy prices are compelling breweries to look far into the future when planning their investments. Moreover, social pressure and legislation relating to climate change will mean that new installations have to be chosen with a view to their CO_2 emissions. Today's brewhouses achieve consumption figures of 6.2 kWhr/hL of cast wort cold for thermal energy, 3.0 kWhr/hL for electricity, and 1.4 hL/hL (1.4 US bbl/US bbl) for water. This development status constitutes the yardstick—one where any improvement seemed well-nigh impossible. The autarchic brewery, then, was thought to lie far off in the future. Thanks to a revolutionary design enhancement, the consumption of primary energy in the brewhouse, however, has been reduced by approximately another 30%. To achieve this, the production of superfluous hot water at the wort cooler is avoided, and instead, energy is recovered at a high temperature level in an additional heat exchanger stage. A pressure, less stratified, storage tank is used as a buffer between the energy source and the energy sink. This energy, in the form of hot water, suffices for heating the entire mashing process. The heating medium temperature here is only slightly higher that that the mash being heated. But, the requisite heat-up rates are achieved nonetheless. This is made possible by the specially shaped heating surfaces, which as pillow plates feature a cushion-shaped surface on the product side. This means that in mash kettles heat transfer efficacies hitherto unprecedented are being achieved, up to 100% above those of conventional heating surfaces. This invention enables alternative forms of energy to be efficiently deployed at a temperature level of <95°C (203°F). The very low heating medium temperature creates not only energy-economy gains, but technological advantages as well. Fouling in the mash kettle is avoided entirely, and the natural enzymes are treated gently. The thermal stress on the mash has been significantly reduced. Thanks to the reduced steam consumption in the brewhouse, new installations in particular can be designed with a smaller steam boiler and smaller steam and condensate valves. Now that less cooling output is required in the conventional stages of the wort cooling process, less iced water is needed too, which means that less electricity is consumed in producing it. This energy recovery system brings CO_2-neutral, media-friendly beer production within feasible reach.

Peter Gattermeyer graduated in 1996 as an engineer for brewing and beverages at TU Munich-Weihenstephan. After that he started his career at Steinecker. In 2003 he became head of the Brewhouse Technology Section, and in 2008 he was appointed head of the Technology Center in Krones' Process Technology Division. In this position he is responsible for commissioning and developments in beverage technology. He has published scientific articles and is member of various committees.

60. Monitoring of the mashing process by viscosity measurements. Presenter: Simon Henke, TU München, Chair of Process Engineering of Disperse Systems, Weihenstephan, Germany. Co-author(s): Jens Voigt and Karl Sommer, TU München, Chair of Process Engineering of Disperse Systems, Weihenstephan, Germany.

During mashing the conversion of starch to fermentable sugars is the most important result. Over the last decades brewhouse technology and malt quality have improved substantially, so mashing time is very short. Nevertheless the success of mashing is controlled mainly after the mashing procedure by an iodine test or laboratory analysis of the Congress wort. A proper in-line measurement hasn't been established. A procedural approach gives the opportunity to control the mashing procedure in-line. The measurement parameter of the presented method is the viscosity of the mash suspension. The viscosity is a sensitive parameter that shows changes in the fluid phase of the mash as well as in the disperse phase of the mash. A torque measurement of the agitator in the mash tun provides the data to calculate suspension viscosity. With this measurement and further knowledge about the performance characteristic of the agitator, the development of mash conversion is detectable. A thoroughly developed performance characteristic is independent of the test suspension and only has to be acquired once. This work gives detailed information about the experimental way to set up the required power characteristic and the resulting viscosity calculation. The presented procedure is possible for every agitator system independent of its scale. Different experiments were conducted varying the grinding parameters and water/grist ratio. The influence of these parameters was monitored by the viscosity measurements and is presented in this work. The gelatinization point, as well as the saccharification of the mash, were detectable. So, the measurement technique offers an easy way to better understand and control mashing procedures.

Simon Henke graduated from Technical University Munich in 2009 with an engineering degree in brewing sciences and beverage technology. In 2010 he started his work at the Chair of Process Engineering of Disperse Systems, TU Munich, as a research associate. His

fields of activity are mass transport phenomena and procedural aspects of the mashing process. He is responsible for the pilot plant brewery at the Chair of Process Engineering.

Technical Session 18: Microbiology II

36. Comparative genomics enables a genetic barcode to discriminate and score beer-spoiling and non-spoiling *Lactobacillus brevis.* Presenter: Rudi Vogel, Technische Universität München, Freising, Germany. Co-author(s): Patrick Preissler, Angel Angelov, and Wolfgang Liebl, Technische Universität München, Freising, Germany.

Beer is an uncomfortable environment for many bacteria. Nevertheless, specific bacteria, mainly lactic acid bacteria, are able to grow in beer and spoil it. In this group of bacteria, *Lactobacillus brevis* is the most common beer spoiler found in breweries. Within the large biodiversity of this genus, different ecotypes exist, some of which exhibit stress responses enabling survival under the antimicrobial conditions in beer. Thereby, the tolerance to hop compounds, which are mainly responsible for inhibition of growth in beer, is a multifactorial process. For this reason, any approaches to predicting the physiological differences between beer-spoiling and non-spoiling strains on the basis of a single marker gene are limited. In addition, most known genetic determinants that are potentially useful for PCR detection of beer-spoilage bacteria are widely spread in strains with no reference to high hop tolerance. Comprehensive and strain specific information about the ecotype beer spoiler compared with non-spoiler strains reside in their genomes. In this study we aimed to identify genes related to the ability to grow in stronger hopped beers (e.g., pilsner beer) via comparative genomics of four different strains of *L. brevis*. The genomes of two beer isolates (*L. brevis* TMW 1.313 and 1.465) and one strain isolated from feces (*L. brevis* TMW 1.6T) were determined by next generation pyrosequencing. A fourth sequence of a published genome (*L. brevis* ATCC 376, silage isolate) was included in the genome comparison. Redundant information, which resides in the core genome of all *L. brevis* ecotypes or strain-specific sequences were removed, and gene fragments exclusively occurring in beer-spoiling strains were identified, as well as ecotype-specific DNA sequences of non-spoiling strains. Subsequently, targeted arrays derived from these sequences were established and hybridized with DNA from a bigger set of different *L. brevis* strains to identify discriminative marker sequences for the ecotype "beer-spoiler" or "non-spoiler." As a result, 34 oligonucleotides could be identified that are able to differentiate the ecotype "beer-spoiler" and are useful for predicting beer-spoiling potential. Furthermore, four oligonucleotides specific for the ecotype "non-spoiler" were found that can be used as negative markers for beer-spoiling strains. The cumulative detection of more than one of these marker sequences to a score enables the establishment of a genetic barcode that can be used by brewers to predict the beer-spoiling potential of *L. brevis* isolates. For practical applications, a multiplex PCR targeted toward a further reduced set of selected marker sequences proved effective.

Rudi F. Vogel is a biochemist interested in food microbiology and biotechnology. As head of Technische Mikrobiologie at the Technische Universität München he conducts research on starter culture development, high pressure in food, and biosciences, as well as control of unwanted microbes in food. A clear focus is on lactic acid bacteria, their metabolism and genetics, pre- and probiotic functionality, and mechanisms of stress response and adaptation. In this context beer-spoiling lactobacilli are used as models to understand molecular mechanisms of hop resistance.

63. Investigating the possibility to control brewery biofilms by inhibiting quorum sensing. Presenter: Erna Storgårds, VTT Technical Research Centre of Finland, Finland. Co-author(s): Outi Priha and Riikka Juvonen, VTT Technical Research Centre of Finland, Finland; Kaisa Tapani, Sinebrychoff, Finland.

Bacteria are no longer regarded as undifferentiated cells focused on multiplication. Cell-to-cell signaling, known as quorum sensing, is common both within and between bacterial species. Quorum sensing has also been shown to participate in biofilm formation. Because quorum sensing is not involved in bacterial growth, inhibition of signaling provides a potential means to control microbial biofilms without development of resistance. A multitude of compounds that inhibit quorum sensing signaling in bacteria have been found, many of them non-toxic secondary metabolites of fungi, plants, or algae. This study investigates the possibilities to control brewery biofilms by inhibiting quorum sensing signaling in bacteria, and it is part of a larger project aiming at reducing microbial attachment on brewery surfaces using novel methods. Several signaling molecule groups exist among bacteria. Acyl homoserine lactones (AHLs) are produced solely by Gram-negative bacteria, whereas autoinducer-2 (AI-2) molecules are produced and detected by both Gram-positive and Gram-negative bacteria. AI-2 is described as the universal signaling molecule for interspecies communication. Signaling molecules are produced and active in very low concentrations, which is why they are generally detected by bioassays. Production of AI-2 was detected from bacteria isolated from brewery filling machinery surfaces by measuring changes in the bioluminescence of *Vibrio harveyi* BB170, a reporter bacterium. Altogether 9 out of 20 screened strains produced AI-2. The biofilm formation ability of isolates producing AI-2, or isolates previously found to produce AHLs, was screened with a microtiter plate crystal violet assay. Fourteen strains had significant biofilm formation capability. In summary, 11 strains both produced AHL or AI-2 signaling molecules and had biofilm formation capability. Subsequently, inhibition of quorum sensing signaling with arctic berry extracts, resiniferous extracts of conifer trees, and hop extracts were studied with reporter bacteria and microtiter plate assays. Two hop extracts inhibited AI-2 mediated quorum sensing, but also the growth of the reporter bacterium at concentrations ranging from 1 to 10 mg L^{-1}. The principle of quorum sensing inhibitors is that they should only affect the signaling of bacteria, not their growth. Studies on the effect of berry extracts and resiniferous extracts on the detection of signaling molecules by bacteria and on their biofilm formation capability are ongoing and will be reported. This work demonstrates that AHL- and AI-2-producing bacteria are common on brewery process surfaces, and quorum sensing inhibitors could be potential means to control them. The objective is to find compounds that are suitable for incorporation into functional coating materials in brewery production plants. Incorporation of quorum sensing inhibitors into washing chemicals would be another option. Quorum sensing inhibitors have also been found to have synergistic effects with existing biocides. Eventually the breweries could employ quorum sensing inhibitors as part of novel synergistic means to control production hygiene in a sustainable and efficient way.

Erna Storgårds holds a Ph.D. degree in microbiology from Helsinki University. She joined the VTT Technical Research Centre of Finland in 1988. From 1988 to 2007 she worked with brewery microbiology and process hygiene, first as a research scientist or senior scientist, later as group manager and team leader. In 2008 she took over re-

sponsibility for the VTT Culture Collection; in addition to that, she also takes part in projects in her field of expertise. She has been a member of the EBC Microbiology Group, later the EBC Brewing Science Group, since 1992 and its chair (2004–2008); chair of the EBC Microbial Contaminants Subgroup (1993–2004); and a member of the Microbiology Subcommittee of the EBC Analysis Committee (1998–2008). She has been a member of ASBC since 2004.

175. Quantitative evaluation of biofilm composition using real-time PCR. Presenter: Robert Riedl, Research Center Weihenstephan for Brewing and Food Quality, TU Muenchen, Freising, Germany. Co-author(s): Jennifer Koob, Mathias Hutzler, and Fritz Jacob, Research Center Weihenstephan for Brewing and Food Quality, TU Muenchen, Freising, Germany.

Biofilms are a serious problem in breweries and beverage bottling plants. Biofilms are associations of various species of bacteria, yeasts, and molds. In contrast to planktonic microorganisms, a layer of extracellular substances protects the cells in biofilms, which makes them much more resistant against cleaning and disinfection solutions. Most biofilm starter organisms, such as acetic acid bacteria (AAB) or *Enterobacteriaceae*, are considered to not be product spoiling. For this reason, most breweries do not use cultivation media that are designed to detect them. Therefore a biofilm will not be detected until product spoiling organisms colonize it. Additionally, established cultivation media methods such as the NBB-B-AM swab test, according to Prof. Back (1994), do not specify the associated organisms. The composition of the associated organisms is very important for evaluation of the level of maturity and potential product spoiling risk of biofilms in breweries or beverage plants. The rather long incubation time of 5–14 days for nutrition media tests is another disadvantage. With molecular biological screenings, the cultivation time can be reduced to 3 days using real time-PCR systems to detect different target fractions of microorganisms. Most commercial real time-PCR kits, established in brewing microbiology, focus on the detection of beer spoiling bacteria. In this study a modular PCR-screening assay was designed and evaluated to detect a wide spectrum of bacteria and yeasts involved in the growth of biofilms. The first screening step detects product specific, defined groups of organisms that can be used as indicator organism groups for the state of maturity in the biofilm development. The second step identifies the organisms within the groups. The identified organisms were linked with data about the organisms, containing metabolic products, product risk, and typical locations. Maturity, as well as the potential for product spoiling of the biofilm, can be measured by typical indicator organisms detected using the real time-PCR screening system.

Robert Riedl was born in 1983 in Munich. He studied brewing and beverage technology at the Technische Universität München and graduated with a Dipl.-Ing. degree in 2011. Since July 2011 he has been a scientific assistant at the Research Center Weihenstephan for Brewing and Food Technology and is working on biofilm development in beverage plants.

Technical Session 19: Outside the Box

65. Putting science to work in the brewery. Presenter: Alastair Pringle, Pringle-Scott LLC, St. Louis, MO, USA. Co-author(s): Anthony Cutaia, Science Source Consulting LLC, St. Louis MO, USA.

There are two philosophies for obtaining knowledge: deductive thinking and inductive thinking. In deductive thinking knowledge is reasoned from existing facts without the inconvenience of having to design experiments or make measurements. However, in inductive thinking abstract thought and reasoning are supported by real world findings. Inductive thinking is the basis for the scientific method that we know today. In applying the scientific method to brewing there are several pitfalls that need to be considered, including imprecise measurements, raw material variability, process variability, etc. In this paper we will discuss a step-by-step process to reliably gain knowledge. The initial steps include selecting suitable small scale experimental systems, gathering variables, and testing variables in screening experiments. Once the most important variables have been identified, then the interactions can be explored using such techniques as response surface methodology in further lab scale experiments. Finally the effects of the most important variables can be confirmed in a pilot plant and at full scale. Evolutionary operations (EVOP) protocols are an effective way to test two variables while eliminating the inherent noise of production brewing.

Alastair Pringle was educated in England, where he earned undergraduate and graduate degrees in microbiology. He joined Anheuser-Busch in 1984 following five years of post-doctoral research in the United States. At Anheuser Busch Alastair held a number of technical management positions, including director of brewing research, where his responsibilities included all aspects of the brewing process. He is currently the principal consultant at Pringle-Scott LLC, a science-based consulting company that works with craft breweries on process control and quality. In addition, Alastair teaches microbiology at Maryville University in St. Louis, MO, and is a member of the IBD Board of Examiners.

66. Oat: Substrate for malted cereal fermented beverages. Presenter: Alicia Muñoz Insa, Lehrstuhl für Brau- und Getränketechnologie, Technische Universität München, Freising, Germany. Co-author(s): Martina Gastl, Martin Zarnkow, and Thomas Becker, Lehrstuhl für Brau- und Getränketechnologie, Technische Universität München, Freising, Germany.

Fermentation of food is a worldwide technique applied for preservation of a wide range of raw materials. It also provides a natural way to increase the nutritive value and appearance of the raw material and leads to a general enhancement in the shelf-life, taste, and aroma of the final product by destroying undesirable components. During cereal fermentation with lactic acid bacteria, synthesis or degradation of some components, such as phytine or vitamins, and increase in digestibility provides higher nutritional quality to the final product. Also the formation and removal of several volatile compounds contributes to acceptability. In recent years, interest in oat as a food ingredient for beverage production has increased due to its high dietary fiber content and health benefits. Oat soluble dietary fibers are not digested in the human intestine and pass through to the colon, where they are available as prebiotic materials for microbial fermentation. Moreover oat exerts antioxidant activity. But, compared to other foods such as milk, cereals, and its by-products it sometimes has an inferior or poor nutritional value and sensory properties. Although oat is a suitable substrate for fermentation, through malting and mashing the availability of some components, acceptability, and nutritional value, not only for lactic acid bacteria (or yeast) but also for the human body, increases. This project attempted to produce high quality wort from malted oat to ensure good fermentation and to increase the availability, acceptability, and nutritional value of the final beverage. The influence during malting, mashing, and fermenting parameters on final product quality was investigated. Finally, regimes tailored to the physical and chemical properties of oat are proposed. Every criteria was

based on malting and brewing standard values. At the final stage of this research, four LAB species were selected to independently ferment oat wort. As a result, four different beverages with different taste profiles were developed. Generally the produced novel beverages were characterized as sour refreshments.

Alicia Muñoz Insa studied at the Technical University of Madrid, Spain, and carried out her diploma thesis at the Technical University of Munich. In 2009 she began working as a Ph.D. student at the Institute of Brewing and Beverage Technology (TUM) under the supervision of Thomas Becker. Her research is currently focused on field alternative cereal-based beverages.

124. Energy conservation decisions germane to the small brewery. Presenter: Jaime Jurado, Susquehanna Brewing Company, Pittston, PA, USA.

America's craft brewing heritage is enhanced by breweries investing in technical solutions to improve their environmental stewardship. The largest craft breweries may enjoy some advantages due to scale, as do large national breweries, but much can be done in small craft breweries using traditional tools of cost-benefit and payback evaluation. Focusing on possible energy decisions for the small artisanal brewery, an exploration of the choices for a new brewery versus a brewery evaluating a change or retrofit. Areas discussed include decisions addressing steam production and consumption, compressed air, refrigeration, cleaning and sanitation, microbiological beer stability, and packaging. Sample calculations are presented for a brewery initially built for 17,500 bbl/year but with a second phase of 40,000 bbl/year and final phase of 100,000 bbl/year.

Jaime Jurado is masterbrewer/operations partner for Susquehanna Brewing Company. His engineering degrees are in chemical and electrical engineering at the undergraduate and graduate levels; he also did additional post-graduate work in medical engineering. He was educated in brewing as a Praktikant in the Bavarian breweries of Patrizier-Brau AG under the guidance of Peter Hellich and U. Ost and has been in professional brewing since 1983. Jaime served as director of brewing operations at The Gambrinus Company breweries for nearly 15 years until the end of 2011 and was at The Stroh Brewery Company prior to Gambrinus. He is a senior member of the AIChE, past chair of a 440-strong ACS section, and past president of MBAA.

Technical Session 20: Finishing and Stability

67. Analysis of the control factor concerning beer filterability and establishment of the method for controlling filterability. Presenter: Tomoyuki Nakahama, Suntory Liquors Ltd., Ohra-gun, Japan. Co-author(s): Seisuke Takaoka, and Haruyoshi Sotome, Suntory Liquors Ltd., Ohra-gun, Japan.

Beer filterability is one of the most important indicators concerning productivity, cost, and quality for most breweries. Although analysis for improving beer filterability has been carried out for a long time, the technique for completely controlling beer filterability has not yet been established. Thus, we have analyzed the factors concerning beer filterability in our brewery looking for the critical control factor. As a result of a long-term investigation, we have finally found that the critical factor is the amount of micro-particles (under 3 μm in diameter) in lager beer. If other factors, for example beta-glucan, etc., are controlled, beer filterability can be explained by the amount of micro-particles. In the analysis of the behavior of micro-particles in the brewing process, normally micro-particles are decreased during cooling to –1.0°C after VDK rast, but in the case of bad filterability, most micro-particles did not decrease at all. Furthermore, from the result of component analysis, it was found that the micro-particles consisted of not only beta-glucan but also protein. In order to maximize filterability we designed the malt recipe and mashing conditions considering both cytolysis and proteolysis. We also constructed the filterability prediction technique from the amount of micro-particles by utilizing the advantage of the simple and rapid measurement method. Using the predictive results, filterability can be stabilized by optimizing the filtration design so it contributes to the improvement of productivity and the reduction of production costs.

Tomoyuki Nakahama is a senior assistant brewmaster in the Suntory Tonegawa brewery. The main subject of his work is development of brewing technology. He graduated from Tokyo University with an M.S. degree in agricultural chemistry, and he was engaged in functional analysis of unique genes and proteins (sPLA2s) in a Japanese traditional mold, A. oryzae. He joined Suntory Ltd. in 2006. He now works on improvement of the filtration process, especially for the optimization of brewing conditions.

68. The foaming properties of pale and specialty malts. Presenter: Alexander Combe, University of California, Davis, CA, USA. Co-author(s): Charles Bamforth, University of California, Davis, CA, USA.

Dogma holds that the use of certain specialty malts is to the advantage of beer foam stability. A diversity of such materials have been investigated for their foam stabilizing capabilities compared to reference pale malts. Substantial differences were observed between the various materials, and it seems that the ability to enhance foam is very different between malts. Indeed some appeared to have a foam-destabilizing effect. The reasons why will be explained.

Alex Combe is originally from New Orleans, LA, but he spent his high-school years in Houston, TX. He obtained his B.S. degree (with honors) in mechanical engineering from Louisiana State University and Agricultural & Mechanical College in 2009. He is pursuing his M.S. degree in food science at UC Davis.

69. Thiols during production and storage of beer. Presenter: Marianne Lund, University of Copenhagen, Denmark. Co-author(s): Signe Hoff, René Lametsch, and Mogens Andersen, University of Copenhagen, Denmark.

Thiol-containing proteins have been suggested to play a role together with sulfite in the antioxidative mechanism controlling the oxidative stability of beer. Thiols may react with H_2O_2, which is hereby removed. The resulting thiol-containing oxidation products may be regenerated to thiols if they are reduced and may then again react with H_2O_2 for further removal. A method for quantification of sulfite and free thiols in beer was developed based on derivatization with the fluorescent reagent ThioGlo1, separation of sulfite- and thiol-ThioGlo derivatives by high-performance liquid chromatography (HPLC), and subsequent fluorescent detection. Quantification of sulfite and free thiols was performed by preparing standard addition curves in each beer sample with sulfite and glutathione (GSH), which is a tripeptide containing cysteine. The obtained method was used to quantify sulfite and free thiols in wort and beer samples from different storage experiments in order to investigate the correlation of sulfite and thiols with oxidative stability (determined by lag phase measurements using electron spin resonance [ESR] spectroscopy), volatile compounds (analyzed by gas chromatography-mass spectrometry [GC-MS]), protein concentration and profile (determined by the Bradford method and characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] with subsequent MS identification of relevant protein bands), and content of transition metals (determined by inductively cou-

pled plasma [ICP]-MS). Results showed that sweet wort did not contain any detectable thiols. In fact, addition of GSH to sweet worts during the standard addition protocol used for thiol determination caused consumption of GSH resulting in a "thiol-removing capacity." Contrary to sweet wort, detectable amounts of thiols were quantified in beer, indicating that thiols are either secreted by the yeast or that oxidized thiols are reduced during fermentation. A positive correlation between content of sulfite and oxidative stability (length of ESR lag phase) was confirmed, but the content of thiols was also found to be positively correlated with oxidative stability. Pasteurization improved the oxidative stability of beer during storage for one year at room temperature compared to unpasteurized beer, and pasteurized beers also contained more soluble protein after one year of storage than unpasteurized beers, indicating that an increased concentration of proteins positively influences the oxidative stability of beer. Furthermore, the thiol-containing protein, LTP1, was still present in beers with good oxidative stability after storage for one year at room temperature, while beers with poor oxidative stability did not contain any LTP1, suggesting that LTP1 is either involved in oxidative changes during storage of beer or is a marker for oxidation. Determining the content of transition metals did not provide any conclusive results in relation to the effect of pasteurization on the oxidative stability of beer, but the profile of volatile compounds showed that the content of Maillard-derived compounds, such as certain Strecker aldehydes, increased when beer was pasteurized, which is likely due to the introduction of heat during pasteurization.

Marianne Lund (Lametsch) is an associate professor. She received an M.S. degree in food science and technology in 2003 from the University of Copenhagen, Denmark (UCPH), where she also obtained her Ph.D. degree in 2007 based on studies of protein oxidation in meat, with a focus on characterization of radical formation in meat proteins and the consequences of protein oxidation on meat quality. In 2007 she was awarded the IMS Prize at the International Congress for Meat Science and Technology for scientific excellence among those under the age of 40 engaged in research on red meat. In 2008 she began work as a post-doc in the Department of Food Science at UCPH, working with oxidation in beer and the influence of thiol groups on the oxidative stability of beer (with pauses for two maternity leaves) and was appointed associate professor in 2012. She now works on a collaborative project between UCPH and Novozymes A/S on flavor stability of beer. She has published 16 peer-reviewed scientific papers.

70. Evaluation of pre-isomerized hop extracts and their influence on the long-term stability of beer by using a charge titration method. Presenter: Jean Titze, National University of Ireland, University College Cork, School of Food and Nutritional Science, Cork, Ireland. Co-author(s): Jörg Kaspar, Technische Universität Berlin, Institute of Technology, Chair of Brewing Sciences, Berlin, Germany; Pedro Oliveira, National University of Ireland, University College Cork, School of Food and Nutritional Science, Cork, Ireland; Christina Schönberger, Barth-Haas Group, Barth Innovations, Joh. Barth und Sohn, Nuremberg, Germany; Vladimír Ilberg, Hochschule Weihenstephan-Triesdorf, Fakultät Gartenbau und Lebensmitteltechnologie, Freising, Germany; Elke K. Arendt, National University of Ireland, University College Cork, School of Food and Nutritional Science, Cork, Ireland.

Assuring a constant beer quality is one of the biggest challenges in the brewing industry. One important parameter is the physico-chemical stability of the product. This is the resistance of filtered beer against haze formation. One of the main ingredients of beer is hops. Hop bitter acids are the major bitter compounds in beer. They contribute to more than 85% of the overall bitterness. Humulones (alpha-acids) that are present in hops undergo an isomerization reaction to isohumulones (iso-alpha-acids) after thermal treatment. Usually the yield of iso-alpha-acids derived from the dosed hops is not more than 30%. This low yield is caused by numerous factors. Therefore, the use of pre-isomerized hop products such as isomerized kettle extract is a possible approach to achieve higher yields (>50%). Besides the reduction of losses, downstream products are used to produce light stable beers or to improve foam stability. The question remains, do the different downstream products have a positive effect on the long-term stability of filtered beer? With the help of a wort granulate a standardized beer was brewed. After fermentation and maturation different pre-isomerized hop extracts containing purely iso-alpha-acids (IAA), rhoiso-alpha-acids (RIAA) and tetrahydroiso-alpha-acids (THIAA), as well as a mixture of RIAA and THIAA were added just before the final filtration. The dosage of acids was adjusted to the amounts usually used in commercial breweries. To prevent the unhopped wort from boiling over (especially at the beginning of boiling), as well as to avoid an infection with unwanted microorganisms from the wort and/or green beer, beta-extract was added to the pre-boiled wort. As a comparison a traditionally hopped beer with hop pellets was brewed using the same wort granulate to determine the influence of the polyphenol fractions on haze stability. To predict the time until haze becomes visible, two methods were used: 1) determination of the particle surface charge with titrimetric analysis (charge titration method), and 2) a modified forcing test performed according to MEBAK (artificial aging). With the first method the prediction of haze formation was already possible in the fresh bottled beer using the calculation of a stability factor. When performing the forcing test long-term stability was represented by the haze increase after a certain time of artificial aging. Both methods revealed the same results. While the beer hopped with THIAA showed the smallest haze increase, which means the best long-term stability, the beers with IAA or combination of THIAA and RIAA were less stable. In between was the beer with RIAA. However, all the beers with pre-isomerized acids showed a significantly higher physico-chemical stability than the traditionally hopped beer. It should be noted that long-term stability is only one parameter of beer quality. Overall quality in terms of flavor was not investigated.

Jean Titze studied the technology and biotechnology of food at the Technical University of Munich, as well as food and feed law at the Academy of Food Law, Philipps-University of Marburg. He worked several years as a brewery consultant for the Research Center Weihenstephan for Brewing and Food Quality and later as a senior consultant for Deloitte, focusing on the food and beverage industries. Since March 2011 he has been a senior research scientist at UCC, focusing his research on colloidal chemistry and particle analysis. For his research in the area of colloid science he received the 2011 Research Award from the German Brewing Industry. Since winter term 2011/2012 he is also a lecturer for food law at the University of Applied Science Weihenstephan-Triesdorf.

Technical Session 21: Spent Grains

71. A new approach for sustainable utilization of spent grains to develop a profitable process. Presenter: Benjamin Haeffner, TU München, Chair of Process Engineering of Disperse Systems, Weihenstephan, Germany. Co-author(s): Jens Voigt and Karl Sommer, TU München, Chair of Process Engineering of Disperse Systems, Weihenstephan, Germany.

Energy from waste materials of the brewing process is playing a rising role in breweries. Today and in the future, it will

become very difficult for the brewing industry to dispose of their waste materials. Due to new regulations and higher standards for waste treatment, producers are being forced to find new methods of cost neutral discharge of their biological waste that also meet regulatory requirements. Brewery wastes with a high content of cellulose, ligno-cellulose, and hemicellulose, like spent grains, have a high potential to become a key factor in cost neutral brewery waste discharge if you know how to gain access to this stored energy. Thus, the use of spent grains as an energy source through anaerobic fermentation is a good solution. The key to solving this problem is the combination of two well-known unit operations: milling and fermentation These application areas are focused on at the Chair of Process Engineering of Disperse Systems. A current research project is biogas fermentation in a multi-step solid bed process to optimize the dwell-time, which is supported by AiF (German Federation of Industrial Research Associations). The grinding process is performed with an agitator ball mill. This task is achieved by changing the parameters of the milling process to reach the highest possible energy utilization. Another advantage is an increased area of particles, resulting in a higher degree of bioavailability, which is expected to have a positive effect on the degradation rate and residence time distribution. In the fermentation process a fixed bed reactor containing filling materials is used, increasing the contact surface between substrate and bacteria and leading to a higher fermentation rate. With these improvements the hydraulic retention time of the reactor can be reduced without changing the performance parameters (biogas yield, degree of degradation). With the results from the work packages, we can achieve the best energy efficiency of the overall process.

Benjamin Haeffner was born in 1979. He apprenticed as a specialist in food technology at the Döhler company. He received a diploma engineer (M.S.) degree in food and beverage technology from TU München-Weihenstephan, Germany, in 2006. After that he started his career at the Chair of Process Engineering of Disperse Systems at TU München as a technical engineer in wet/dry grinding and fermentation processes. He is an expert in comminution of organic materials and gives lectures on engineering science fundamentals of apparatus. Since 2011 he has been working as a Ph.D. student at the Chair of Process Engineering of Disperse Systems and has edited his own project ("Biogasfermentation in Multi-step Solid Bed Process as Optimization for Dwell-Time"), which is supported by the German Federation of Industrial Research Associations (AiF) and the Research Group of the German Food Industry (FEI).

72. Ultrasonic treatment of brewer's spent grains for bioethanol production. Presenter: Jason Bennett, University of Abertay, Dundee, Scotland. Co-author(s): Graeme Walker and David Bremner, University of Abertay, Dundee, Scotland.

Bioethanol (fuel ethanol derived through fermentation) is now the leading biofuel alternative to fossil-based liquid transportation fuels. Current production is dominated by U.S. corn-based and Brazilian sugarcane-based processes. However, more sustainable future bioethanol production needs to be based on non-food substrates that use lignocellulosic biowastes. The brewing and distilling industry sectors are uniquely placed to exploit the conversion of lignocellulose to bioethanol, through the utilization of spent grains. Bioconversion of brewer's spent grains (BSG) to fuel alcohol represents an attractive but challenging opportunity for sustainable bioethanol production. In addition to the technological and scientific challenges in bioethanol production from spent grains, there are also constraints relating to economics and energy balances. For example, enzyme costs need to be lowered, particularly considering cellulolysis of feedstock. Any innovations to decrease cellulase enzyme dosage are a distinct advantage. We have evaluated the influence of ultrasonic irradiation (at varying frequencies between 382 and 1,174 kHz) on cellulolytic enzymatic digestion of pre-treated BSG. Results have shown that ultrasonic irradiation during enzymolysis increases the total sugar release rate from BSG. In particular, results from exposure of enzymolysis to ultrasound at a frequency of 998 kHz shows that ultrasound holds the potential to significantly reduce the dosing rates of cellulose enzyme required for the hydrolysis of lignocelluloses. Different yeast species, including *Saccharomyces cerevisiae*, *Pichia stipitis*, *Kluyveromyces marxianus*, *Pachysolen tannophilus*, and *Candida shehatae*, have been evaluated for their ability to ferment the mix of five and six carbon sugars liberated following ultrasonic pretreatment and during enzymatic hydrolysis of BSG. Results have indicated that while *Saccharomyces cerevisiae* can ferment hexose sugars within BSG hydrolysates, it lacks the ability to ferment pentose sugars. *Pichia stipitis*, *Kluyveromyces marxianus*, *Pachysolen tannophilus*, and *Candida shehatae* exhibited the ability to ferment the full range of both hexose and pentose sugars within BSG hydrolysates. However, sugar utilization between species varied greatly, with *Pichia stipitis* and *Kluyveromyces marxianus* displaying the best fermentation performance. Research conducted during this study has shown that the application of ultrasonic technology during the enzymolysis of BSG has the potential to significantly reduce the costs associated with cellulolytic enzyme dosing during the bioconversion of lignocellulosic substrates to bioethanol.

Jason Bennett graduated with a B.S. degree in biotechnology from the University of Abertay Dundee in 2008, with a thesis titled "The Application of Ultrasound in Yeast Biotechnology." He is currently completing his Ph.D. degree with a thesis titled "The Application of Ultrasound in Bioconversion of Brewer's and Distiller's Spent Grains to Bioethanol." In January 2012 he commenced a new post within the university, focusing on developing sustainable solutions for dealing with the co-products produced during malt whisky distillation.

73. Treatment of spent grains by hydrothermal cleavage to purify dietary fibers. Presenter: Julia Steiner, TU München, Freising, Germany. Co-author(s): Martin Zarnkow and Thomas Becker, TU München, Freising, Germany.

Seventy-five percent of all organic residues originating from brewing are spent grains, which constitute the most important and energy-rich by-product from the brewing process. Refining spent grains rich in beneficial ingredients is of great interest for valuable preservation of human nutrition. Although they are a remnant, they still contain high-quality dietary fibers (i.e., arabinoxylan and beta-glucan), which are difficult to utilize due to their structure and the preceding process steps. Therefore, the use of hydrothermal cleavage was investigated, and possible fields of application for beverage technology have been developed. It was the purpose of this study to break down the complex insoluble polysaccharides to educe and transfer the cleavage products to a soluble state in order to add them to new beverages as an ingredient with health beneficial attributes. With regard to an increase in health consciousness and the shift in consumption habits toward soft drinks, the brewing industry faces new opportunities and challenges. Based on the current trend, beverages rich in dietary fiber have emerged. These successful innovations receive distinctive appreciation and sustained acceptance by consumers. In particular, beta-glucan offers a comprehensive potential for functional beverages, due to its origin in natural raw materials and its scientifically proven positive and health-promoting effects. This paper

presents an innovative way to produce novel fiber-based drinks using lactic acid fermentation. Dietary fibers are purified using hydrothermal cleavage, and subsequently the hydrolysates are fermented by selected strains. The resulting fermentation products are mixed with different beverages and carbonated, resulting in well-balanced refreshment. Laboratory-scale trials have been carried out to select the best process parameters in order to gain maximum dietary fiber content. The evaluation of the novel utilization technology is based on analytical attributes measured using HPLC. To ensure the hydrothermal reaction conditions and to determine the influence of temperature and residence time, the cleavage process was conducted at temperatures ranging from 170 to 230°C and varying residence times. Consequential rising decomposition products such as hydroxymethylfurfural and furfural, which may not exceed certain concentrations due to possible health risks, are undesired. By contrast, a preferably high proportion of arabinoxylan and beta-glucan is definitely desired. Suitable approaches to and treatment intensities for an improved yield of dietary fibers and a low yield of undesired substances (under the critical limit) are shown in this research. Particular attention is directed toward functional ingredients, with a focus on beta-glucan, which was shown to be stable in the produced beverages. With an adequate concentration of beta-glucan, specific application examples could be offered as a reward for functional food with an additional benefit by the EFSA and FDA.

Julia Steiner was born in 1984 in Munich, Germany. In 2009 she graduated from the Technische Universität München as with a Dipl.-Ing. degree in technology and biotechnology of food. Since 2010 she has been working as a Ph.D. student at the Institute of Brewing and Beverage Technology in Weihenstephan, TU München. Julia is investigating complex spent grain components, pursuing the aim to preserve this brewery by-product, which is valuable for human nutrition. The intent of this research project is the transfer of insoluble dietary fiber fractions into a soluble state in order to add them to novel beverages as an ingredient with health benefits.

74. From spent grain to "bio-coal"—Is hydrothermal carbonization (HTC) an unvalued key technology? Presenter: Heinz Dauth, Münster University of Applied Sciences, Steinfurt, Germany. Co-author(s): Juergen Mueller, Tobias Bosse, and Peter Dettmann, Münster University of Applied Sciences, Steinfurt, Germany.

HTC was described for the first time in 1913 by a chemist named Bergius and gained increasing interest for several applications during the last decade. What does hydrothermal carbonization mean? In easy to understand terms, it stands for a thermo-chemical process for the conversion of solid biomass at an elevated temperature and pressure in the presence of water. The achieved product differs significantly in its chemical and physical properties in comparison to the starting material. During the HTC process mainly water and carbon are dissociated from the biomass. Thus, the energy density is raised significantly and the heating value is approximately that of dry, high quality brown coal. As mentioned previously the HTC process takes place in an aqueous reaction medium so that wet biomass ,like spent grain, can be easily used and is actually preferred. Initial experiments with spent grain show that this biomass is an ideal substrate for conversion into "bio-coal." The advantages of spent grain are its particle size distribution, chemical properties due to the composition of the biomass, excellent mechanical compactibility, which results in a high load capacity (mass loading) in the autoclave, and a nearly homogeneous distribution of the biomass in the liquid phase inside the autoclave. Due to the chemical composition of the substrate the required energy input into the system after initializing the process is significantly lower compared to other examined substrates. After a treatment of 5 hr under mild process conditions with respect to temperature and pressure the resulting product is a coal-water slurry. The coal fraction can easily be separated and dried. The "bio-coal" gained from spent grain has a heating value of approximately 27.000 kJ/kg (which is higher than the heating value of brown coal) and has an ash content of just 6% in relation of dry "bio-coal." The crop of "bio-coal" from 1 kg of "wet" spent grain is approximately 12 mass percent. Furthermore, the process can be enhanced in a way that other biomass from the brewery, like label residues or sewage sludge, is mixed with the spent grain. This will be the next step for experimental examinations. This "bio-coal" from spent grain can serve as a CO_2 neutral substitute for fossil coal because by burning "bio-coal,, only that amount of CO_2 which was trapped in the plants by means of photosynthesis is released, making the HTC process a perfectly climate neutral and sustainable energy process. In summary, the HTC process provides a porous, brittle, and partly dust-like product that is considerably easier to dry and convert to thermal energy in a brewery than the original biomass. This is a clear conceptual advantage for energy use compared to, for example, the burning or gasification of untreated biomass like spent grain.

Heinz Dauth graduated with a Dipl.-Ing. degree in food technology and biotechnology from the Technische Universität München-Weihenstephan in 1993. Afterward he was appointed as a scientific researcher at the Chair of Process Engineering (Karl Sommer) in Weihenstephan. His doctoral thesis was completed in 1999 in the field of mechanical process engineering. He served the Chair of Process Engineering, TU München, as scientific assistant and university lecturer from 2003 until 2011. His main research interests are bulk solids technology, dispensing technology, and hygiene, as well as process engineering for specific problems in the food and beverage industries. During this time he has also been working as an assistant professor at the Weihenstephan University of Applied Sciences, lecturing on mechanical and thermal process engineering. Since October 2011 he has been a professor at the Münster University of Applied Sciences in the Faculty of Chemical Engineering.

Technical Session 22: Yeast IV

76. Sub-genomic cooperation in the hybrid lager yeast *Saccharomyces pastorianus*. Presenter: Brian Gibson, VTT, Espoo, Finland. Co-author(s): Virve Vidgren, VTT, Espoo, Finland; Jari Rautio, Plexpress, Helsinki, Finland; John Londesborough, VTT, Espoo, Finland.

The hybrid *Saccharomyces pastorianus* genome consists of two diverged genomes believed to be those of *S. cerevisiae* and the recently discovered *S. eubayanus*. To clarify the functional relationship between these sub-genomes and its contribution to fermentation performance, molecular probes were designed to monitor differential transcription of *S. cerevisiae*- and *S. eubayanus*-type genes of *S. pastorianus* under different fermentation conditions. The TRAC (transcriptional profiling with the aid of affinity capture) system was used, as it has the advantage of allowing reliable differentiation of orthologous genes in large numbers of samples (10 samples were taken in the first 24 hr). Samples were taken from 2-L, 15°P, all-malt wort fermentations conducted at different temperatures (10–20°C), and the TRAC system was used to monitor the expression of genes involved in sugar import, including *MAL×1* (maltose transport) and *MAL×2* (alpha-glucosidase). Sugar transport is known to be strongly temperature-dependent. As expected, peak expression of *MAL×1* and *MAL×2*, both the *S. cerevisiae* and *S. eubayanus* versions, occurred later in fermen-

tations at lower temperatures. It also lasted longer (about 2 days at 10°C compared to half a day at 20°C). Unexpectedly, the *S. cerevisiae MAL×1* and *MAL×2* genes were activated clearly (up to 12 hr) before their *S. eubayanus* versions. The results give insight into the independence and inter-dependence of the *S. cerevisiae* and *S. eubayanus* sub-genomes in *S. pastorianus*. The different timing of responses may have practical importance regarding monitoring of yeast activity during fermentation. Results are discussed in relation to the activity of other orthologous genes in *S. pastorianus*, including *MAL×3* (MAL activation), *AGT1* (alpha-glucoside transport), and *HXT* genes responsible for high or low affinity glucose transport.

Brian Gibson was awarded a Ph.D. degree from University College Dublin, Ireland, in 2004, where he had specialized in fungal stress responses. On completion of his studies he joined the brewing science research group at Oxford Brookes University and later at Nottingham University, England, where his research covered a range of subjects, including brewing yeast stress responses, yeast transcriptomics during industrial fermentation, genetic stability of brewing yeast, and molecular identification of brewery contaminants. Since 2009 he has been employed as a senior scientist and project manager at VTT, Finland, with responsibility for yeast physiology and fermentation research.

77. Large-scale systems biology approach to select and create novel yeast strains with superior fermentation characteristics. Presenter: Kevin Verstrepen, CMPG Laboratory for Genetics and Genomics, Leuven, Belgium.

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 spent relatively little attention on the selection and optimization of brewing yeasts. This is at least partly due to the fact that each brewery often uses one or a few particular, proprietary yeasts, whereas wine and bread yeasts are often produced by large, specialized companies. This implies that may breweries are using sub-optimal yeasts and that there is a vast potential for selection and breeding of superior beer yeasts. In the 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, or directed evolution) with improved or combines 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., Kevin joined the lab of Gerald Fink at MIT. 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, Kevin joined Harvard University as a Bauer Fellow. In 2007, he was promoted to lecturer and started teaching industrial microbiology to undergraduate students. Meanwhile, Kevin 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 associate professor and research director at the Flanders Institute for Biotechnology (VIB). His team continues to investigate eukaryotic genetics and epigenetics, with specific interest in industrial microbiology.

78. Genetic drift and variation in brewing yeast cultures. Presenter: Chris Powell, University of Nottingham, UK. Co-author(s): Thien-Khiem Nguyen, University of Nottingham, UK.

Once a brewery fermentation has reached completion, it is common practice to harvest the yeast from the fermentation vessel and use the recovered biomass to inoculate a fresh batch of wort in a process known as serial repitching. Repitching yeast often results in a reduction in yeast quality over time, although the extent to which this occurs depends on the individual yeast strain and the number of serial repitchings (generations). It is well known that some yeast strains are able to be reused many times with little apparent effect on product quality. However, other strains are less tolerant to repitching, and these populations can accumulate mutant cells that ultimately influence the capacity of the population to produce acceptable beer. While process and product parameters may play a significant role in the number of times a strain can be reused, it is also possible that some industrial strains are simply more genetically malleable than others. Previous studies have shown that chromosomal rearrangements manifest themselves in laboratory *S. cerevisiae* yeast after 30–50 generations under nutrient limited conditions. However, the rate of mutation in polyploid industrial brewing strains under sub-lethal but stressful conditions has not been investigated. Here the relationship between brewery process conditions, associated stress factors, and genome stability is investigated. Furthermore, we explore the potential for selection during full scale beer production and the significance of this on population dynamics. It is anticipated that the data will provide a greater understanding with regard to the number of times which a yeast culture can be expected to perform to its optimum capacity.

Chris Powell holds a Ph.D. degree on the subject of yeast cellular aging and fermentation performance from Oxford Brookes University, U.K. Chris has also occupied research positions at Bass Brewers (now Coors UK) and more recently at Lallemand, based in Montreal, Canada. During his six years at Lallemand, Chris was responsible for the R&D laboratory for the molecular identification and characterization of micro-organisms utilized within the food and beverage industries, in addition to research focused on brewing yeast. In 2010 Chris returned to the United Kingdom to take up his current position as lecturer in yeast and fermentation at the University of Nottingham. Chris is presently involved in research in the areas of both brewing science and sustainable bioenergy. Chris is the author or co-author of more than 40 scientific publications and is a regular reviewer for several scientific journals. Chris has also served on the ASBC Technical Committee since 2005 and the ASBC Board of Directors since 2010. Outside of work, Chris is a keen soccer player and spends a significant portion of his time running, hiking, and exploring different parts of the world.

228. A novel method of inducing and retaining cell cycle synchronization in cultures of *Saccharomyces cerevisiae*. Presenter: Johnathon Layfield, NC State University, Raleigh, NC, USA. Co-author(s): Lucas Vann and John Sheppard, NC State University, Raleigh, NC, USA.

In conventional batch and continuous fermentation, the cell cycles of individual yeast are randomized within the population, and the observed metabolic performance is the result of an averaging effect. Synchronous cellular growth is

characterized by cells in a population aligned with respect to their metabolic processes traversing the cell cycle and dividing mostly in unison. Thus, synchronized populations of cells can be used as a tool to reveal more precisely how an individual cell reacts under different environmental conditions (Sheppard et al, 1999). *S. cerevisiae* is a unique organism in that it serves as a model eukaryote for academic and industrial research. Thus, a method for inducing and storing a synchronous yeast culture for rapid use in metabolic studies is advantageous to both academia and industry. In this study, a novel method for inducing and retaining cell cycle synchronization in yeast cells (diploid- and polyploid-type cells) was developed. This technique is derived from the continuous phased-culture induction method (Dawson, 1969). The original induction method was based on a cyclical process in which one-half of the cell culture was harvested and a fresh nutrient solution added to replace the harvested volume at a period corresponding to cell doubling. This replenishment of sufficient nutrients only for cell doubling resulted in the growth and division of a single division of cells prior to the beginning of a new cycle. After about six such cycles, the cells became aligned with respect to their cell cycles and began dividing synchronously. Our new method begins with a small volume and doubles it each cycle by periodically adding fresh nutrient solution, without having to remove any cells. This adaptation is better suited for industrial applications, such as seed expansion, due to its relative simplicity and equivalent effectiveness in producing cell synchrony. This was demonstrated by measuring the synchrony index of both *S. cerevisiae* 288C (diploid) and the brewing strain London ESB 1986 (polyploid), which matched that produced using the conventional continuous phased method (71 and 83%, respectively). We have also shown that synchronized cells can be stored for later use in glycerol at –80°C for at least 2 weeks without significant loss in synchrony. Small volumes (1.5 and 10 mL) of both *S. cerevisiae* 288C and London ESB 1986 showed no loss of synchrony from the original synchrony procedure. However, as the volume of a synchronous stock increased to 50 mL, certain aspects of synchrony (depending on the strain) seemed to degrade. The extra time required for both freezing and thawing the larger synchronous stocks is thought to be the cause. However, for most metabolic studies, freezing at –80°C is a viable approach for retaining cell synchronization in *S. cerevisiae*.

Johnathon Blake Layfield received a bachelor's degree in food science (2003) and master's degree in food science, with a minor in biotechnology (2009), from North Carolina State University in Raleigh, NC. He is currently pursuing a Ph.D. degree in food science at NC State University under John D. Sheppard. Johnathon has interned for Smithfield Foods Ltd. (quality assurance) and Novozymes (biofuel R&D) and was a co-op student with Campbell Soup Co. (beverage product development). He is a member of both the Institute of Food Technologists (IFT) and the American Society of Brewing Chemists (ASBC). Johnathon has published in JASBC, where his work on desiccation tolerance in lager yeast was selected as an "Editor's Pick" (August 2011). He also gave an oral presentation at the 2009 ASBC Annual Meeting in Tuscon, AZ.

Posters

Analytical

79. A glimpse of craft beer over the past 6 years through large scale analytical testing. Presenter: Kara Taylor, White Labs, USA.

For the past six years, White Labs, Inc. has once a year performed large scale laboratory testing on craft beer with our Big QC Day program. This analytical testing of beer provides the customer with values such as ABV, diacetyl, bitterness units, attenuation values, color, calories, and microbiological contaminants. Each year over 500 samples are submitted for analysis from craft breweries all over the United States and some internationally. Questions regarding increases in quality control, sanitation, and adherence to style guidelines can be answered through analysis of six years of craft beer laboratory testing. Additionally, correlations between analytes can be drawn, such as the affect of pH, alcohol, IBU, and microbial contaminants. By collecting data from every region and for a long span of time, we can evaluate how craft beer has changed stylistically, regionally, and overall.

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 January 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.

80. A new and improved method for monitoring beer vicinal diketones as maturation markers. Presenter: Greg Rahn, Hamilton College, USA. Co-author(s): Tim Elgren, Hamilton College, USA; Mike Adler, The Matt Brewing Company, USA.

Vicinal diketones (specifically 2,3-butanedione [diacetyl] and 2,3-pentanedione) are chemical compounds mainly produced during fermentation of wort. They are produced as a by-product of valine synthesis, when alpha-acetolactate and alpha-acetohydroxybutyrate are decarboxylated and are later enzymatically reduced to acetoin and 3-hydroxy-2-pentanone. However, diacetyl can also arise due to bacterial contamination without the development of 2,3-pentanedione. Having quantification of both compounds completed quickly and easily is much more useful than the standard 7 days that a complete microbiological examination would take. They can play a role in beer maturation indices as they are among the last processes that occur during fermentation. Current decisions as to adequate beer aging and storage are typically time-based. If evaluation of these maturation markers occurred in real time, adjustments in temperature or holding time in a fermentation vessel could be made to both enhance the quality of the finished product and to reduce the time that the beer needs to mature. The end result of both is a superior product and better tank utilization, which makes this an invaluable tool in adjusting fermentation profiles. In order to assess potential bacterial contamination of newly fermented wort and monitor these same chemical markers as proxies for beer maturation, accurate and precise updated methodology was sought. Existing methods (EBC Method 9.24.2) based on gas chromatography and electron capture detection had labor-intensive sample preparation steps and potential interferences from endogenous compounds. It also suffered in that an experienced scientist was needed to carry out the procedures. Our goal was to simplify the method, especially the sample preparation portion, make it less technical so someone less skilled (undergraduate students) could perform it, yet ensure the integrity of the results through direct comparison of samples analyzed using both methods. This updated method uses automated static headspace electron capture detection (ECD) with "dilute-and-shoot" sample preparation and monitors low ppb levels of both vicinal diketones using 2,3-hexanedione as an internal standard. The use of this internal standard ensures quantitative accu-

racy since it compensates for any and all unanticipated method shortfalls. When the virtues of this new method were realized through direct sample read-back comparisons, a 3-day validation of it was performed. This validation comprised the statistical evaluation of sample results from student prepared standards, quality control samples, and spiked and real samples. In addition, stability assessments of stored, in-process, and prepared samples were made to further ensure the accuracy of sample result reporting. This poster will detail the updated methodology, discuss the accuracy and precision of it through an in-depth evaluation of the sample results generated during the course of the validation, and demonstrate how analysis of real process samples leads to informed decisions regarding beer maturity.

Greg Rahn is currently an instrumentation specialist in the Department of Chemistry at Hamilton College in Clinton, NY. He received a B.S. degree in chemistry in 1981 at the State University of NY College at Cortland. He joined the Hamilton staff in 2008 after more than 25 years of developing and implementing analytical services in the environmental and pharmaceutical industries. With general expertise in analytical methodologies, his primary expertise is in the area of mass spectrometry.

81. A novel gas chromatographic system to characterize hop aroma. Presenter: Andrew Tipler, PerkinElmer, Shelton, CT, USA. Co-author(s): Lee Marotta and Tom Kwoka, PerkinElmer, Shelton, CT, USA.

The flavors of many beers are greatly affected by the addition of hops at different stages during the brewing process. Aroma plays a very important part in the tasting experience. Hops contain many volatile organic compounds (VOCs) that contribute to the aroma and, hence, the taste of beer. A gas chromatographic system has been developed to assist in the objective characterization of hop aroma. The first component of this system is an equilibrium headspace sampler with an integral adsorbent trap. A hop or beer sample is placed in a sealed vial and maintained at an elevated temperature for a fixed period of time. During this time, VOCs from the sample migrate into the vapor (headspace) phase inside the vial. This vapor is then vented into an cooled adsorbent trap to focus and concentrate the VOCs. The VOCs in the trap are thermally desorbed and delivered to a gas chromatograph (GC) for component separation. The chromatographic column used for the separation is a 60 m × 0.32 mm × 0.5 µm Carbowax column (same stationary phase as used in ASBC Method Hops-17). The effluent from the chromatographic column is split between a mass spectrometer (MS) and an olfactory port (OP). The splitting device is fabricated using chemically deactivated laser-etched micro-channel wafer technology to ensure minimum dispersion and adsorption of compounds eluting from the GC column. The MS system enables the detection, identification, and quantification of each VOC component. The MS used in this work is a new single quadrupole designed specifically for GC use and has an enhanced sensitivity to enable spectral identification of hop VOCs at very low levels. The olfactory port is a new design that enables the operator to smell each component as it elutes from the GC column in relative comfort. In this way, the chemical profiles generated by the MS may be correlated against the subjective organoleptic information obtained from the olfactory port. This presentation will describe the design and application of this system. A wide variety of hop types have been examined using this system, including American West Coast strains, English strains, and noble strains. Both leaves and pellets have been examined. A variety of beers have also been examined. This system can be used for the quality control of hops prior to brewing and to troubleshoot beer after production.

Andrew Tipler is the chromatography R&D manager at PerkinElmer in Shelton, CT. He is English, obtained a degree in pure chemistry at the University of Manchester, and worked for many years in various laboratories in England. Since joining PerkinElmer at their English site in 1983, he has been involved in the development and application of nearly all the company's GC products. He moved to the company's site in the United States in 1993 and continued to work on new GC technology and applications. He has been granted a total of 25 patents and gives papers at many key GC conferences around the world. He is also a keen home brewer and has won awards in regional competitions, particularly for English bitters. He is studying to become a judge in the BJCP program.

82. Analysis of volatile thiols in beer with on-fiber derivatization and GC/MS determination. Presenter: Minoru Kobayashi, R&D Laboratories for Brewing, Asahi Breweries, Ltd., Moriya-Shi, Japan. Co-author(s): Nana Yako, Susumu Masuda, and Masayuki Aizawa, R&D Laboratories for Brewing, Asahi Breweries, Ltd., Moriya-Shi, Japan.

Volatile thiols in beer are known to cause off-flavors, even at low concentrations. However, the determination of volatile thiols at low levels in beer is particularly complicated and difficult. A fast and automated method for analysis of volatile thiols, such as 3-methyl-2-butene-1-thiol (MBT), to the nanogram per liter level was developed in this study. We adapted the method used for wine to the analysis of beer. Briefly, a sample is poured into a 20-mL vial, and the headspace gas is extracted with a solid-phase microextraction (SPME) fiber pretreated by exposure to vapors of pentafluorobenzyl bromide. The derivatized compounds are subsequently desorbed in GC-MS. As a result, good analytical precision and linearity can be achieved for MBT, benzenemethanethiol, and 2-mercapto-3-methyl-1-butanol. These volatile thiols can be repetitively detected in beer at concentrations $\leq$0.1 ng/L. The proposed procedure is much simpler than the present methods from the viewpoints of processing and time requirements. Therefore, the new method can be used as an alternative to the existing methods, and it is an effective method for brewers to use when evaluating off-flavors in beer. Through the introduction of this analytical method, it has become possible to reduce variation in the amount of thiol formation in the brewing process and improve stabilization of beer flavor quality. In this presentation, we will show some examples of the control of off-flavors in our brewery.

Minoru Kobayashi is a scientist at brewing research and development laboratory, Asahi Breweries Ltd. He received his M.S. degree in applied biological chemistry from Tokyo University in Japan, where he majored in analytical chemistry. He has been engaged in the analytical technology laboratory since 1998, especially in the analytical chemistry section. Since 2003 he has worked in the brewing science section, especially in beer flavors.

83. Assessment of instruments for use in breweries. Presenter: Catharine O'Shaughnessy, Campden BRI, Nutfield, UK. Co-author(s): Karin Pawlowsky and Gordon Jackson, Campden BRI, Nutfield, UK.

There are many new instruments being developed for the chemical and microbiological testing of beer. It is difficult to know which of these are suitable. Currently brewers have to arrange in-house tests before purchase, and this is time-consuming and expensive. This paper describes the Campden BRI instrument assessment service for the alcoholic drinks industry, which can provide an independent evaluation of new and existing equipment. Instruments are tested against existing

methods to determine their suitability for use in the brewing sector. The results from these assessments can be published on the Campden BRI instrument website (www.compareinstruments.com), which brings together information about equipment (particularly new instruments) that are available for the brewing industry. This service is free to staff in breweries. This paper provides data from recent evaluations of new instruments for chemical and rapid microbiological testing of beer. It presents data on ease of use, repeatability, reproducibility, and comparison with existing methods.

Catharine O'Shaughnessy graduated from Birmingham University in 1994, having obtained a B.S. degree in biochemistry with biotechnology and an M.S. degree in biochemical engineering. After finishing at Birmingham, Catharine joined Campden BRI (then known as Brewing Research International) as a research engineer on their Process Innovation Team and worked in the areas of process optimization, cross-flow microfiltration, and waste minimization. She gained her diploma in brewing in 1995. In 2003 she was awarded the IBD Cambridge Prize for her work at Campden BRI in the areas of waste minimization, malt and cereal roasting, malt flavor chemistry, and the sensory modeling of malt flavor. In 2007 she joined the Sensory team, where she managed the expert sensory malt profile panel and their expert technical taste panel. At the beginning of 2011, Catharine was appointed manager of the Instrument Assessment Programme. She is author and co-author of a number of scientific publications in international journals.

84. Beverage antioxidative index (BAX)—An advantageous tool for the evaluation of beer flavor stability. Presenter: Frank-Jürgen Methner, Technische Universität Berlin, Department of Biotechnology, Chair of Brewing Sciences, Berlin, Germany. Co-author(s): Thomas Kunz and Christian Müller, Technische Universität Berlin, Department of Biotechnology, Chair of Brewing Sciences, Berlin, Germany.

For the prognosis of the flavor stability of beer, electron spin resonance (ESR) spectroscopy has been used for determination of the so-called lag time. Our former investigations demonstrated that the lag-time measurement used until now falsifies the results of oxidative flavor stability due to an increasing pH value during the analysis caused by the spin-trap reagent (PBN). The developed EAP determination excludes the falsifications due to the use of a different spin-trap reagent (POBN) in lower concentrations, which results in a beer matrix dependent, linear correlation between the SO_2 content and the EAP value. For this reason, the EAP determination offers a new beneficial index number for the evaluation of flavor stability, the beverage antioxidative index (BAX), which provides additional information about the anti- and pro-oxidative properties of the beer matrix independent of SO_2 content. BAX is affected by the content of metallic ions, pH value, polyphenols, proteins, intermediate Maillard reaction products, etc. and gives information about the consumption rate of the existing antioxidative potential during storage. In addition, EPR spectroscopy standard analyses performed according to MEBAK and CFA (continuous flow analyzer) (SO_2, polyphenols, etc.) were used to obtain additional information about the influences of the different beer ingredients on the radical generation and oxidative stability by application of the described EAP and BAX determination. The investigations clearly demonstrate that lower pH values improve oxidative beer stability, which is reflected by higher EAP, lower radical generation, and higher BAX values. Iron entry caused by raw materials and kieselguhr filtration deteriorates oxidative stability, although polyphenols do not change the EAP and BAX significantly. Furthermore, it could be illustrated that hop ingredients like alpha- and beta-acids can act as chelating agents and can significantly influence radical generation and reduce oxidative processes. The latest results showed that specific intermediate Maillard reaction products with reductones/endiol structure formed during kilning of the malt and wort boiling decreased the oxidative stability by the acceleration of the Fenton-Haber-Weiss reaction system. In this context, brewing with raw barley leads to improved oxidative stability, as indicated by a higher BAX and decreasing radical generation. The optimized lag-time measurement, called EAP determination, makes an unbiased examination of flavor stability possible. In combination with BAX, based on a beer matrix dependent linear correlation of the EAP value and the SO_2 content of a beer, it is possible to obtain a deeper insight into the influences of different beer ingredients on flavor stability. An additional advantage of the BAX determination is the indirect determination of the SO_2 content.

Frank-Jürgen Methner conducted studies in brewing science at Berlin Institute of Technology (TU) from 1975 to 1981. After the studies, he began working as an operating supervisor at the Schlösser Brauerei, Düsseldorf. From 1982 to 1986, he was a scientific assistant with teaching duties. Research projects and Ph.D. thesis, "Aroma Formation of Berliner Weissbier with Special Focus on Acids and Esters," were further tasks. For 18 years, starting in 1987, he held a leading position as a director at the Bitburger Brauerei, Bitburg, Germany, with responsibilities in fields such as research and technology, as well as quality assurance. Beginning with the winter semester 2004/2005 he took over the Chair of Brewing Science within the Department of Biotechnology at Berlin Institute of Technology (TU Berlin).

85. Carbohydrate analysis using HPLC with PAD, FLD, CAD, and MS detectors. Presenter: David Thomas, Thermo Fisher Scientific, Chelmsford, MA, USA. Co-author(s): Paul Ullucci and Ian Acworth, Thermo Fisher Scientific, Chelmsford, MA, USA.

Carbohydrates are a structurally diverse group of compounds that can be categorized as monosaccharides, disaccharides, oligosaccharides, glycoproteins, etc. Carbohydrates are difficult to analyze because they have similar physical and chemical characteristics and do not have a suitable chromophore for UV detection. Several different HPLC methods using various detector strategies (pulsed amperometric electrochemical, fluorescence following derivatization, charged aerosol detection, and mass spectrometry) were developed to help study carbohydrates, and examples for each approach are presented. Although fluorescent tags improve chromatographic resolution and detector sensitivity, they can lead to increased assay variability. Various HPLC modes can be used for carbohydrate separations, with ion exchange, hydrophilic interaction liquid chromatography (HILIC), and reversed phase (RP) on porous graphite column (PGC) being the most common. HPLC provides for simple chromatographic methods; direct detection using PAD or mass detectors such as ELSD, MS, and CAD are employed. CAD is an ideal detector when combined with HILIC or RP/PGC for measuring different carbohydrates. It is a mass-sensitive detector that can measure any non-volatile, and many semi-volatile compounds, typically with low ng sensitivity. Unlike ELSD, it shows high sensitivity, wide dynamic range, high precision, and more consistent inter-analyte response independent of chemical structure. For the analysis of glycans liberated from glycoproteins, the utility of the LC-MS-CAD platform is presented; CAD is used for quantitative analyses, while MS provides structural verification. The advantage of this approach over methods using fluorescent tags is discussed.

Dave Thomas received a Ph.D. degree in analytical chemistry from the University of Nebraska-Lincoln in 1994 for his work developing

high-performance immunoaffinity chromatography. In post-doctoral appointments at Midwest Research Institute-California Operations and Sandia National Laboratories, he worked to implement HPIAC and HPLC approaches on miniaturized electrochromatographic separation and analysis platforms. Later, he spent several years developing a variety of IC, HPLC, and LC/MS applications at Dionex Corporation and Thermo Fisher Scientific, where he also served as manager of the HPLC and LCMS applications laboratory. After a few years in vaccine analytical development at Wyeth and Pfizer, Dave returned to Thermo Fisher Scientific, where he continues to develop applications for HPLC with charged aerosol and electrochemical detection.

86. Comparing optical versus traditional measurement technology in the brewery. Presenter: Daniel Gore, Anton Paar, Graz, Austria. Co-author(s): Keyvan Ghanaviztchi, Anton Paar, Graz, Austria.

Optical measurement technology is becoming more and more common in process environments, especially in the brewing and beverage industries, and offers many benefits, such as ease of installation and cleaning, compact size, reduced maintenance, etc., but does it also meet the high expectations of the brewmaster and quality personnel in terms of accuracy, repeatability, and stability in daily use? This paper describes the results of a head-to-head test to compare ease of use in daily routine, measurement performance (accuracy and repeatability) of the test instruments compared to the lab reference methods, performance during product changes, start-up procedures, and measurement response time. The test location for this comparison was a 60.000 hL brewery with 14 different beers and multiple product changes every day. The test candidates were the VS-3000BM optical sensor with the VS-300 sensor management station and the beer monitor, comprised of the DSRn427S, Carbo 510 Smart Sensor, and mPDS 5 evaluation unit. The VS-3000BM is an optical, mid-infrared ATR (attenuated total reflectance) spectrometer that measures the components of liquids. A specific, modulated, infrared emitter emits a signal that reflects through the ATR crystal, where some wavelengths are absorbed by the product and then strikes various detectors, each with a narrow band pass interference filter. Specific wavelengths are used to measure alcohol, extract, and CO_2 and as a background reference. The classic beer monitor is a measuring system combining two well-established sensors. The combined density and sound velocity sensor DSRn427S measures density according to the oscillating U-tube principle. The U-shaped tube is made of Hastelloy C276 and is excited to a continuous oscillation at its natural frequency by means of a magneto-electrical excitation system. The oscillation frequency is directly related to the density of the product flowing through the tube. The sound velocity is measured by an ultrasonic transmitter and receiver located on one side of the U-tube. The electronics measure the propagation time of the ultrasonic pulses through the product and calculate the sound velocity. These two sensors are responsible for alcohol and extract measurement. The Carbo 510 Smart Sensor combines the classic method of CO_2 analysis according to Henry's law with the volume expansion method, which makes use of the fact that the solubility of CO_2 in beverages is much higher than the solubility of air.

Daniel Gore received his B.A. degree from the University of Maryland, College Park, including two years of study in Germany. After graduating in 1995 he returned to Germany and began an apprenticeship as a brewer and maltster at the Lammbrauerei Hilsenbeck. After successfully finishing his apprenticeship he worked in multiple breweries throughout Germany, including the Uerige Obergärige Hausbrauerei and Quenzer Bräu before moving back to the United States to assume the role of head brewer at the Long Trail Brewing Company. In 2006 he changed focus to work as a technical sales representative for Anton Paar, USA and continued to put his 12 years of practical brewing experience to good use serving the beverage industry. During this time Daniel was a member of MBAA and ISA and enjoyed working with local chapters in the Northeast. In 2010 he moved to Graz, Austria, to become Anton Paar GmbH's application specialist, supporting Anton Paar's existing applications in the beverage industry, as well as developing new beverage applications and technologies.

87. Complex evaluation of technological changes—Impact on foam. Presenter: Adam Broz, Budejovicky Budvar, n.p., Ceske Budejovice, Czech Republic. Co-author(s): Petr Kosin and Jan Savel, Budejovicky Budvar, n.p., Ceske Budejovice, Czech Republic.

Traditional brewers are facing the pressure of being fully competitive in the beer market. Their more difficult position comes from the use of traditional recipes and natural raw materials. The traditional brewing philosophy brings higher production costs due to higher energy demands and the higher price of raw materials. The traditional brewer has to be very careful about any technological changes. Possible savings in this case could be found by the increase of process effectiveness and minimization of extract loss in the production pathway. All steps to higher effectiveness must be proved in quality tests. Either analytical specifications or complex characteristics, and the sensory profile of a beer must be kept constant. One of the complex characteristics is beer foam, which is very sensitive to any technological changes and could indicate not only foam problems. A special method has been developed that allows measurement of foam potential in brewing intermediates. The method could be used as well for intermediates without any carbon dioxide content or sample filtration to obtain results immediately. Using this unique method, a study was implemented that tests the qualitative impact on beer when two extract loss decreasing techniques are used: wort recovery from sediment in a whirlpool and beer recovery from yeast after main fermentation, as well as after maturation. Different process modes of wort or beer recovery were tested to obtain a saving solution without any quality damage in the conditions of a traditional industrial brewery (volume 650 hL per brew, two-mash process, two-phase fermentation). A decanter separator installed after the whirlpool was tested in production scale. Separated wort was added into the wort line before cooling, followed by two-phase fermentation. Next to matrix foaming potential, amounts of wort recovered, removed solids, fatty acids, tannoids, nitrogen of MW > 5,000 in wort, wort concentration, dry matter, color, pH value, colloidal stability of beer, and beer sensory profile were analyzed. A cross-flow micro-filtration device was installed and run for recovery of beer from yeast. Recovered beer was added to young beer before maturation. Matrix foam potential, original extract, real extract, alcohol, color, pH value, bitterness, colloidal stability of beer, and beer sensory profile were analyzed. A new approach to judging the suitability of brewing intermediate recovery methods was used, with a focus on the complex evaluation of foaming potential. Using a very sensitive method of matrix foaming potential could reveal beer foam problems in early production phases. Results were compared with NIBEM and foam developed using a pouring test in final beer. Evaluation of such an important beer characteristic accompanied by stability and sensory measurements in final beer allows brewers to select optimal solutions for savings with no risk to quality.

Adam Broz received a Dipl.-Ing. (M.S. equivalent) degree in brewing and malting from the Institute of Chemical Technology

Prague, Czech Republic, in 1999. He has been employed for Budweiser Budvar N.C. in Ceske Budejovice, Czech Republic, since his graduation. He worked as a technician (1999–2001), a brewhouse chief (2001–2004), a plant technologist (2004–2006), and a deputy brewmaster (2006–2008). Since 2009, he has been working as a technical and production director. He received a Ph.D. degree in biotechnology from the Institute of Chemical Technology Prague in January 2011.

88. Determination of isoxanthohumol, xanthohumol, alpha and beta bitter acids, and *trans*- and *cis*-iso-alpha-acids in beer using HPLC with UV and electrochemical detection. Presenter: David Thomas, Thermo Fisher Scientific, Chelmsford, MA, USA. Co-author(s): Paul Ullucci and Ian Acworth, Thermo Fisher Scientific, Chelmsford, MA, USA.

Beer is the most widely consumed alcoholic beverage in the world and the third most popular drink after water and tea. Beer is brewed from four basic ingredients: water, a starch source (e.g., malted barley), brewer's yeast, and a flavoring agent such as hops. Many varieties of beer result from differences in these ingredients, the additives used, and the brewing process followed. While there are many different types of beer, all have one characteristic in common—bitterness. Hop or hop extracts are added during the boiling of the wort. During this time the virtually insoluble alpha-acids (humulones) are isomerized into the more soluble iso-alpha-acids, the main bittering substances in beer. In addition, beta-acids (lupulones) also add to the bitterness in beer. The analysis of hop acids in hops and beer is important for quality control of the beverage. Many HPLC methods have been applied to the determination of bitter acids in beer. HPLC techniques using UV detection typically require a concentration step in the analysis to be able to determine low levels of bitter acids. Application of HPLC with electrochemical detection allows the determination of bitter acids even in light beers without the need for preconcentration. Chromatographic separation was performed on a Dionex Acclaim C30 column (3 µm, 3.0 × 150 mm) at 35°C with gradient elution and simultaneous UV (270 nm) and EC detection (500 and 800 mV). Sample preparation involved extraction with acidified acetonitrile and centrifugation. All calibration curves showed good linear regression ($r^2 > 0.996$). RSDs over a 20-hr run are as follows: isoxanthohumol and xantholhumol, 1.2%; alpha and beta bitter acids, 2.5%; and *trans*- and *cis*-iso-alpha-acids, 2.4%.

Dave Thomas received a Ph.D. degree in analytical chemistry from the University of Nebraska-Lincoln in 1994 for his work developing high-performance immunoaffinity chromatography. In post-doctoral appointments at Midwest Research Institute-California Operations and Sandia National Laboratories, he worked to implement HPIAC and HPLC approaches on miniaturized electrochromatographic separation and analysis platforms. Later, he spent several years developing a variety of IC, HPLC, and LC/MS applications at Dionex Corporation and Thermo Fisher Scientific, where he also served as manager of the HPLC and LCMS applications laboratory. After a few years in vaccine analytical development at Wyeth and Pfizer, Dave returned to Thermo Fisher Scientific, where he continues to develop applications for HPLC with charged aerosol and electrochemical detection.

89. Determining flavors and "defects" in beer by headspace trap/gas chromatography/mass spectrometry (HStrap/GC/MS). Presenter: Lee Marotta, PerkinElmer, USA. Co-author(s): Andrew Tipler and Tom Kwoka, PerkinElmer, USA.

Beer is a popular beverage produced by the fermentation of hopped malt extracted from barley and other grains. Some compounds (flavors) have a positive effect on aroma (attributes) and some have a negative effect (defects). This presentation focuses on a new method that enables the investigation and characterization of flavors and defects of beer in one analysis using HStrap/GC/MS. Classically, this analysis is performed on four separate detectors. This new method employs one detector (MS) to provide the solutions required for the production and testing of beer. The outcome is a more cost-effective, accurate means to ensure the validity and quality control of the product. Other benefits include enhanced productivity, attaining more information from a single analysis, and requiring less bench space. The following experiments and results are discussed: quantitation of dimethyl sulfide (DMS), 2,3-butanedione (diacetyl), 2,3-pentandione, and t,2-nonenal; characterization of several types of beers; fermentation profiling; analysis of raw materials; and aging studies.

Lee Marotta has been employed by PerkinElmer LAS for 20 years as a GC, GC/MS application specialist. Throughout the years, she has helped customers select the appropriate instrumentation for their specific needs and has provided application solutions for both internal and external customers to satisfy stringent analytical requirements. PerkinElmer has provided her the opportunity to work with people in all industries who utilize the technique of gas chromatography/mass spectrometry, assisting customers with method development and troubleshooting support. In addition, she has expertise in sample introduction techniques such as thermal desorption and headspace. Prior to PerkinElmer, Lee worked at Exxon Corporate Research and Hoffman-LaRoche as a method development chemist for gas chromatography.

90. Development and validation of an assay method for phenolic flavor compounds in beer flavor standards. Presenter: Boris Gadzov, FlavorActiV Limited, Chinnor, UK. Co-author(s): Mark Powell, Quay Pharmaceuticals Limited, Deeside, UK; Dale Smith, FlavorActiV Limited, Chinnor, UK.

Flavor standards are a well-established means of training professional sensory panels for the beer and beverage industries. The utilization of cyclodextrin encapsulated flavors provides a more representative and consistent sensory experience than raw flavor materials. Although sensory evaluation of the taste of flavor standards is an important aspect of quality control, using instrumental methods is equally important. Data from instrumental methods of analysis afford a more traceable and less subjective means of assuring quality and batch-to-batch consistency compared to sensory evaluation techniques. This study describes the development and validation of an assay method for seven phenolic flavor compounds (2-methylphenol, 4-ethylphenol, 2,6-dichlorophenol, 2-bromophenol, 2,4,6-trimethylphenol, methoxy-4-vinylphenol, and 2-isopropylphenol) complexed with cyclodextrin for encapsulation as beer flavor standards. An isocratic reversed-phase HPLC method was developed that was capable of separating all seven compounds, using a mobile phase comprising 47% aqueous phosphoric acid (0.1%) and 53% methanol at a flow rate of 0.5 mL min^{-1}. The column was an ACE C18 (150 mm × 4 mm, 3 µm d.p., Advanced Chromatography Technologies) maintained at 35°C. Detection was performed by UV absorbance at either 225 or 261 nm, and the run time was 20 min. Adequate specificity was established for all seven flavor compounds. Over the range of application, correlation coefficient (R^2) values were equal to or better than 0.9995, and *y* intercept values were close to zero. Recovery values for cyclodextrin-complexed samples ranged from 97.4 to 106.9%, and the poorest value for method precision was 3.2% RSD. The method's performance is considered to be more than adequate to control these seven compounds at concentrations relevant to their use as flavor standards.

Boris Gadzov is director of taster management at FlavorActiV Limited in the United Kingdom. Boris provides professional sensory training to taste panels throughout the world for FlavorActiV and to many of the biggest brewers and beverage producers. Boris holds a Ph.D. degree in food molecular microbiology and holds a doctorate in veterinary medicine. A talented linguist, Boris is fluent in a number of languages, including Macedonian, Russian, Croatian, Bosnian, Serbian, Bulgarian, Polish, and German.

91. Development of a fast and reliable microwave-based assay for measurement of malt color. Presenter: Yin Li, Malteurop North America Inc., Milwaukee, WI, USA. Co-author(s): Mary-Jane Maurice, Malteurop North America Inc., Milwaukee, WI, USA.

The ASBC color method (Wort-9) has been widely accepted and used in the malting and brewing industries to determine wort color and is based on the measurement at a single wavelength (430 nm) using a spectrophotometer. However, this method is time-consuming and needs clarified wort generated from Congress mash. It is impossible for satellite laboratories without mashing baths to obtain results. Therefore, the major challenge is to set up a fast and reliable wort color assay method without a routine mashing procedure. In this study, we successfully established a novel and quick method for the measurement of wort color without Congress mashing based on the simulation of mashing by microwave technology. Three important factors involved in the new method, including microwave power, heating time, and grist to water ratio, have been optimized by a randomized complete block statistical design (RCBD). The new method is able to obtain a color result within 30 min. The standard deviation of the new method ranged from 2.4 to 2.8%, suggesting the reproducibility of the new method is very reliable. Forty-five malt samples were measured by both the novel and Wort-9 methods. The results suggested that the new method showed a good correlation ($r = 0.95$) with the ASBC color method, and the difference between the two methods was less than 10%. We feel the method would be of interest to maltsters for quickly checking their shipment samples to brewers and would be useful for those laboratories without mashing equipment. Microwave mashing also utilizes less expensive equipment, making it suitable for more laboratories.

Yin Li is a quality assurance manager at Malteurop North America, Inc. Before joining Malteurop, Yin was a research assistant professor in the Department of Plant Sciences at North Dakota State University (NDSU). He received his Ph.D. degree in fermentation engineering from the School of Biotechnology at Southern Yangtze University in Wuxi, China, working on research in the area of malting and brewing, and did his post-doctoral research work with Paul Schwarz at NDSU. He has published more than 40 papers in international peer-reviewed journals in malting and brewing areas, as well as one book chapter, "Malting and Brewing Uses of Barley." He is the winner of 2007 AACC International Bruce Wasserman Young Investigator Award. Yin has served as a reviewer for more than 10 journals in cereal and food sciences and is an editorial board member of the Journal of the Institute Brewing.

92. Development of a microplate FAN method—Not always as straightforward as expected. Presenter: Mark Schmitt, USDA Agricultural Research Service, Madison, WI, USA. Co-author(s): Allen Budde, USDA Agricultural Research Service (retired), USA.

Many malting quality analysis methods have several versions available, often an original manual method and a newer automated version. For determination of wort free amino nitrogen (FAN) concentration, a long-established manual method (ASBC Wort-12) is available but infrequently used, and an automated segmented flow analysis (SFA) version has recently been recommended for acceptance as an approved method (MacLeod et al, JASBC 69(4):295, 2011). A third format, a 96-well microtiter plate-based assay, is attractive in certain situations. In this study, we compared the manual Wort-12 method for FAN analysis, a standard SFA method (plus several variations), and two microplate versions, using chemistry derived from either the manual or the SFA version. We examined a number of variables, including reagent (ninhydrin) source and grade, incubation time and temperature, and reductant concentration, for the two microplate versions. In general, the adaptation of the SFA reagent yielded a more robust assay with results that matched manual and SFA assays. In contrast, direct adaptation of the chemistry from Wort-12 to a microplate format generated results that did not consistently match those from the other methods. In addition, the Wort-12 process in a microplate format required longer incubations (up to 15 min) at higher temperatures (up to 99°C) for full color development, indicating the assay could be more susceptible to variations in color development. The assay derived from the SFA reagents worked well, generated results that matched those from existing manual and automated methods, and is recommended for FAN analysis in a microplate format.

Mark Schmitt received a Ph.D. degree in plant physiology/plant biochemistry from the University of Wisconsin, Madison. He joined USDA's Agricultural Research Service in Madison in 2003. His research emphasis is on malting quality.

93. Ensuring product quality, efficiency, consistency, and safety through advanced process analytics. Presenter: John Morgan, Mettler Toledo, Bedford, MA, USA. Co-author(s): Brian Vaillancourt, Mettler Toledo, Bedford, MA, USA; Stefan Bardeck, Mettler Toledo, Urdorf, Switzerland.

Implementing proper in-line measurement of DO, CO_2, pH, haze, and conductivity methodologies at specific points in the brewing and packaging process creates opportunities to save money, improve product quality, reduce maintenance time and cost, and improve safety. For example, measuring conductivity in the filling tank and lines has been demonstrated to be an effective method for determining when the water used to clean and rinse the tanks and pipes has been drained away. This measurement technique replaces manual processes that rely either on visual inspection of the flow through a sight glass or simple timing to ensure the water has been flushed away. Being able to detect the true phase separation between water and beer saves labor time and minimizes product waste. Product quality can be enhanced in several ways, such as measuring pH during mashing and wort boiling to maximize yield and consistency. Measuring dissolved oxygen prior to bottling ensures a long shelf life and prevents spoilage. A new technique for monitoring and stabilizing ppb oxygen measurements using optical sensors increases operational uptime with minimal maintenance, improves measurement accuracy, and enhances speed of response. Product safety and cost savings can be achieved through the use of pH sensors to monitor caustic cleaning cycles. When cleaning piping with caustic, it is imperative to ensure that all the cleaning solution is rinsed away before filling the lines with beer. The use of in-line pH measurement can determine precisely when all the caustic has been rinsed away, saving time, reducing operational costs, and assuring product safety. Having sensors in-line creates a need to maintain them to ensure accurate and reliable measurements. With ever-decreasing operating budgets, it is imperative that sensor installation be

simple and that maintenance be predictive. New intelligent "plug and measure" sensors minimize calibration and setup time and offer sensor wear information and alarms to alert maintenance crews to the need to change or maintain sensors before they break down, thus preventing disruption in the production flow.

John C. Morgan is a product manager for Mettler Toledo North America and is responsible for its family of process analytical instruments used to measure dissolved oxygen, pH, conductivity, turbidity, and carbon dioxide. John received his B.S. degree in chemical engineering from Clarkson University in Potsdam, NY.

94. Fast GC-FID method for the analysis of primary hop essential oils. Presenter: Cheryl Ermey, John I. Haas, Inc., Yakima, WA, USA. Co-author(s): Joyce Carr, John I. Haas, Inc., Yakima, WA, USA.

A modified rapid method has been developed for the analysis of hop essential oils by GC-FID based on rapid characterization of hop essential oils using gas chromatography–time-of-flight mass spectrometry. This method, which was published in the *Journal of the American Society of Brewing Chemists* (60(3):116, 2002), is able to successfully identify 40 key common essential oils. The author's modification, which utilizes GC-FID, provides a rapid method for the identification of the four primary hop essential oils: myrcene, humulene, caryophyllene, and farnesene. The current ASBC method for GC analysis of hop oils results in a run time of 86 min. The fast GC method utilizes a smaller and more efficient DB-5 column with a run time of less than 9 min, resulting in a decreased GC analysis time of nearly 10-fold. This modified method works very well for the primary hops oils myrcene, humulene, caryophyllene, and farnesene. However, because of the dilute nature of the sample in the fast GC method, essential oils that occur at less than 1% (e.g., geraniol, linalool) may not be reliably quantitated. An adjustment in the sample dilution protocol may make it possible to include these lesser compounds.

Cheryl Ermey has a bachelor's degree in biology, with a minor in chemistry, from Central Washington University in Ellensburg, WA. She has been employed by John I. Haas, Inc. in the Quality Assurance laboratory since 2008. Prior to working at Haas, Cheryl worked as a microbiology technician for Ag Health Labs in Sunnyside, WA, and as a chemistry technician for Battelle Toxicology Northwest in Richland, WA. She has several years of experience working with gas chromatography, both at Haas and at Battelle. Cheryl also spent five years as a volunteer firefighter with the West Valley Fire Department in Yakima County.

95. Fate of mycotoxins during beer brewing. Presenter: Yasushi Nagatomi, Research Laboratories for Food Safety Chemistry, Asahi Group Holdings, Ltd., Moriya, Japan. Co-author(s): Tomonori Inoue, Atsuo Uyama, and Naoki Mochizuki, Research Laboratories for Food Safety Chemistry, Asahi Group Holdings, Ltd., Moriya, Japan.

Mycotoxins are frequent contaminants of grains and are considered critical risk substances for brewers. Meanwhile, in response to recently increasing consumer demands for food safety assurance, brewers have to present any risks substances that can contaminate their products, based on experimental data. In this study, the fates of mycotoxins in the course of beer brewing were investigated with the view to ensuring improved risk control. Ground malt was artificially contaminated with 14 mycotoxins that are well known and often found as food contaminants and was brewed via steps such as mashing, boiling, and fermentation on a laboratory scale. Analytical samples were taken from wort, spent grain, and beer produced at certain key points in the brewing process. The samples were extracted and purified with the SPE (solid phase extraction) method, and concentrations of the mycotoxins in the samples were analyzed by LC-MS/MS using a multi-residue method. In the results, half of the mycotoxins clearly showed a reduction in concentration in the unhopped wort and were adsorbed onto the spent grain after mashing. In addition, some of the mycotoxins diminished during boiling and fermentation. This suggests that the risks of contaminating beer with mycotoxins was reduced remarkably after the entire brewing process.

Yasushi Nagatomi received a B.S. degree in pharmaceutical science and a Ph.D. degree in synthetic organic chemistry from Osaka University. He began his career in 2000 at Merck Research Laboratories Tsukuba Research Institute (Banyu, Japan), where he was engaged in the development of anticancer substances for eight years. In 2008, he joined Asahi Breweries, Ltd. and currently works in Research Laboratories for Food Safety Chemistry at Asahi Group Holdings, Ltd. As a chief researcher at the laboratories, he is responsible for developing technologies for food analysis for the assurance of food safety.

96. Free and oxidized fatty acids: Comprehensive strategies for separation and quantification from hops, malt, wort, and beer. Presenter: Nils Rettberg, TU Berlin/VLB Berlin, Germany. Co-author(s): Leif Garbe, TU Berlin/VLB Berlin, Germany.

For decades fatty acids (FA) and their oxidation products have attracted the attention of brewers. They strongly influence beer foam and fermentation, are closely linked to beer flavor (in)stability, and may promote beer gushing. Data from free and oxygenated hydroxy fatty acids (HFA) are known from the literature. However, analytical approaches differ considerably in time and chemicals required. In the current paper we present two user-friendly sample preparation procedures for low-level quantification of C12-C20 fatty acids and their abundant oxidation products. The aim of the study was a significant reduction in the amount of sample, chemicals, and costs required. We compare a modified and miniaturized Bligh and Dyer extraction with a solid phase extraction (SPE) assay. Additionally, we introduced a mild and efficient derivatization procedure that enables simultaneous methylation of all free organic acids. Quantification of FA and HFA can be done by GC-FID/internal standard methods. Here, we present a convenient GC-MS route with stable isotope dilution techniques (SIDA). Lab synthesis of deuterium labeled FA and O-18 labeled HFA was performed. Selected ion monitoring mass spectrometry (SIM-MS) strongly increased the sensitivity and selectivity of our analysis. We analyzed wort, beer, and several intermediates to validate our experimental setup and to underline its advantages. High recovery rates and excellent repeatability prove that both sample preparation procedures are very suitable and useful. Compared to assays that have been presented in the past we could strongly reduce solvents, waste, and harmful reagents.

Nils W. Rettberg is a trained brewer and maltster from Radeberger Gruppe, Germany. In 2011, he received a diploma in biotechnology from the Berlin Institute of Technology (TUB) and started as a Ph.D. student at the TUB Chair of Bioanalytics. In addition, Nils is employed at the Research and Teaching Institute for Brewing in Berlin (VLB), Department for Special Analyses. His work includes courses for students of biotechnology and brewing science ranging from basic chemical-technical analysis to more sophisticated modern analytical techniques. As a member of Leif-Alexander Garbe's research group his scientific work focuses on brewing-relevant special analyses using mass spectrometry and stable isotope dilution assays. Initiated by his diploma thesis on "Flavor Active Epoxydecenals," he has developed a deep interest in lipid oxidation, beer staling, and trace analysis in brewing.

97. Hop Aroma analysis in beer using PDMS-stir bar sorptive extraction-GC-MS. Presenter: Yanping Qian, Oregon State University, Corvallis, OR, USA. Co-author(s): Tom Shellhammer and Michael Qian, Oregon State University, Corvallis, OR, USA.

The development of stir bar sorptive extraction (SBSE) allows for simple sample preparation to establish a volatile spectrum. Automated SBSE extraction coupled with gas chromatography-mass spectrometry (GC-MS) is a valuable technique to analyze a wide range of volatile aroma compounds in alcoholic beverages with minimum sample preparation. The PDMS phase is particularly useful to selectively extract hop aroma compounds in beer in the presence of a high concentration of alcohol with high sensitivity and reproducibility. Hop aroma compounds such as alpha-pinene, beta-pinene, myrcene, limonene, alpha-terpineol, linalool, alpha-humulene, and many other compounds can be reliably quantified at the ppb level or even lower. Simultaneous MS full scan and selective mass monitoring enabled quick identification and sensitive quantification. Comparison of beers using different hop varieties showed the Citra variety had the highest concentration of linalool.

Yanping Qian received her B.S. degree in agro-chemistry from Huazhong Agricultural University, M.S. degree in agro-chemistry from the University of Illinois, Ph.D. degree from the University of Minnesota, and MBA from Metropolitan State University (Minnesota). She has been working on many areas related to agriculture and agricultural products, natural resources, and environmental sciences. More recently, she has been working on the flavor chemistry of beer and hops.

98. Indirect detection of microbial contamination in beer by chemical fingerprints. Presenter: Jennifer Koob, Research Center Weihenstephan for Brewing- and Food-Quality, TU Muenchen, Freising, Germany. Co-author(s): Robert Riedl, Mathias Hutzler, Mehmet Coelhan, and Fritz Jacob, Research Center Weihenstephan for Brewing- and Food-Quality, TU Muenchen, Freising, Germany.

A few bacteria, especially species of the genera *Lactobacillus* and *Pediococcus*, are able to spoil beer. Procedures to detect and identify these bacteria are numerous, but nearly all of them are based on the presence of living or augmentable cells. This study attempts to detect beer spoiling microorganisms indirectly using chemical fingerprints. Nine different, as determined by real time-PCR (polymerase chain reaction) and DNA-sequencing, strains of beer spoiling bacteria were inoculated in bottled beer and incubated for a period of at least 2 weeks. As reference samples, uncontaminated blank beers were processed and analyzed in the same way as the contaminated samples. After the formation of turbidity by the beer spoiling bacteria the sample was chemically analyzed. Several chromatographic methods were applied to detect the differences between the contaminated media and the blank samples. The accumulation or degradation of compounds had to be caused by microbial growth. The analyses performed were, for example, the determination of the fatty acid content and the concentrations of aging-relevant substances by GC and flame ionization detector (FID) after steam distillation. In addition, the contents of amino acids, dimethyl sulfide, organic acids, and fermentation by-products were determined by chromatographic methods. The major differences (±60% of the initial values of the blank samples) were collected and transferred to a profile that is specific for every bacteria strain used in this study. The results for *L. brevis* and *L. backi* differed particularly with regard to organic acid, fatty acid, and aging-relevant compound contents. The profiles or chemical fingerprints can be regarded as a novel tool to identify a certain beer spoiling microorganism by recovering them in contaminated beer samples that don't have to contain living cells. This work establishes the basic experimental setup and analysis for further studies on the chemical detection of microbial infections in unfiltered and filtered sections in breweries.

Jennifer Koob was born in Bad Kissingen, Germany, in 1986. From 2005 until 2010 she studied brewing science and beverage technology at TU München and graduated with a Dipl.-Ing. degree. After her studies she worked as an assistant manager for the Lang Brewery, Waltershausen. Since October 2011 she has been a scientific assistant at the Research Center Weihenstephan for Brewing and Food Quality. Her main research topics are beer spoiling microorganisms and their chemical identification.

99. Matrix effect and practical considerations for accurate quantification of acetaldehyde and higher alcohols in beer using headspace GC-FID. Presenter: Qin Zhou, Oregon State University, Corvallis, OR, USA. Co-author(s): Yanping Qian and Michael Qian, Oregon State University, Corvallis, OR, USA.

Acetaldehyde, ethyl acetate, isobutyl alcohol, isoamyl alcohol, and isoamyl acetate are the major volatile compounds in beer fermentation; they are considered important quality indicators for brewing. The determination of these compounds helps to evaluate whether complete and proper fermentation has taken place. Therefore, it is critical for brewers to build a simple and reliable method to analyze these compounds. The ASBC headspace GC-FID protocol was re-evaluated in different beer matrices and alcohol concentrations. Sample size and incubation temperatures were also tested to determine the sensitivity of the compounds. Sample preparation, including acetaldehyde standard preparation, was modified. The result showed that the alcohol content in beer affected the recovery of analytes, as well as the internal standard; however, the alcohol content in typical beer range (less than 7%) did not affect the quantification of these compounds as long as an internal standard calibration method was used. Methyl propionate turned out to be a better option as an internal standard than 1-butanol, since it has much less interaction with the beer matrix. Calibration correlation coefficients for all compounds were better than 0.997, and good repeatability was also obtained. Detection limits were below the normal ranges of concentrations in beer and also below the odor thresholds. Recoveries were nearly 100%. The improved procedure could be a more accurate alternative to the ASBC standard procedure for analyzing these compounds in beer.

Qin Zhou received a B.S. degree in chemistry from Wuhan University in China in 2006, and her M.S. degree in fermentation engineering from China National Research Institute of Food and Fermentation Industries in 2009. In 2010, she began pursuing 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 the flavor chemistry of alcoholic beverages, including beer and wine.

100. "Just shoot"—Quick and easy determination of hop iso-alpha-acids in beer. Presenter: Michael Heidorn, Thermo Fisher Scientific, Germering, Germany. Co-author(s): Markus Martin and Frank Steiner, Thermo Fisher Scientific, Germering, Germany; Rainer Bauder, Thermo Fisher Scientific, Chelmsford, MA, USA.

Sample preparation is a crucial point in the workflow of beer analysis, especially during the brewing process where very complex matrices form the basic samples. Various sample

preparation techniques are used to remove the beer matrix or enrich analytes of interest, with solid-phase extraction (SPE) being commonly used when HPLC is applied for analysis. Typically SPE is performed manually, thus causing a significant bottleneck as well as error source in laboratory workflows. Furthermore, samples need to be analyzed in time due to the ongoing process in the brew kettle. However, conventional analysis takes about an hour due to sample pretreatment steps, a manual SPE procedure, and RP-HPLC separation. An automation of this sample cleanup and analyte enrichment process eliminates all the issues described above, while the high-speed capabilities of UHPLC technology can significantly reduce the time required for chromatographic separation. Hence, direct injection of untreated beer samples becomes feasible, ensuring higher confidence in the analytical result and higher throughput by an unattended operation. In this presentation, the UHPLC separation of hop iso-alpha-acids in beer with an automated on-line SPE solution is demonstrated. An untreated beer sample is injected directly, all SPE steps are performed automatically, and the whole analysis lasts only 9 min. Thus, no manual sample pretreatment is needed, and the result of the high-speed separation reflects the content of iso-alpha-acids in the beer virtually in real time. Using this automated on-line SPE RP-UHPLC approach, the bitterness, foam, and stability of a beer can be controlled in a quick and easy way.

Michael Heidorn, born in 1980, completed an apprenticeship as a chemical laboratory assistant at Honeywell Specialty Chemicals GmbH, Seelze, Germany, and thereafter worked for the Research and Development Department of this company. Afterward, he studied analytical chemistry at the University of Applied Science in Luebeck, Germany. He completed his diploma thesis on the influence of frictional heating on column efficiency in UHPLC at the Research and Development Department of Dionex-Softron GmbH, now part of Thermo Fisher Scientific, and obtained a graduate chemical engineer degree. He then began working as a solution specialist in the HPLC Marketing Department of Thermo Fisher Scientific.

102. Near real-time monitoring of carbohydrates during beer processing by a microchip capillary electrophoresis technology. Presenter: Dale Willard, Carbo Analytics, LLC, Fort Collins, CO, USA.

Sugars are the fundamental intermediary in the brewing process as barley is broken down into sugars before being fermented into ethanol, and monitoring them throughout the process is critical to flavor profile and process efficiency. If an on-line sugar measurement solution was available, brewing facilities could make production adjustments during the process to rapidly dial in batches to within specifications, flag problem variations at an early stage, and quickly pursue corrective actions. Our market research suggests an average facility will save nearly US$1 million annually. Because we hold the only demonstrated technology for combining capillary electrophoresis and pulsed amperometric detection on a microchip format, we can uniquely offer fast, simple, and reliable sugar analysis. A core team of researchers have come together to form Carbo Analytics, LLC (CARBO) with the sole mission of adding value to carbohydrate-based commodities through reliable process monitors. Funded through a U.S. Department of Agriculture Small Business Innovative Research Phase II award (no. 2011-33610-31198), CARBO is currently transforming a proof-of-concept prototype into demonstration units (breadboard and production instruments) meeting end-user specifications and addressing performance, reliability, longevity, and manufacturability. Instruments will be field tested with three key industry leaders. On-line instruments automatically sip, filter, and deliver samples to our microchip for analysis. The instrument design calls for automated analysis of a five sugar panel with a measurement time from sip to a reported result in <15 min. Bench-top instruments, about the size of a brief case, provide results in <2 min with minimal sample pretreatment by the user.

Dale Willard is founder and president of Carbo Analytics, LLC. He was formerly founder and principal scientific investigator for Advanced MicroLabs, LLC. He received his Ph.D. degree in analytical chemistry from Colorado State University and B.S. degree in chemistry from the University of California, Davis. He has served as principal investigator for 11 projects (3 SBIR Phase II awards) and developed 2 commercial prototypes. He has 13 peer-reviewed scientific papers to his credit, is an expert reviewer for several scientific journals and NIH and NSF grant panels, and author of 12 funded scientific research proposals. He is a member of the American Society of Brewing Chemists and the American Chemical Society.

103. New insights on preservation of beer with a high oxygen reduction potential. Presenter: Frank Verkoelen, Pentair Haffmans, Venlo, Netherlands.

Preventing oxygen (O_2) pick up during the production and packaging of beer is essential to preserve product quality (freshness) and taste stability and lengthen shelf life. As a result, an increasing focus is being placed on beer's O_2 content during the production and packaging processes. As part of bottle-fermented beer production, O_2 is added to the bottles. The addition of yeast for this type of beer reduces the O_2 and brings the beer to specification, which should preserve the beer quality during its shelf life. The O_2 behavior in beer with high O_2 reduction potential, as well as bottle-fermented beer and standard beer was investigated by measuring total packaged oxygen (TPO). The classical *Z* method of TPO measurement based on measured dissolved oxygen (DO) is compared with TPO based on the differentiated measurement of headspace oxygen (HSO) and DO. The application of differentiated TPO measurement provides valuable new insights into the oxidation/reduction dynamics of beer with high O_2 reduction potential; the weaknesses in the classic *Z* method; and how well the quality of this beer is preserved.

Frank Verkoelen studied mechanical engineering at HTS Venlo and finished in 1982. He has worked for Haffmans BV since 1984, where he started as a project engineer for CO_2 recovery. He joined the R&D team in 1987 and became the R&D manager. In 2001 he moved into the quality control product manager position and became the senior product manager responsible for sales of quality control and in-line equipment.

104. Owlstone's FAIMS-based ("field asymmetric ion mobility spectrometry") chemical analyzer quantifies diacetyl, contaminants, VOCs, and much more in real-time right at the point of need. Presenter: Steven Freshman, Owlstone Inc., USA.

Owlstone's portable instrument quantifies concentrations of in-process chemicals of interest (such as diacetyl), raw material contaminants, CIP residues, flavor mal odors, VOCs, and much more in real time (within seconds) at ppb/ppm levels. The instrument platform is Owlstone's FAIMS-based system ("field asymmetric ion mobility spectrometry"). FAIMS is a detection technology that separates and identifies chemical ions based on their mobility under a varying electric field at atmospheric pressure. Utilizing its proprietary FAIMS-based platform, Owlstone Inc. has created Lonestar, a portable rapid detection instrument. The heart of the Owlstone FAIMS detec-

tion technology and the Lonestar instrument is a dime-sized, silicon chip spectrometer. Multiple analytes can be identified and quantified within seconds at low trace concentrations. The instrument is portable and is designed to be operated by non-technical personnel. At the push of a button, non-technical production staff is able to identify and quantify chemicals of interest right at the point of need in the brewery. Some of the applications discussed include the real-time detection of diacetyl, amyl acetate, acetaldehyde, raw material contaminants, flavor mal odors, and water residues.

Steven Freshman, global GM for Owlstone, is responsible for FAIMS partnering and installation efforts globally for Owlstone's Industrial Process Control applications across the brewing, food, pharmaceutical, and oil and gas industries.

105. Rapid determination of high molecular weight 1,3/1,4-beta-D-glucan by a novel photometric method. Presenter: Liisa Otama, Thermo Fisher Scientific, Vantaa, Finland. Co-author(s): Sari Tikanoja, Sari Hartikainen, and Annu Suoniemi-Kahara, Thermo Fisher Scientific, Vantaa, Finland.

In the beer malting and brewing process, one important analyte is beta-glucan. Beta-glucans are polysaccharides of D-glucose monomers linked by beta-glycosidic bonds. Beta-glucans are present in the cell walls of cereals and are capable of clogging process filters. Excessive amounts of beta-glucan may cause haze in the end product and impair the taste of beer. For these reasons it is important to determine the concentration of beta-glucan, in particular the part of the beta-glucan polymer that has a molecular mass of about 10,000 Da or more. The aim of this study was to provide a robust automated photometric analysis method that is suitable for liquid samples derived from processing of cereals. A rapid two reagent method was developed for automated discrete analyzers. The method is easily adapted to a manual spectrophotometer as well. The use of blank buffer eliminates sample color interference. In preliminary testing of beer and wort samples, this method correlates well with the results obtained by a fluorometric method using Calcofluor fluorescence dye. The Calcofluor method is recommended by the European Brewery Convention in EBC Methods 8.13.2, 4.16.2, and 3.10.2 and by the American Society of Brewing Chemists in ASBC Method Wort-18. Thus, this study presents an alternative rapid method especially suitable for routine use. A method performance study was done by Thermo Scientific Gallery and Arena discrete analyzers at wavelength of 405 nm. Method linearity was determined between 15 and 500 mg/L with aqueous beta-glucan standard solutions. Beer and wort samples tested showed excellent repeatability and reproducibility, with typical variation being 2% or less. Total analysis time for 9 samples with 10 replicates (total 90 results) was less than 40 min. As an improvement on the existing fluorometric method, the open on-board stability of these novel non-hazardous reagents was tested to be at least 30 days.

106. Resonance light scattering technique for the determination of proteinase A activity. Presenter: Qun Song, Jiangnan University, Wuxi, China. Co-author(s): Jinjing Wang and Qi Li, Jiangnan University, Wui, China.

A simple and sensitive method had been developed for the determination of proteinase A activity using a resonance light scattering (RLS) technique. The method was based on the principle that TCA and trace protein can form association particles and the particles' concentration proportioned resonance scattering light intensity under certain conditions. In this study, several methods were compared for proteinase A activity measurement. In the meantime, the influence of temperature, pH value, and TCA concentration were investigated. This new method was fast and convenient and had a low detection limit (0.595 g/mL) and wide linear range (1–200 g/mL). The system had better tolerance against interfering substances, and the reaction system remained stable for at least 150 min. The method was satisfactorily applied to the determination of trace proteinase A activity.

Qun Song, a master's degree student in biotechnology at Jiangnan University in China, is mainly engaged in research on detection methods and secretion of proteinase A.

107. SBU—A new and rapid method for determining bitterness in beer. Presenter: Philip Wietstock, Technische Universität Berlin, Fachgebiet Brauwesen, Berlin, Germany. Co-author(s): Thomas Shellhammer, Department of Food Science and Technology, Oregon State University, Corvallis, OR, USA.

Determining a beer's bitterness is an important parameter for beer quality. The international bitterness units (IBU) method, the archived ASBC iso-alpha-acids (IAA) method, and examination of hop bitter acids concentration using high performance liquid chromatography (HPLC) are prone to operator error, are time-consuming, and require expensive equipment, respectively. In this study, a solid phase extraction technique was modified for the spectrometric determination of beer bitter units (SBU). Using 30 different commercially available beers, the new SBU method was compared to the IBU method and the archived ASBC IAA method. The same data were correlated with hop acid concentrations as determined using HPLC and sensory bitterness as perceived by a taste panel of 11 trained panelists. The method's repeatability and reproducibility were examined in a collaborative study (six collaborating labs) with three sample pairs differing in hop bitter acid concentration and level of dry-hopping. The repeatability and reproducibility was then calculated according to ASBC *Methods of Analysis*. Plotting all data from IBU, IAA, and SBU against iso-alpha-acid concentration as measured via HPLC yielded the highest coefficient of determination for the IAA method (R^2 = 0.97), followed by the SBU method (R^2 = 0.96) and the IBU method (R^2 = 0.90). Sensory bitterness data displayed the highest linearity with the new SBU method (R^2 = 0.93) compared with data from the IBU method (R^2 = 0.90) and IAA method (R^2 = 0.90). Variance analysis indicated that the SBU method had the lowest variance (Var = 226) followed by the IAA method (Var = 286) and the IBU method (Var = 371). Repeatability and reproducibility coefficients of variation for the SBU method ranged from 1.12 to 3.32% and 4.61 to 15.56%, respectively. Taking all the data together, there is an indication that the SBU method is a repeatable and precise measurement for determining both concentration of iso-alpha-acids and sensory bitterness. This new method features elimination of trimethylpentane and is relatively simple to execute using solid phase extraction media.

Philip Wietstock is a scientific assistant at the Technische Universität Berlin, Germany. After graduating from his biotechnology studies with a diploma in engineering from the Technische Universität Berlin (2009), he worked for one year as an intern at the Department of Food Science and Technology at Oregon State University, Corvallis, OR. In 2011, he transferred to his present position, where he is working on his dissertation which focuses on the investigation of the influence of hops on oxidative beer stability.

108. Stale aldehyde analysis by in-solution PFBHA derivatization and SPME-GC-ECD. Presenter: Qin Zhou, Oregon State University, Corvallis, OR, USA. Co-author(s): Michael Qian, Oregon State University, Corvallis, OR, USA.

Aldehydes are important flavor compounds that are widely distributed in every food system, contributing positively or negatively depending on their concentration and the food system. Stale aldehydes, including Strecker and saturated and unsaturated aldehydes with chain lengths of C8–C10, are generated from degradation of amino acids and oxidation of lipids, which are typically associated with beer oxidation during storage. Some of these compounds, such as (*E*)-2-nonenal and (*E,Z*)-2,6-nonadienal, can cause a "sawdust" off-flavor in beer at concentrations as low as 0.1–0.3 ng/mL^{-1} (ppb). However, the aroma contribution of these aldehydes to beer flavor is poorly understood due to the technical difficulty in measuring these compounds at such low concentrations. In this study, a sensitive method for the determination of stale aldehydes has been developed. Aldehydes were derivatized with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) in solution at 50°C for 40 min. The corresponding oximes were extracted using DVB-PDMS solid phase microextraction (SPME) and analyzed by gas chromatography–electron capture detector. The limit of quantification for most compounds was as low as 0.1 µg/L (0.1 ppb), and the linearity held at least up to 50 µg/L with R^2 in the range of 0.991 to 0.999. The method was successfully applied to analyze stale aldehydes in beer samples.

Qin Zhou received a B.S. degree in chemistry from Wuhan University in China in 2006, and her M.S. degree in fermentation engineering from China National Research Institute of Food and Fermentation Industries in 2009. In 2010, she began pursuing 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 the flavor chemistry of alcoholic beverages, including beer and wine.

109. The effect of hop processing and exposure time on dry hop aroma extraction. Presenter: Peter Wolfe, Oregon State University, Corvallis, OR, USA. Co-author(s): Thomas Shellhammer and Michael Qian, Oregon State University, OR, USA.

The rate of hop aroma compounds extracted from Cascade hops during dry hopping was studied using both an unhopped beer and a model beer system devoid of malt and yeast aromas. Cascade hops pelletized by four different processors yielded different particle size distributions and pellet densities. These pellets, as well as whole hops, were dosed into a degassed medium (water, 6% [v/v] ethanol, pH 4.2), and the hop aroma extraction was measured periodically over a 1 week period. Solid phase micro-extraction (SPME) followed by gas chromatography (GC-FID) was used to analyze the levels of aroma compounds in the extraction medium. Variation in hop pellet physical properties did not significantly impact the extraction rate of hop volatiles such as linalool, geraniol, limonene, and myrcene, with one exception. One treatment showed an increased absolute concentration of geraniol. Separately, dry hop aroma extraction was measured over a short time (1 day) at room temperature in the unhopped beer. Irrespective of the hop form (whole or pellet), the concentrations of hydrocarbon terpenes peaked between 3 and 6 hr and subsequently declined, while the concentrations of terpene alcohols continued to increase throughout the 24 hr dry hop extraction. The rate of hop aroma extraction appeared to be significantly influenced by hop pellet properties and occurred rather rapidly regardless of hop form.

Peter Wolfe received a B.S. degree in physiology from the University of Oregon in 2007. After graduation, he worked as a researcher at the University of Oregon, publishing work on construction ergonomics. He began working toward a master's degree at Oregon State University in 2010 in the Food Science and Technology Department. Working under Tom Shellhammer, his work focuses on hop aroma in beer and aroma extraction during the dry-hopping process.

110. Thermodynamic properties of primary gushing of beer. Presenter: Guy Derdelinckx, KU Leuven, Department of Microbial and Molecular Systems (M^{2}S), Malt and Beer Sciences (MBS), and Leuven Food Science and Nutrition, Belgium. Co-author(s): Mohammadreza Khalesi, Sylvie Deckers, and Kurt Gebruers, KU Leuven, Department of Microbial and Molecular Systems (M^{2}S), Malt and Beer Sciences (MBS), and Leuven Food Science and Nutrition, Belgium; Vladimir Ilberg, Hochschule Weihenstephan-Triesdorf, Fakultät Gartenbau und Lebensmitteltechnologie, Freising, Germany; Jean Titze, National University of Ireland, University College Cork, School of Food and Nutritional Science, Cork, Ireland; Christina Schönberger, Barth-Haas Group, Barth Innovations, Joh. Barth und Sohn, Nuremberg, Germany; Hedwig Neven, Brewery Duvel-Moortgat, Puurs, Belgium; Jean-Marie Rock, Orval Brewery, Villers devant Orval, Belgium.

Primary gushing of beer consists in overfoaming of beer out of the bottle. Nowadays, the mechanism of this phenomenon is rather well understood, and it is possible to tackle exact aspects of the explosion. The problem is studied by considering the volumetric and the barometric parameters of CO_2, temperature, and shaking effects and their influences on the quantity of energy transferred to the bottle. By considering that the bottle opening at atmospheric pressure takes place under adiabatic conditions, that a closed beer bottle is an isochoric thermodynamic system, as well as that all the procedures take place at low pressure, the ideal gas law can be applied. With the theoretical calculation it could be shown why differences exist when applying the same methods at different analytical labs on identical malt samples. This is the case for results observed for gushing sensitive malt samples analyzed by the modified Carlsberg method The calculation model was tested for malt samples in order to determine an interval of confidence regarding the analytical lab conditions and the risks of industrial use of contaminated grains.

Guy Derdelinckx (1954) has been teaching specialized microbial aspects of barley, malt, and beer at KULeuven-Belgium since 2002. After obtaining an M.S. degree in tropical and subtropical sciences (1978), he successively obtains a master in brewing sciences degree (1979) and a Ph.D. degree (summa cum laude, 1985) on the flavanoid issue and boiling. After starting his professional work at the Université Catholique de Louvain, assuming the responsibility of the assay station, he became the scientific advisor for different Belgian breweries. He joined KULeuven in 1994 and for eight years he used his expertise internationally. In 2005, he went back to research and, more precisely, to exact science and focused his work, together with his research group and with the support of industry friends, on understanding the fundamentals of the mechanisms of beer gushing.

111. Turbidity and haze identification in beer—An overview. Presenter: Martina Gastl, Lehrstuhl für Brau- und Getränketechnologie, Freising, Germany. Co-author(s): Elisabeth Wiesen, Barth Innovations, Nürnberg, Germany; Thomas Becker, Lehrstuhl für Brau- und Getränketechnologie, Freising, Germany.

Turbidity provides the consumer's first visual impression of beer quality. Consumers expect a filtered beer to be a clear, bright, non-hazy product that remains so during its shelf life. Hazy products are often regarded as defective and perhaps even potentially harmful. Therefore, controlling haze formation is an important problem in beer production. For breweries not only costs from rejected turbid beers and therefore an "image problem" arise, but also increased costs due to the

higher use of filter aids have to be considered. It is well known, that beer is a complex mixture of over 450 constituents. In addition, it contains macromolecules such as proteins, nucleic acids, polysaccharides, and lipids. Proteins influence the entire brewing process with regard to enzymes, which degrade starch, beta-glucans, and proteins. Protein-protein linkages stabilize foam and are responsible for the mouthfeel and flavor stability of beer. Together with polyphenols, proteins are thought to cause haze formation. With this complexity, problems in processability are as various as the constituents. Several substances in beer are responsible for haze formation. Organic compounds such as proteins, polyphenols, and carbohydrates (alpha- and beta-glucans) are known to form haze. In addition, inorganic particles such as filter aids and label residues can cause increased turbidity. In the brewery it is necessary to have methods not only to identify the haze, but also to determine the source of the haze formation. A simple, reproducible, and low cost analysis procedure that can be carried out with basic laboratory equipment demonstrates that the source of haze particles in beer (raw material, yeast, etc.) can easily be determined and technological factors during the brewing process of haze formation can be monitored step by step. This study presents an overview of several research studies (haze formation and haze identification), as well as analytical methods for haze formation, protein analysis, and haze identification, including dyeing methods, microscopic analyses, and size exclusion chromatography.

Martina Gastl apprenticed as a brewer and maltster from 1994 to 1996 in Klosterbrauerei Andechs, Germany. She studied brewing and beverage technology at the Technische Universität München-Weihenstephan, Germany. She graduated as an engineer in 2002. From 2002 until 2006 she completed her Ph.D. concerning the "Technological Influence on Lipid Degradation in Terms of Improvement of Beer Flavor Stability." She is currently assistant professor and head of the laboratory, as well as the raw material and beverage design research group, at the Lehrstuhl für Brau- und Getränketechnologie in Weihenstephan. Since 2008 she has been working on her postdoctoral lecture qualification. Her research interests involve characterization and interaction of flavor active taste and aroma compounds in cereal-based beverages influencing beverage harmony.

258. Identification of yeast by MALDI–TOF MS. Presenter: Jana H Gierds, Research and Teaching Institute for Brewing, Berlin, Germany. Co-author(s): Isil Baki, Research and Teaching Institute for Brewing, Berlin, Germany; Christina Quandt, NovaBiotec Dr. Fechter GmbH, Berlin, Germany; Erik Pollmann, Johannes Bader, Roland Folz, and Diedrich Harms, Research and Teaching Institute for Brewing, Berlin, Germany.

Reproducible production of beer and bakery products is based on the application of specific culture yeast strains. Microbiological purity and physiological fitness are strongly required to ensure controlled processes and the production of desired products including the aroma profile. Fast and reliable detection methods are required to achieve process control. The presented detection method is based on the determination of exact molecular masses of yeast proteins using matrix assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS). For this purpose, a yeast sample is solubilized and mixed with an appropriate organic matrix followed by a co-crystallization directly on a sample plate. A laser beam (e.g., nitrogen laser) is focused and hits the sample in pulses. The absorption of the photonic energy of each laser pulse leads to the desorption of the crystal and the formation of partly ionized matrix and protein molecules and the ionization of analyte molecules by charge transfer. Ionized molecules are accelerated in an electromagnetic field, which is the start of the separation process that is the basis of the time-of-flight principle. This velocity depends on the mass of the ions with heavier molecules having a higher moment of inertia and hence a lower velocity. The mass spectrum can be used for yeast fingerprinting and process monitoring. With the achieved spectra a database will be built to enable fast and reliable identification of baker's and brewer's yeasts. Furthermore, the detection of wild yeast or undesired bacteria is a goal. This method is underpinned by PCR, a reproducible reference analysis, based on the alignment of specific selected DNA sequences. The described detection system is completed by a newly developed sampling kit enabling safe shipping and conservation of the samples. This enables small- and medium-sized enterprises to improve their process control without having their own MALDI-TOF system.

Food chemist Jana H. Gierds studied at the Technical University of Berlin. She started work as a scientific assistant in the Central Laboratory of the Research and Teaching Institute for Brewing in Berlin in 2010. Since September 2011 she has been working on the presented project "Identification of Yeast by MALDI–TOF MS." (ZIM [zentrales innovationsprogramm mittelstand] KF2132320SK1).

Brewhouse Operations

112. Compact brewhouse for up to ten brews/day and 250,000 hL/year. Presenter: Fred Scheer, Krones Inc., Franklin, TN, USA.

The individual configurations and characteristics of brewing vessels allow customized solutions for particular requirements and optimum flexibility for brew sizes and brewing processes. Three to five vessels can be combined in a modular framework concept, depending on the mash process involved (infusion or decoction) and the daily output needed. These modules can be arranged in a row, at an angle, or in a square. The space required is only about 110 m^2 for a complete four-vessel brewhouse. All media connections and pipe systems are standardized for all variants; extensions are easy to implement. The framework concept allows high flexibility in the combination of the vessels and is also standardized. The modules are supplied completely installed in two to four parts, while the vessels are inserted into the modules during final assembly. This modular design allows minimized installation and commissioning times. With four optional components, all needs can be satisfied: our well-known wet mill (Variomill 5.2), a combined trub and weak wort tank, a single tank CIP system, and for energy recovery a vapor condenser for production of hot water. All vessels are equipped with our approved Steinecker technologies for maximized wort quality. The heating process, for both mashing and wort boiling, is implemented with ShakesBeer Pillow plates, which generate a turbulent mash flow directly on the heating surface for ultra-effective heat transfer. The lauter principle is based on the field-proven level control of Pegasus, and the size decision criterion is the specific false bottom load in dependence on the targeted brewing rhythm. The Stromboli Venturi nozzle is integrated into the vessel for the wort boiling variants. During the boiling phases, the wort can be circulated with an external pump and the Venturi nozzle alone, without any heating. Stromboli allows the circulation of the wort to be separated from the evaporation for a reduction in free DMS content with reduced energy input. Hot sludge separation is effected by a state-of-the-art whirlpool with the right ratio between the wort level and vessel diameter. The wort cooling system can be fitted with a one or two-stage heat exchanger, depending on the customer's re-

quirements. The fully automatic brewing and cleaning processes are managed by the batch-oriented technology software BOTEC. This software has an integrated order and recipe management system, batch logging and trend image recording, and a remote maintenance capability. In a word—big business in small vessels.

Fred Scheer graduated in 1976 as brew and malt master from the Doemens Brewing Academy in Munich, Germany. After that, he worked in several breweries and alcohol-free operations in Europe. In 1985 he immigrated to the United States and started and operated several microbreweries. Currently he is director of brewing and process technology for Krones Inc. in Franklin, WI.

115. New results of procedural analysis methods for mash characterization. Presenter: Johannes Tippmann, TU München, Wissenschaftszentrum Weihenstephan, Lehrstuhl für Brau- und Getränketechnologie, Freising, Germany. Co-author(s): Simon Henke, Jens Voigt, and Karl Sommer, TU München, Wissenschaftszentrum Weihenstephan, Lehrstuhl für Verfahrenstechnik Disperser Systeme, Freising, Germany; Thomas Becker, TU München, Wissenschaftszentrum Weihenstephan, Lehrstuhl für Brau- und Getränketechnologie, Freising, Germany.

Technological aspects of the mashing process have been well investigated. Parameters like pH, saccharification, and enzyme activity are used in standard analyses to judge the quality of the wort. The influences of raw material quality, fineness of the ground malt, and temperature profiles during mashing have also been well explored. A new view of the status of mash is provided by procedural analytical methods, which additionally can present new possibilities for optimizing the lautering process. Four analytical methods have been investigated and explored in the past last years. One very quick method is particle analysis with laser diffraction. It very clearly shows the status of the particles in the mash depending on the temperature and enzyme activity. The second method is picture analysis in a microscope mash tun, where the starch particles can be observed directly during the mashing process. The third method is photon density wavelength analysis, a laser measurement of the whole particle status in a suspension like mash. The particle status of the mash can be observed inline using this method. The fourth method is analysis of the flow potential of mash. This can provide important information about the filterability of mash in the lauter tun. This poster gives an overview of the four methods and the most important results. The paper is a review concerning the methods and new results.

Johannes Tippmann graduated from university in 2004 as a diploma engineer for brewing sciences and beverage technology. In 2005 he started his Ph.D. thesis with Karl Sommer at the Lehrstuhl für Verfahrenstechnik Disperser Systeme, TU München, on solids handling in the brewhouse. He collected wide experience with the procedures in beer production during his studies, conducting student research projects and his diploma thesis on this topic. In 2012 he changed his affiliation and is now working for the Lehrstuhl für Brau- und Getränketechnologie, TU München. He is group leader for the work group Brewhouse Processing and Dispense Systems. Since 2000 he has worked as a student research assistant with dispensing systems and has collected much experience in this subject area. Since 2006, he has been responsible for research issues in dispense systems. He is also a member of the Dispensing Systems Technical Committees of the government association for the food and catering industry (BGN) and of the DIN German Institute for Standardization. He is working for the MEBEK dispense group and has published a number of papers.

116. The false bottom's free passage area—Important feature or negligible? Presenter: Simon Henke, TU München, Chair of Process Engineering of Disperse Systems, Weihenstephan, Germany. Co-author(s): Jens Voigt and Karl Sommer, TU München, Chair of Process Engineering of Disperse Systems, Weihenstephan, Germany.

The lautering process is the most time-consuming step in wort production. Besides mash filters, the lauter tun is still the most common device used for mash filtration in the brewhouse because no other separation techniques have been established. For this reason equipment suppliers have done a lot of development work on existing lauter tuns that has led to acceleration of this filtration step. Nevertheless, there are still many open questions regarding the performance of mash filtration in the lauter tun. Specifically, causes of filtration problems during this unit operation have not been fully investigated. High flow rates through the filter cake often lead to increased compaction of the compressible filter cake. This work investigates how false bottoms with different free passage areas influence the lautering performance and composition of the grain cake. For this reason a pilot scale glass lauter tun was constructed, offering the opportunity to make an image analysis of the grain cake during the whole process. The lauter tun is equipped with four different false bottoms with defined free passage areas between 6 and 20%, which cover the range of available industrial scale systems. Besides optical analysis the most important physical parameters of lauter wort are recorded in-line. The presented filtration equations allow the determination of filter cake permeability and the development of permeability during filtration with these measurements. The experiments conducted provide an answer to the introductory question whether the false bottom's free passage area is important for filtration.

Simon Henke graduated from Technical University Munich in 2009 with an engineering degree in brewing sciences and beverage technology. In 2010 he started his work at the Chair of Process Engineering of Disperse Systems, TU Munich, as a research associate. His fields of activity are mass transport phenomena and procedural aspects of the mashing process. He is responsible for the pilot plant brewery at the Chair of Process Engineering.

117. The mechanical principles of the whirlpool. Presenter: Udo Funk, GEA Brewery Systems, USA.

To optimize the fermentation process it is necessary that the yeast cells have a well-functioning substance exchange through the cell wall. Due to this, approx. 95% of the hot trub accumulated during boiling must be removed. After discussing the formation and properties of hot trub, the available methods of separation, filtration and sedimentation, are described. The sedimentation speed of the hot trub particles determines the process performance of the settling tank, centrifugation, or whirlpool separation. The most commonly practiced means of trub separation in wort handling is the whirlpool. Very large amounts of pellet hops or whole hops require one to individually customize the whirlpool design in order to achieve good hot trub separation at minimum extract losses. Other aspects of trub handling such as reuse and disposal are also discussed.

Udo Funk graduated from the Technical University of Munich (Weihenstephan) as a brewing engineer in 1994. Since 1995 he has worked in the brewery supply industry, with a focus on the process side, from malt handling to beer filtration. He has worked as a commissioning engineer, process engineer, and project manager in Europe, Latin America, and North America. Since 2007 he has lived with his family in St. Paul, MN, and represents GEA Brewery Systems as the senior sales engineer for North America.

Cleaning/Sanitation

118. Clean—What does it mean? CCP control with ultraviolet: Where, when, how? What are the controls and solutions gained? Presenter: Troy Smith, Radiant Industrial Solutions, Houston, TX, USA.

Throughout the brewing process we have seen an increase in the critical control points, as well as the need to improve processing through contamination control. In air, surface, and water the effort to use UV technology is growing; however, the method to integrate this technology as a reliable means within CCP is changing through sensing technology. We now have the ability to control via UV the sanitary state of storage tanks, compressed air, air cooling, water, and water waste. UV technology is available to control cleaning methods through microbial measured disinfection directly to bottle, cap, storage tank, filling rooms, and product transport. While the technology of UV is vast in its ability accurate measurement and control of this technology is often not utilized. We have learned over many years of working with UV that air, surface, and water quality can be controlled through UV measurement. Performance standards have been established by the EPA, Ashrae, FDA, and other associations. While the EPA has provided guidelines, it is still in the hands of equipment owners to maintain a working system that will meet application requirements. Third party service groups such as Radiant Industrial Solutions, Nalco, and others work with end-users to establish working standards that are site-specific. The end-user focus should be on the reliance of a reporting system that indicates performance information critical to the operation, such as energy levels, temperature, oxygen levels, degradation, transmittance, and application operational points. With this information the UV system becomes an asset to operation through performance and data points. The data collection offers management of CCP steps through reporting differences in air, surface, and water quality. Through proper management of UV systems, processes become intelligent, with the ability to interlock and start/stop processes based on environmental conditions. The focus of technology sensing is designed to monitor the air, surface, and water quality passing through a UV system from the feed source. Performance is measured on UV penetration through air, water, and surface exposure. Within each system attributing factors are designed that will determine performance set points. Further definition of air or water quality both in application and reporting will determine how to maintain the process. The measurement ability of UV is providing the industry with the ability to offer solutions to CCP points that have not been seen in the brewhouse to date. UV sensing provides the ability to control disinfection to a level of microbial contamination control. This technology advancement is providing the industry with control methods not previously seen when discussing "Clean—What does it mean?" It is now realistic to control air, surface, and water contamination in-process to include bottle, cap, storage both in dry and liquid form, water process, waste, and recycle. The advancements in UV technology are changing processes through contamination removal that directly improves process and cleaning downtime.

Troy Smith is the president of Radiant Industrial Solutions, LLC based in Houston, TX. Troy has been in the ultraviolet air, surface, and water markets for more than 25 years. Prior to Radiant Industrial Solutions, Troy has worked with Trojan Technologies, Aquafine, Technical Connections, and Ultraviolet Systems and Equipment, as well as filtration companies. Troy has been involved in regulatory compliance, as well as organizations, including, IBWA, ISBT, Ashrae, IUVA, SGIA, Radtech, and other technical committees. Over the past 10 years Troy has been involved with product patents and process improvements, as well as providing training seminars and educational training on topics surrounding ultraviolet technologies throughout various industries and tradeshows.

119. Sanitation challenges for the growing brewery. Presenter: Dirk Loeffler, Loeffler Chemical Corporation Atlanta, GA, USA.

As smaller breweries grow larger in output, new and used equipment, as well as changing conditions and processes, continuously generate new sanitation challenges. This presentation looks at commonly found problems and challenges in a growing brewery while showing approaches and solutions. Among other subjects, the effect of temperature differential in various size tanks is discussed, as well as changing fluid mechanics in process piping and what to avoid. Field studies from various areas in the brewery will be presented and practical solutions for any size brewery given. Growing can be challenging, but knowing what to look out for and maintaining proper sanitation will arm you with the knowledge to successfully take on the challenge.

Dirk Loeffler is the technical director of Loeffler Chemical Corporation, a chemical company specializing in sanitation products and chemical automation for breweries with corporate offices in Atlanta, GA. In his position, Dirk continuously develops and implements new products, product applications, and cleaning technologies. Born and raised in Cologne, Germany, Dirk came to the United States in 1992 to lay the groundwork for the U.S. operations of Loeffler, resulting in the incorporation of Loeffler Chemical Corporation in 1994. Dirk graduated in 1989 with a degree in business administration. After his military service, he worked for Chemische Fabrik Kalk GmbH in Cologne before joining the family business, where he worked in technical sales and research and development. Dirk lives in Atlanta, GA, with his wife Alexis and their dog Elvis. He has been an active member of the Master Brewers Association of the Americas since 1993, and he is also an active member of the American Society of Brewing Chemists and the Brewers Association.

120. The Food and Drug Act of 2010—What effects can we expect on the brewing industry? Presenter: David Radzanowski, Radzan Associates, Madison WI, USA.

With the passage of the U.S. Food and Drug Modernization Act of 2010, Congress has dictated stronger regulations for the food industry, with more frequent inspections demanded as well as product submission to independent laboratories on a regular basis to check for food safety. The costs of the inspections and laboratory submissions are to be borne by the company. If we add to this the breakup of ATF leaving TTB as the oversee agency of the Treasury Department, will the FDA return to the heavy demand for regulations and labeling requirements the agency tried to impose on the brewing industry during the latter half of the last century? We will attempt to evaluate the possible effects on the brewing industry.

David Radzanowski began his brewing career in 1962, joining the Duquesne Brewing Company of Pittsburgh, PA, after studying chemical engineering at Carnegie Tech (now Carnegie Mellon University). After graduating with the 1970 Siebel Institute Diploma Class, David was named the associate supervising master brewer, sharing production responsibilities with the vice president of production. With the closing of the Duquesne brewery, David joined the Joseph Huber Brewing Company of Monroe, WI, in 1973 as director of QC and assistant master brewer, eventually becoming master brewer and vice president of production. In 1992, David joined the Siebel Institute of Technology as vice president of educational services, becoming president of the institute in 1998, serving until 2000. In 2000, David and his colleagues joined Alltech Inc., resulting in the formation of the

Alltech Institute of Brewing and Distilling (AIBD) in conjunction with Herriot-Watt University. With Alltech, David had a dual role as technical manager for Asia-Pacific, covering brewing, ethanol production, and distilling, and as administrator of educational services of AIBD. He is now president of Radzan Associates, offering services to the brewing, distilling, and ethanol industries.

Engineering

121. A guide to understanding the brewery flash pasteurization process, determining the most appropriate operational requirements, and selecting the equipment that best fits your brewery application. Presenter: J. David Duff, FleetwoodGoldcoWyard, USA.

A brewery flash pasteurization system can be customized for each brewer's specific requirements. Finding the right flash pasteurization system requires a good overall knowledge of the beer pasteurization process. This paper covers the general design, description, features, and capabilities of flash pasteurization technology and is followed by an awareness and evaluation of the criteria brewers need to take into consideration when designing their flash pasteurization process and selecting the right pasteurization equipment. The outcome of this review offers the brewer a better understanding of this technology, a mechanism for determining their required operational specifications, and optimizes the brewer's ability to select the right system for their specific application.

David Duff has been a member of MBAA since 1982 and has held positions in his local district, as well as contributed on MBAA Technical Committee as an organizer and session moderator. He has presented at MBAA Conferences on several occasions, covering brewery pasteurization technologies. David began his career with Labatt Brewing Company in Canada. In 1997 David left Labatt to join forces with the Stroh Brewing Company as director of packaging operations. He now holds the position of North American sales executive with FleetwoodGoldcoWyard, part of the Barry-Wehmiller Group of Companies; his role specializes in brewery pasteurization and process.

122. A small brewing plant for product development whose initial cost could be reduced dramatically by using recycled equipment. Presenter: Atsushi Suzuki, Orion Breweries, Ltd., Nago-city, Japan.

A "small brewing plant" for product development, which could dramatically reduce the initial cost, was created. The cost saving was mostly achieved by utilizing recycled products for most the equipment. Although the estimated cost of the 200 L-scale plant we originally planned to install was 250 million yen, we succeeded in reducing the total cost by 99% by downsizing the volume by 90%, using recycled products, and assembling them on their own. Furthermore, the smaller plant enabled us to produce five times as much product at once than the previous plant. The chemical compositions of wort, immature beer, and filtered beer produced in this small plant according to the common brewing recipe were almost identical to those made in our main brewery. Furthermore, none of 10 inspectors could distinguish the brews produced in the small plant from those in the preceding ones. Six kinds of products developed using this small plant have been launched, and all of them are produced appropriately and have sold well. This report provides information to brewers who may be planning to purchase a low-cost small plant for product development. One example of the component of the small plant is obsolescent stainless kegs, which are reused as fermentation tanks. The stainless kegs satisfy almost all the primary requirements for fermentation tanks: 1) high sealing performance, 2) baro tolerance, 3) autoclavability, 4) ease of putting in and taking out liquids while maintaining sterility, and 5) ease of increasing the number of tanks.

Atsushi Suzuki received a master's degree in science from Ryukyu University in Okinawa, Japan. He began employment with Orion Breweries in 2004 as a manufacturer in the Product Development section. Since 2007, he has functioned as manager of new product development.

123. Beer clarification with modern centrifugal separators. Presenter: Alexander Gertsman, Flottweg, Independence, KY, USA.

Disc separators have been used in breweries for approximately 100 years, with an automatic type for over 60 years. Installation of these machines in the early years affected beer quality with issues such as oxygen pick up, consistent discharges, product heating, and hygiene. The issue of oxygen pick up was solved by several methods. One design offered a bottom fed hermetic separator where the feed was introduced via hollow spindle. The bowl of the machine with this design is pressurized, and the clarified beer is discharged with a corotating pump. The problems with this design are the use of expensive mechanical seals and requirement for high inlet pressure to the machine. The typical design of these bottom-fed separators incorporates use of a steep cone angle of the disc stack, which does not allow for maximum solids compaction. The best alternative was found with the use of a conventional top-fed separator with a shallow cone angle and hydrohermetic sealing to prevent oxygen pick up. For a while to ensure actual zero oxygen pick up it was still necessary to provide CO_2 blanketing, but a new design from Flottweg eliminates its need. With new hygienic features separators can be effectively cleaned in place rather than requiring disassembly. Design must incorporate high grade finishes and avoid trapping areas. Using automation during cleaning is important to avoid personnel presence during use of chemicals. Product heating was largely addressed with the use of a water jacket. This concerns particularly the discharged yeast. With little space between the bowl and its housing and high rotational bowl speed, the air in this space is heated and consequently the bowl and the housing. When discharged yeast comes in contact with a hot surface it dries very quickly and requires more cleaning time. In colder conditions the yeast remains wet and can be flushed away easily. Effective water jacket design provides good sound insulation. In the early years of automatic separators the bowl piston used a hydraulic system that incorporated an operating slide, valve plugs, and springs to assist the return of the piston to closing position. This system, largely seen as obsolete, is still being used by some centrifuge manufacturers. It can't deliver reproducible discharges due to the lag in the closing mechanism. The system uses lots of parts and is hard on maintenance. The most up-to-date modern design is Flottweg's SoftShot, which uses two simple valves mounted to the side of the bowl for easy access. Working together with modern turbidity meters this system will produce consistent discharges maximizing beer yields. Modern control systems allow for programming of different recipes when processing a variety of beers and assigning different turbidity standards to any given brand. When processing green beer tanks or running tank bottoms one can also rely on a turbidity meter installed on incoming feed that prevents overloading of the centrifuge. Another turbidity meter can be used on feed to centrate bypass for ensuring consistent turbidity of wheat beer.

Alexander Gertsman received a B.S. degree in chemical engineering from New Mexico State University. He has been working with centrifuges for 16 years, including employment with Alfa Laval and

currently Flottweg, both in North America. Alexander has been responsible for brewery applications and sales for Flottweg in North America since 2005. He is also a profound crafter of homemade kvass, a Russian national malt beverage.

125. Removal of volatiles from beer by gas (N_2) stripping coupled with high-vacuum. Presenter: Luis Castro, Washington State University, USA. Co-author(s): Carolyn Ross, Washington State University, USA.

In order to study flavors and their interactions, these volatile compounds often need to be removed from their matrix. The removal of volatile compounds from complex beverage matrices like beer remains a challenge, due to the difficulty of separating these compounds from the non-volatile beverage matrix without altering their properties. The aim of this study was to develop a novel method for the removal of volatile compounds from beer. The new technique, designated nitrogen gas stripping coupled with high vacuum (NSHV), applies a vacuum to the beer sample while using forced gas (nitrogen) stripping without the application of heat. Application of NSHV to beer samples spiked with known amounts of selected volatile compounds commonly isolated from beer (ethanol, isoamyl acetate, ethyl hexanoate, myrcene, benzaldehyde) resulted in higher reduction percentages for each compound when compared to commonly used rotary evaporation. Four of the five volatile compounds studied showed >85% concentration reduction following 90 min of treatment by NSHV compared to only one compound (myrcene) showing a >70% reduction using the rotary evaporation technique. The new method was demonstrated to be a promising method for volatile compound removal from beer samples and possibly other liquid samples as well.

Luis Castro received a B.S. degree in chemistry from the University of Costa Rica in San Jose, Costa Rica. After two years of working in both industry and academia, he moved to the 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 that he started research on the impact of beer matrix components and their interactions on 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.

126. Wort stripping based on thermal desorption supports the classic boiling process with a more efficient evaporation and without using additional thermal energy. Presenter: Roland Feilner, Krones AG, Neutaubling, Germany.

Wort stripping after wort boiling or the whirlpool process is an efficient way to reduce the amount of unwanted volatile substances, which are formed post-boil primarily during the whirlpool rest period. The focus lies mainly on free dimethyl sulfide (DMS) and volatile Strecker aldehydes and reduced fatty acids. Due to high temperatures present in the whirlpool, reactions like degradation of DMS precursor and aroma formation are maintained. Immediately before cooling the wort stripping system evaporates newly formed DMS. Thus it is not necessary to evaporate the total amount of undesired volatile substances, because stripping is one more system in the process, which can reduce these reaction products. A new kind of stripping system is based on a specific evaporation process, which is based on the desorption of volatile substances without using primary energy. The innovative design using a special inlet leads to the total covering of the entire internal surface area of the stripping vessel by an even but turbulent trickle film. Besides the generation of an efficient, uniform, and turbulent layer, the stripping gas keeps up the driving gradient between the gas and liquid phase. Based on this, the quantity of the expelled substances is controlled via the stripping gas volume. To evaluate the efficiency of evaporation, it is important to understand the evaporation behavior of single unwanted aroma compounds under reduced influencing parameters. Therefore the big matrix of wort is reduced to pure water, and the temperature dependent volatilities of these aroma compounds are determined. With this knowledge a basis for the description of evaporation efficiency is generated. In the course of this work, small-scale laboratory trials were verified by an upstream pilot trial, where also the geometry and volume of the stripping gas of the system was varied. While these investigations describe a reproducible quantitative reduction of unwanted aroma compounds, further investigations of this work are dealing with the rate of the reduction reactions. Thereby correlations to the current dynamic parameters can be arranged for further optimization of the brewhouse process. The stripping gas control function of the new stripping system enables the operator to react specifically based on the terms of malt quality like DMS-precursor content and wort parameters at a very low energy level combined with the same beer quality and an unchanged beer character. A significant reduction in energy input with thermal treatment and a simultaneously better wort quality could be realized. Reasons for this are a highly efficient stripping process, which uses the existing thermal energy of the wort instead of adding primary thermal energy.

Roland Feilner (born in 1981) finished his apprenticeship as a brewer and maltster. After that he studied food science technology in Weihenstephan and graduated in 2006 as an engineer. His career entry with Krones AG, Germany, started with membrane filtration of beer. At the same time, he worked as a process and development engineer for thermal product treatment. Additionally the degassing of beverages and juices was one of his main development areas. Currently he is responsible for new developments in wort treatment and process technology as a technical specialist in the Krones R&D Division. Furthermore he has been a post-graduate at the Technical University of Berlin since June 2011. His area of research is the reduction of unwanted flavors in wort, with a desorption-based stripping process.

Enzymes, Extracts, Other

127. Brewing with unmalted barley and Ondea Pro® enzyme technology: The science and the economic potential. Presenter: Kevin Redd, School of Plant Science, University of Tasmania, Hobart, Australia. Co-author(s): Evan Evans and Anthony Koutoulis, School of Plant Science, University of Tasmania, Hobart, Australia; Gordon MacAulay, GrainGrowers Australia, North Ryde, Australia; Elvig Niels, Novozymes A/S, Bagsvaerd, Denmark.

Malting, mashing, fermentation, and maturation are enzymatic processes. Barley, the main raw material, contains extract components such as starches and proteins, as well as enzymes such as amylases and proteases. As malted barley is a relatively expensive raw material and the enzymes produced by malting have become commercially available on a large scale, brewers have taken an interest in developing methods to substitute the malt with unmalted raw materials by using these exogenous enzymes. It is already common practice to use 25–50% unmalted cereals (adjunct) in conjunction with malt, and therefore the idea grew to substitute an even higher proportion of the malt with unmalted raw barley by processing the adjunct with exogenous enzymes. Brewing good quality beer directly

and entirely from barley is now a practical reality with the development of Novozymes Ondea Pro® enzyme technology. We outline the results from a pilot barley brewing trial using the Novozyme Ondea Pro® enzyme technology and provide the fermentability and process efficiency data for a range of Australian-grown barley grains from different varieties, geographic locations, and growing conditions. We used a small scale laboratory test to demonstrate that Ondea Pro® barley brewing is very efficient and comparable to malt-based brewing. The current malting varieties and Hindmarsh (a food variety) appear to be the most suitable for barley brewing as there is at least a 3% extract advantage with such varieties. The barley varieties associated with improved levels of extract and fermentability include "high" fermentability Flagship; "intermediate" fermentability Hindmarsh, Buloke, and Commander; and "low" fermentability Fitzroy, Schooner, Gairdner, and Baudin. We emphasize that good quality barley is required for optimal barley brewing results and recommend that the purchase of cheaper feed grade barley for barley brewing will result in less consistent brewing outcomes in terms of efficiency (extract, lautering, filterability, fermentability) and beer quality. In addition, cheaper feed grade barley will reduce the opportunities to optimize the use of the Ondea Pro® enzyme product, therefore costing brewers substantially more in the long term. Optimization of the use of Ondea Pro® during mashing has considerable potential to further reduce the cost of barley brewing. In addition to the laboratory results, we provide economic modeling to illustrate a scientific and economic assessment of the potential benefit from the use of enzymes on different Australian barley varieties, with a focus on the gains to be made in emerging markets.

Kevin Redd received a bachelor's degree in biology from the University of California, Santa Cruz, and then began a series of assignments in Alaska and British Columbia for the National Marine Fisheries Service and International Pacific Halibut Commission. He moved to Australia to undertake a Ph.D. program in forensic molecular ecology at the University of Tasmania before commencing work on a range of GRDC-funded malt- and barley-related projects.

128. Development of 100% wheat brewing by optimizing the selection of wheat raw materials and the enzyme composition. Presenter: Katsuya Sasaki, Asahi Breweries, Ltd., Japan. Co-author(s): Nami Matsumura, Koichiro Takahashi, Kazuhiko Uemura, and Masayuki Aizawa, Asahi Breweries, Ltd., Japan.

Wheat raw materials give a distinct flavor to beers like *Weizen* beer. In contrast to barley, which has a husk, the lack of a husk in wheat lengthens the lautering process. For this reason, it has been difficult to raise the proportion of wheat used beyond a certain level. By optimizing the selection of wheat raw materials as well as the enzyme composition, we successfully developed a 100% wheat brewing method (200- and 3,000-L scale), the first of its kind in modern breweries. We report our experience herein. Regarding selection of wheat raw materials, we found that hard (i.e., having a high protein content) whole-meal flour was the most suitable for lautering. As for enzyme composition, we found that a raw material composition of 50% wheat malt and 50% wheat flour greatly affected wort filterability due to cellulase and lipase and that alpha-amylase and protease also play increasingly important roles as the proportion of wheat flour used increases. Based on these findings, we also successfully brewed 100% wheat flour (no malt) beer. In addition, in terms of physical stability, primary gushing was suppressed by increasing protease levels, while haze was suppressed by using proline-specific protease. These types of 100% wheat beer were both brewed using bottom fermentation yeast. Compared to 100% barley beer, the flavor components of 100% wheat beer were characterized as being high in isoamyl acetate and low in astringency-conferring components hordatinA and polyphenol.

Katsuya Sasaki is a research worker at the Research & Development Laboratories for Brewing, Asahi Breweries, Ltd. in Ibaraki, Japan. He received his M.S. degree in engineering from the Tokyo Institute of Technology in 1998 and joined Asahi Breweries, Ltd. He has researched malting and brewing technology.

129. Enzymatic production of gluten-free beers from conventional grains. Presenter: Aaron Hanson, BunsenBrewers, Estacada, OR, USA.

Gluten sensitivity affects an estimated 6% of the general population, leaving many unable to drink beers fermented from conventional grains. Gluten sensitivity is caused by a T-cell driven intolerance to wheat gluten epitopes. Gluten epitopes are proline rich and are vulnerable to enzymatic degradation by prolyl oligopeptidases. *Aspergillus niger* prolyl endoprotease (AN-PEP) has been shown to efficiently degrade gluten by post-proline cleavage. Studies have also shown AN-PEP to be active at pH ranges from 2 to 8, with peak enzymatic activity at pH 5–5.5. Enzymatic degradation of gluten in pre-boil wort by AN-PEP may be achieved, producing gluten-free beers with conventional grains. Gluten degradation occurs by cooling the wort to 37°C as it is transferred from the mash tun to the kettle, and whirlpooling the 37°C wort with the AN-PEP enzyme until gluten degradation is complete. The wort is then boiled as normal, with the AN-PEP denaturing before fermentation occurs. The end result is gluten-free beer with the flavor profile of a conventional grain recipe.

Aaron Hanson received a B.S. degree in biochemistry from the University of Minnesota in Minneapolis, MN. He is currently a brewer in the Portland area.

130. Optimization of the application of commercial enzymes in sorghum mashes. Presenter: Birgit Schnitzenbaumer, School of Food and Nutritional Sciences, National University of Ireland, University College Cork, Cork, Ireland. Co-author(s): Jean Titze and Elke Arendt, School of Food and Nutritional Sciences, National University of Ireland, University College Cork, Cork, Ireland.

Brewing with unmalted sorghum involves the addition of exogenous enzymes such as alpha-amylase, beta-amylase, protease, and hemicellulase. High levels of commercial enzymes usually improve both extract content and processability of sorghum worts. However, a balance between product quality and production costs has to be established. The aim of this study was to optimize the application of commercial enzymes during mashing (infusion process) when replacing various levels of barley malt with unmalted sorghum. For this purpose, Nigerian white sorghum was fully characterized using standard methods specified by the Mitteleuropäische Brautechnische Analysenkommission, European Brewery Convention, or American Society of Brewing Chemists, as well as lab-on-a-chip capillary electrophoresis and scanning electron microscopy. The optimization of exogenous enzymes added to mashes containing up to 40% sorghum was achieved by monitoring rheological behavior during mashing using a Physica MCR rheometer. In addition, laboratory-scale mashing trials were carried out applying the optimized enzyme treatment for determining the quality of worts produced with various levels of sorghum adjunct. All analyses were done in triplicate. It has

been revealed that the application of a Physica MCR rheometer for optimizing the addition of commercial enzymes to sorghum mashes is highly successful. The optimized use of exogenous enzymes in brewing has not only the ability to significantly improve the quality and processability of mashes and worts containing up to 40% unmalted sorghum, but also to significantly reduce the production costs of beer brewed with commercial enzymes.

Birgit Schnitzenbaumer successfully completed an apprenticeship as assistant tax consultant and worked in this job full-time before she studied brewing and beverage technology at the Technical University of Munich in Weihenstephan, Germany. During her studies, she completed several internships in breweries and did her master's thesis on the effect of malting on the protein profile of proso millet (Panicum miliaceum L.) at the School of Food and Nutritional Sciences of the University College Cork, Ireland. Birgit graduated with a Dipl.-Ing. (M.S.) in brewing and beverage technology in 2009 and started her Ph.D. project on the application of novel and industrial enzymes when brewing with unmalted cereals at the University College Cork in November 2009.

131. Pitfalls and gains from applying xylanases in brewing. Presenter: Lars Boe Larsen, DuPont Nutrition and Health, Danisco A/S, Brabrand, Denmark. Co-author(s): Jens Frisbak Sorensen, DuPont Industrial Biosciences, Danisco A/S, Brabrand, Denmark; Lone Broend Miller, DuPont Industrial Biosciences, Danisco A/S, Brabrand, Denmark.

Limits with respect to mash separation and beer filtration, as well as variations in raw material composition, are constantly being challenged by the brewing industry. Furthermore, variations in climatic conditions can highly impact the consistency of brewing raw materials. This study attempts to clarify some of the potential gains and pitfalls related to applying xylanases in brewing. Studies have been performed to understand the interactions between components in the raw material constituents and enzyme functionality in such a way that application challenges can be overcome without having a negative impact on the process and beer quality. It is well known that cereal consists of starch, protein, non-starch polysaccharides, and lipids. For development of a separation enzyme system the most important components are beta-glucans and arabinoxylans. A high number of xylanases have been screened with respect to relevant parameters that have a significant impact on performance in the application. Various hypotheses have been set up and tested in mash separation studies applying malted barley/raw barley combinations until it has been possible to link the modification of a certain component to enzyme functionality and application performance. Selected candidates have been tested in pilot brewing plant studies. Critical parameters, including filter cake stability at lautering and pressure built up at beer filtration, have been tested applying various raw material compositions. Gains applying combinations with other enzymatic activities have been monitored as well. The presentation summarizes these studies with respect to key screening parameters, test set up, and observations/analyses. The link between substrate selectivity of xylanases on different arabinoxylan fractions and application functionality is described.

Lars Boe Larsen received an M.S. degree in biotechnology from the Technical University of Denmark in 1996 and a master brewer diploma from the Scandinavian School of Brewing in 1998. He began employment with Danbrew (now Alectia) in 1996 as an engineer in the Turn Key Division. From 1998 to 2008 he held a number of positions, including master brewer, process manager, and senior brewing specialist with Royal Unibrew in Denmark and in Eastern Europe. Since 2009 he has been responsible for application brewing with Danisco (now DuPont Nutrition and Health) as the group manager and senior application specialist. He has served as an external lecturer at the Scandinavian School of Brewing and as vice president of the Danish Master Brewers' Guild.

Finishing and Stability

133. Impact of filtration and filter aids on the iron content and haze formation. Presenter: Thomas Kunz, Technische Universität Berlin, Department of Biotechnology, Chair of Brewing Sciences, Berlin, Germany. Co-author(s): Kristian Schubert, Jörg Kaspar, and Frank-Jürgen Methner, Technische Universität Berlin, Department of Biotechnology, Chair of Brewing Sciences, Berlin, Germany.

It is generally known that metallic ions like iron or copper have an impact on oxidative beer stability. Metallic ions can activate oxygen by electron transfer and have an influence on radical generation due to their catalytic effect on the Fenton-Haber-Weiss system. Our prior studies proved that after consumption of the endogenous antioxidant potential the reaction products of the Fenton system (Fe^{3+}, Cu^{+}, OH* radicals) interact and generate metal ion complexes with oxidized, haze active polyphenol-protein complexes, which are significant for visible chill haze formation. The aim was to investigate the influences of kieselguhr (KG), membrane, and Crosspure (CP) on iron content, radical generation, chill haze formation, and oxidative stability. Another focus was the new filter aid Divergan HM (DG HM), which can be used to reduce metallic ions during filtration, to get a deeper view of the specific properties and the possible procedures in application. Besides a turbidity meter, AAS to analyze iron and EPR spectroscopy to determine the influence on radical generation and oxidative stability were used. The lowest iron content and influence on oxidative beer stability resulted from CP, followed by membrane filtration. With a clear distance, KG showed the strongest acceleration in radical generation and the most negative effect on oxidative stability mainly caused by iron entry. In comparison to KG filtration with comparable PVPP stabilization CP showed a lower increase in chill haze formation under storage conditions. DG HM was characterized by a fast reaction rate and sufficient properties to reduce iron during filtration, but this kind of reactions is directly connected to an increase in pH resulting in a negative effect on oxidative beer stability. Additional trials demonstrated that the aggradation of Divergan HM with acids could be a useful process to eliminate the influence on beer pH. The combination of organic acids like lactic and citric acids with DG HM showed the most positive effect on oxidative and colloidal beer stability. Besides slight oxygen entry during KG filtration the most negative effect on oxidative beer stability results from iron entry. In contrast, CP filtration led to a slight decrease in iron, which can be explained by the discharge of iron ions involved in polyphenol-protein complexes. The significantly lower iron content was jointly responsible for lower chill haze formation during storage. DG HM only has advantages for oxidative and colloidal beer stability if the negative effect on pH can be compensated for with by acid aggradations. Additionally, the fast reaction rate of DG HM can improve its application in the brewing process. Our recommendation is to use DG HM with organic acids in a continuous dose during filtration. An alternative procedure would be the addition of a DG HM citric/lactic acid solution in combination with the continuous KG dose.

After qualifying as a certified technician in preservation engineering (1991–1993), Thomas Kunz completed his basic studies in chemistry at the University of Applied Sciences, Isny (1994–1995), and his basic studies in food chemistry at Wuppertal University

(1995–1998), before studying food technology at the University of Applied Sciences, Trier (1998–2002). After graduating, he worked as a chartered engineer in the area of ESR spectroscopy at the Institute of Bio Physics at Saarland University (2002–2004). Since January 2005, he has been employed as a Ph.D. student at the Research Institute of Brewing Sciences, Berlin Institute of Technology (Technische Universität Berlin). His main research focus lies in analyzing radical reaction mechanisms in beer and other beverages using ESR spectroscopy.

134. Influencing factors of hydrogen bonding intensity in beer. Presenter: Qi Li, Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University, China. Co-author(s): Chunfeng Liu, Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University, China; Jianjun Dong, R&D Center, Tsingtao Brewery Co. Ltd., Qingdao, China; Xiangsheng Yin, Cargill Malt, Wayzata, MN, USA.

The hydrogen bonding is prone to be formed by many components in beer. Influencing factors of hydrogen bonding intensity in beer was observed by the nuclear magnetic resonance (NMR) method in this study. Results showed that ethanol content was the primary influencing factor, and its correlation coefficient was 0.629 for correlation analysis (CA). Some factors had a positive correlation with hydrogen bonding intensity, such as the content of original gravity, ethanol, isobutanol, Cl^-, K^+, pyruvic acid, and lactic acid in beer. A mathematical model of hydrogen bonding chemical shift (CS) and the content of ethanol, pyruvic acid, and K^+ was obtained through principal component analysis (PCA) and multiple regression analysis (MRA), with the adjusted R^2 being 0.736 ($P = 0.001$). PCA and RA methods proved that Ethanol content was the most important factor that could impact the hydrogen bonding association in beer. Then, a multiple nonlinearity model could be obtained as follows: hydrogen bonding association intensity (CS) = $4.916 + 0.003[E] - 0.005[P] + 5.031E^{-8}[K^2] + 1.558E^{-5}[K \times P] + 2.369E^{-5}[E^2P] - 2.207E^{-5}[P^3] + 2.244E^{-8}[K^3] - 5.947E^{-7}[K^2P] - 2.037E^{-5}[E^2K] + 4.276E^{-6}[P^2K]$. The average error was 1.34% in the validated experiment.

Qi Li received an engineering doctoral degree in fermentation engineering from Jiangnan University in Wuxi, China. She began employment with Jiangnan University in 1999 as a teacher in the Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangsu Province, China. Since 2009, she has functioned as vice president for the School of Biotechnology in Jiangnan University. Besides serving on the Beer Industry Association subcommittees, she has served on National Professional Standardization Techniques Commission committees, as an adjunct professor for Nanjing University of Technology, and as the specially invited beer judge of China.

135. Laboratory tests of beer aging under aerobic and anaerobic conditions. Presenter: Petr Kosin, Budejovicky Budvar, n.p., Ceske Budejovice, Czech Republic. Co-author(s): Jan Savel and Adam Broz, Budejovicky Budvar, n.p., Ceske Budejovice, Czech Republic.

Beer stability is usually determined by forcing tests based on acceleration of beer aging. The bottles or cans containing beer are placed in heating or cooling bathes, and aging of beer is estimated. Aging is a typical process connected with undesirable changes in beer. They comprise increasing color, haze formation, and/or sensory changes. More procedures are used to accelerate beer aging such as storage at higher temperature, oxidizing agent addition, or light illumination. The substances, which can prevent such changes, are usually called antioxidants, although they can also act as pro-oxidants. Ascorbic acid and sulfur dioxide are examples of such substances. Beer aging can be slowed down by other stabilizers such as PVPP, although they are sometimes considered to have adverse effect, e.g., decrease of polyphenol content. Samples with different amounts of stabilizers are usually tested, but working with many packages is difficult and time-consuming. Oxygen strongly supports beer aging, but its measurement in a package is difficult and inaccurate. Laboratory tests can therefore hardly estimate the influence of oxygen on beer stability. We usually need to prepare many samples with different amounts of tested substances. Indirect measurements, such as reduction power or antioxidant capacity determination, are often used in the presence of air although the results strongly depend on the concentration of oxygen. The solution is to prepare aerated or nitrogenated samples of beer with air or nitrogen in the headspace in the range, which can occur in practice. The ratio between liquid and gas volume in test vials determines the total oxygen content, and their vertical or horizontal position controls the rate of oxygen consumption. The entrance of air after testing enables the beer to reach oxygen saturation again, which is important for measurement of redox potential changes. After sample heating and cooling, the differential spectra or haze are measured to estimate the influence of the addition of various stabilizers. Dyes such as methylene blue or indigocarmine are used as internal standards to recognize the decrease in the reduction power of beer or degradation power of oxygen radicals. 1,2-Diaminobezene is added to compare the reactivity of sugar dicarbonyls to alpha-amino nitrogen. Another strategy is to measure the concentration of traditional stabilizers such as ascorbic acid or sulfite during heating of aerated or deaerated beer. During aging the concentration of inhibitors decreases, so the decay can be used for the measurement of their efficiency. Various analytical methods can be used for this purpose.

Petr Kosin received engineering (M.S. equivalent, 2006) and Ph.D. (2012) degrees in brewing and malting at the Institute of Chemical Technology Prague, Czech Republic. He worked on both of his theses, "Application of Modern Methods for Yeast Activity Control in Brewery" and "Consumer Perception of Beer Qualitative Characteristics," at Budweiser Budvar, N.C. in Ceske Budejovice. He has been working in research and development at Budweiser Budvar, N.C. since his graduation. He has been a member of the EBC Brewing Science Group since 2011.

136. New approaches for kieselguhr-free filtration and characterization of filter aids. Presenter: Alexander Scheidel, Technische Universität München Weihenstephan, Germany. Co-author(s): Jens Voigt, Technische Universität München Weihenstephan, Germany.

The clarity of a beer is the first impression of the consumer and one of the most important quality aspects of beer. The filtration process after a conditioning maturation is needed to reduce haze causing materials (e.g., polyphenols, proteins, yeasts, and carbohydrates) to increase this quality aspect before filling and sale. Conventional kieselguhr filtration with PVPP stabilization (polyvinylpolypyrrolidone) is mainly used as a precoat filtration in breweries to ensure stable beer quality. But, the use of diatomaceous earth (kieselguhr) as a filter aid bears a few problems. It is expensive to dispose of and furthermore the inhalation of SiO_2 dust may be carcenogenic. A few approaches were made to substitute kieselguhr filtration. One of them is filtration with regenerable filter aids; another common method is the selection of membrane filters without

filter aids. The chemical company BASF has developed a regenerable filter aid called Crosspure. This filter aid has comparable characteristics to diatomaceous earth. It is a combined filtration and stabilization tool, is available in two grades of fineness, and can replace kieselguhr in existing plants. Due to the availability of different grades, it allows the brewery to adjust this ratio to achieve an excellent filtration process and final beer quality. The possible separation of the mixture has been investigated successfully, and in a further step the filter cake resistance of the separated filter aid was determined. With knowledge of the filter characteristics of separated Crosspure, breweries are able to adjust an optimal ratio of the two grades of fineness. The separation process was investigated by particle size analysis and characterized by grade efficiency curves. The results of the regenerable filter aid were compared to kieselguhr, and the applicability was investigated by beer filtration tests at the Chair of Process Engineering of Disperse Systems in Weihenstephan. Separation of filter mud consisting of regenerable filter aids has a high innovative potential for kieselguhr-free filtration especially with filter performance comparable to kieselguhr and different sizes for optimal adjustment for different beer types.

Alexander Scheidel was born in 1987. He received degree a diploma engineer (M.S.) degree in brewing and beverage technology from TU München-Weihenstephan, Germany, in 2011. He began employment at the Chair of Process Engineering of Disperse Systems in Weihenstephan as a Ph.D. student. The focus of his work at the university is the filtration process with regenerable filter aids.

137. Recent findings on the mechanism of chill haze—A physico-chemical explanatory approach. Presenter: Jean Titze, National University of Ireland, University College Cork, School of Food and Nutritional Science, Cork, Ireland. Coauthor(s): Antonie Herrmann, Hochschule Weihenstephan-Triesdorf, Institut für Lebensmitteltechnologie, Freising, Germany; Vladimír Ilberg, Hochschule Weihenstephan-Triesdorf, Fakultät Gartenbau und Lebensmitteltechnologie, Freising, Germany.

Is the current understanding of chill haze in beer still correct? Brewing science distinguishes between two forms of haze appearance: reversible chill haze and permanent haze. The composition and formation of both forms is described as being identical in the literature, except chill haze is reversible. Further, haze formation in beer is mostly explained by the interaction of protein and polyphenol. The process usually shows an initially reversible bonding between protein and polyphenol, the so called chill haze. This precipitates as an insoluble complex due to catalysis by metal ions or oxygen. These complexes with covalent bonds will not dissolve if heated, and permanent haze appears. A physico-chemical explanatory approach for the reversibility of chill haze is not given by most authors. In general, there are several types of haze predicting tests available. Forcing tests, for example, involve storing beer at elevated temperatures for a certain time (warm days) to speed up the natural aging process. The results of a long-term forcing test over a period of more than 1 year put the existing theory into question. It could be shown that the assumption that an already formed complex could be initially reversible and later irreversible is wrong. A novel physico-chemical explanatory approach could be found, in which the water retention of proteins (hydration) plays an important role. 1) Colloidal haze (permanent haze) formation in beer is purely a matter of a thermal effect, which is the reason for the following two mechanisms: already denatured proteins in beer agglomerate according to collision (DLVO theory) and native proteins start unfolding. After that, they are able to agglomerate. Hence, the basic requirement for haze formation is thermal energy. With every warm day the amount of denatured proteins, which can agglomerate right away, as well as the number of native proteins, which first unfold and then can agglomerate, increase. 2) Chill haze forms due to cool temperatures; the water gets out of the molecule of the nondenatured proteins with a change in the properties of the hydration hull. Two activities are possible: according to diversification of protein hydration the index of refraction of the molecule changes and the particle becomes visible and due to modification of hydration, hydrophobic groups of the protein can interact with themselves or with already denatured proteins and reversibly accumulate with them. Both phenomena are reversible. Independent of the beer aging status, they lead to an increase in haze due to the cold temperature (chill haze).

Jean Titze studied the technology and biotechnology of food at the Technical University of Munich, as well as food and feed law at the Academy of Food Law, Philipps-University of Marburg. He worked several years as a brewery consultant for the Research Center Weihenstephan for Brewing and Food Quality and later as a senior consultant for Deloitte, focusing on the food and beverage industries. Since March 2011 he has been a senior research scientist at UCC, focusing his research on colloidal chemistry and particle analysis. For his research in the area of colloid science he received the 2011 Research Award from the German Brewing Industry. Since winter term 2011/2012 he is also a lecturer for food law at the University of Applied Science Weihenstephan-Triesdorf.

138. Strategies for dealing with peroxides. Presenter: Kirk Smith, University of California, Davis, CA, USA. Coauthor(s): Charles Bamforth, University of California, Davis, CA, USA.

Research suggests that a key determinant of instability in beer is peroxide. Any mechanism that reduces peroxide levels in beer, thereby preventing their direct damaging effect or their ability to convert to even more damaging species (notably hydroxyl), would be predicted to enhance the shelf life of beer. We have investigated potential enzymic solutions for affording such protection.

Kirk Smith was born in the foothills of North Carolina and lived there on the edge of a family farm for the entirety of his childhood. In 2010, he graduated with a B.S. degree in food science from North Carolina State University, Raleigh. He is currently working toward a M.S. degree in food science at the University of California, Davis. When not conducting, he is likely to be found snowboarding down a mountain.

139. The effectiveness of pre-combined colloidal stabilizers. Presenter: Kenneth Berg, PQ Corporation, USA.

Sometimes, chill haze is harder to prevent in higher malt beer with silica gel alone, which removes mostly just haze-active proteins. Consequently, some brewers find they need to remove haze-active tannoids in addition to haze-active proteins. Treatments with PVPP or with BRITESORB TR are very effective adsorbents of haze-active tannoids. Haze-active protein and tannoid removal are often performed via successive treatments. A dual treatment using a single formulation made by combining agents from the two classes would offer the benefits of reduced labor, inventory, and handling. To determine the feasibility of combining BRITESORB TR with silica gels, a combination with hydrogel was incubated for a month at three different typical storage temperatures. The effect of storage of the combination was determined by treating beer and quantitatively measuring colloidal stability. The incubated combination was compared with components incubated separately but combined at the point of treatment.

Ken Berg received a B.A. degree in biology (biochemistry concentration) from Cornell University in and a Ph.D. degree in biochemistry from Brandeis University in 1981. After a post-doctoral appointment at North Carolina State University, Ken designed protein purifications for Lee Scientific in St. Louis, MO. For the last 26 years he has aided PQ Corporation by inventing new silica-based adsorbents for the food industry, supported PQ's silica gel plants, and contributed to the beer industry both as vendor technical support and as a member of MBAA and ASBC. Ken lives near Philadelphia, PA, with his music teacher wife Shelley.

140. The role of reference standards in modern brewing chemistry. Presenter: John Laferty, ERA A Waters Co., Golden, CO, USA.

For many industries, the use of analytical reference standards has always been a valuable tool for process/product quality control, and the brewing industry is no exception. As technology in the brewing community continues to advance, the use of chemical reference standards is becoming more commonplace for routine quality control, as well as to help solve brewing anomalies impacting flavor, quality, consistency, and stability. This poster presents case studies where chemical reference materials were used to identify and quantify constituents or by-product compounds that impacted the flavor and quality of beer. The importance of using quality chemical reference standards is paramount to a brewing laboratory's success. Chemical reference standards should be obtained from ISO 17025/ISO Guide 34 accredited suppliers.

John Laferty received a B.A. degree in chemistry from the University of Colorado in 1982. He started working as an analytical chemist for Rocky Mountain Analytical Laboratories/Severn Trent Services and continued there until 2002. During this time, he held various positions, including chemist, laboratory group leader, customer service manager, and lab manager. Since 2002, he has worked at ERA, a Waters company, as a product line manager.

141. Use of tannins for beer stabilization during end-filtration. Presenter: Stefan Hanke, Bitburger Braugruppe GmbH, Bitburg, Germany. Co-author(s): Georg Stettner, Bitburger Braugruppe GmbH, Bitburg, Germany.

Stabilization has a long history in beer production. In former times, beer was stabilized by long storage in wooden barrels. During this very traditional and unsystematic method wood derived tannins and beer proteins reacted, and the resulting beer showed a higher colloidal and taste stability. With industrialization of brewing, wooden barrels disappeared, and filtration became a very important step in brewing as distribution channels became more complex and beer was shipped all around the world. Production of a brilliant, clear, and stable beer is the major aim of end-filtration, aside from the removal of yeast and turbidity after fermentation. Nowadays, for these stabilization purposes, the use of silica gel and PVPP is widely spread in the brewing industry. In recent times natural tannin based stabilization aids also came into the market. For these trials a highly purified high molecular weight hydrolyzable commercial tannic acid product was used in-line before end-filtration. Different dosage levels of tannins were applied to industrial brewed beer in semi-industrial scale filtrations. Concentration up to 2 g/hL of tannins were applied and compared to 35 g/hL of silica gel. The impact of these tannin dosages on colloidal and flavor stability were evaluated. All analytics were done according to international standards. It could be proofed that tannins are suitable for beer stabilization. A dosage of 1 g/hL was as efficient as the application of 35 g/hL of silica gel to increase the colloidal stability compared to an unstabilized filtered beer. With an increasing dosage level of tannins from 1 to 2 g/hL the colloidal stability of the final beer could be improved significantly. The removal of proteins was very specific to haze forming proteins because no impact on foam stability could be seen in our trials. The trials also showed that the concentrations of iron, aluminum, and vanadium in the final beer were reduced significantly by using the tannin product. The tannin treated beers came along with a higher antiradical potential (measured by ESR, T600 value), which is good for analytical flavor stability. The removal of the product was very good as no remaining tannins could be detected in the final beer. Also no negative impact on the taste and flavor stability of the fresh beers could be observed. The fresh beers that were stabilized with 1 g/hL of tannins showed a trend to a less lingering aftertaste (with the same analytical IBU) than the silica gel stabilized fresh beers. Besides the quality issues, economical and also ecological (lower filter waste because of lower dosage) savings could be generated by a use of tannins as a stabilization agent. Savings of up to 30% in stabilization costs could be possible. In our trials we showed that a natural tannin based stabilization agent is suitable to increase relevant quality parameters and improves colloidal and analytical flavor stability of filtered beer.

Stefan Hanke was born in 1980. From 1999 to 2004 he studied brewing science and beverage technology at Munich Technical University (Weihenstephan), graduating as an engineer with a Dipl.-Ing. degree. In 2010 he received a Ph.D. degree for his research on the influence of hopping technology on the harmony of beer. During his studies he worked for and received practical training at different German brewing and malting companies. Since 2004 he has been a scientific employee at the Lehrstuhl fuer Technologie der Brauerei I, Freising-Weihenstephan, Germany (Prof. Back). From 2006 to 2007 he headed the institute's Small Scale and Pilot Scale Brewery Department. From 2007 to 2010 he was responsible for the HPLC and GC Laboratory of the Institute for Brewing and Beverage Technology (Prof. Becker) in Weihenstephan. Since 2010 he has been the head of the pilot plant of the Bitburg Brewing Group, Bitburg, Germany, and responsible for R&D issues of affiliated breweries.

Hops

142. A natural foam enhancer from hops. Presenter: John Paul Maye, S.S. Steiner, Inc., New York, NY, USA. Co-author(s): Robert Smith, S.S. Steiner, Inc., Yakima, WA, USA; Richard Wilson (deceased); Harald Schwarz, S.S. Steiner, Inc., New York, NY, USA.

Most consumers view a natural looking foam head as an important part of a good quality beer. For this reason, brewers have been using tetrahydroisoalpha-acids and hexahydroisoalpha-acids to improve the foam and lacing of their beers for many years. Today many brewers seek a more natural foam enhancing hop product. We've investigated and found that alpha-acids, from CO_2 hop extract, can significantly improve the foam stability of a number of commercial beers at low concentrations. Since alpha-acids are not very bitter, they can be added to any beer to improve foam without affecting bitterness. Aqueous solutions of alpha-acids can be made that are both physically and chemically stable. Alpha-acids treated beers showed improved foam stability over an extended storage period demonstrating the foam enhancing potential of this natural product.

John Paul Maye is the technical director at Hopsteiner. He received his Ph.D. degree in organic chemistry from Purdue University in 1994, under 2010 Noble Prize winning chemist Ei-ichi Negishi. John started his work as a hop chemist in 1993 and has worked in hops ever since. He is credited with publishing several papers and patents and in 2000 won the ASBC Eric Kneen Memorial Award for his work

on developing HPLC standards for isomerized and reduced alpha-acids. He is also a founding member of the International Hop Standards Committee and has been an ASBC member since 1994.

143. Analysis of hop-derived flavor compounds in U.S. hops. Presenter: Kiyoshi Takoi, Sapporo Breweries Ltd., Yaizu, Japan. Co-author(s): Yutaka Itoga, Sapporo Breweries Ltd., Sorachi-Gun, Japan; Junji Takayanagi, Takayuki Kosugi, Toru Shioi, and Junji Watari, Sapporo Breweries Ltd., Yaizu, Japan.

In a previous study, we focused on biotransformation of hop-derived monoterpene alcohols (linalool, geraniol, beta-citronellol, nerol, and alpha-terpineol) and their contribution to the flavor of hopped beer. As a result, beta-citronellol was almost absent in hop and wort and gently increased during fermentation, because of the biotransformation from geraniol to beta-citronellol by brewing yeast. The concentrations of geraniol and beta-citronellol in finished beer could be enriched depending on the initial geraniol content in the wort by using a geraniol-rich hop. As a result of sensory evaluation, we found that there was an additive effect among linalool, geraniol, and beta-citronellol and that the flavor impression became lime-like by coexistence of these three monoterpene alcohols. Therefore, we proposed that geraniol metabolism by brewing yeasts contributes part of the hop-derived citrus flavor in beer. In this study, we compared the compositions of monoterpene alcohols in various hops and found several geraniol-rich hops in U.S. hop varieties. Such compositions were almost not observed in European traditional aroma hops. We suggest that a geraniol-rich profile is one of the important characters in U.S. hops. In addition, we discuss the behavior of geraniol and beta-citronellol under various hopping conditions and other factors influencing the citrus flavor of hopped beer.

Kiyoshi Takoi graduated from Tohoku University with an M.S. degree in agricultural chemistry in 1989 and joined the Brewing Research Laboratories of Sapporo Breweries, Ltd. as a biochemist. From 1989 to 2002, he worked on brewing chemistry and mainly investigated beer foam stability. During 2002–2005, he evaluated the brewing properties of malts and hops using the pilot malting and brewing plants in the Production & Technology Development Center. In 2006, he managed product development in the New Product Development Center. During 2007–2008, he worked in the Frontier Laboratories of Value Creation as a lead research brewer and mainly investigated hop-derived flavor compounds. At present, he belongs to the Value Creation Department of Sapporo and develops new products. He received a Ph.D. degree in agricultural chemistry from Tohoku University in 2011.

144. Comparative analysis of North Carolina and Pacific Northwest grown hops by brewing science students at Appalachian State. Presenter: Brett Taubman, Appalachian State University, Boone, NC, USA. Co-author(s): Eric Allain, Seth Cohen, and Shea Tuberty, Appalachian State University, Boone, NC, USA.

Since the hop shortage of 2007, a number of North Carolina farmers have experimented with growing hops to support the burgeoning brewing industry in the region. Now in their fifth year of production, these farmers have learned which hop varieties yield viable harvests in North Carolina, but no scientific assessment has been conducted of their relative quality as bittering and aroma hops when compared to these same varieties grown in Oregon and Washington. This study was conducted to compare beer brewed with North Carolina grown hops to beer brewed with commercially available hops grown in established hop growing regions. Single hop beer recipes were brewed using the pilot scale brewing system at the Ivory Tower Brewery on the campus of Appalachian State University. For each comparison, a recipe was made using an identical grain bill and split into several batches to which North Carolina grown hops or analogous commercially available hops were used to make the beers. The raw hops and finished beers were subjected to a battery of tests including HPLC quantification of hop alpha- and beta-acids, aroma and flavor compound profiling of both the raw hops and finished beer by gas chromatography/mass spectrometry, IBU determination in the finished beer, as well as a tasting panel evaluation. The results of several hop comparisons are presented.

Brett Taubman has been a faculty member in the A.R. Smith Department of Chemistry at Appalachian State University since 2007 engaged in instruction and academic research within chemistry and brewing sciences. He has B.S. degrees in both finance and chemistry and a Ph.D. degree in analytical and environmental chemistry from the University of Maryland. Brett has successfully developed a small-scale instructional brewing facility (Ivory Tower Brewery [ITB]) on the ASU campus that is supported by the university, local industry, and funds from the High Country Beer Fest, which was developed in conjunction with the ITB.

145. Degradation kinetics of iso-alpha-acids. Presenter: Mekonnen Gebremariam, Technical University Munich, Chair of Brewing and Beverage Technology, Freising-Weihenstephan, Germany. Co-Author(s): Yarong Huang, Martin Krottenthaler, and Thomas Becker, Technical University Munich, Chair of Brewing and Beverage Technology, Freising-Weihenstephan, Germany.

The kinetics of the degradation of iso-alpha-acids has been studied at four pH levels between 4.5 and 6.5 at different boiling temperatures (90–130°C) during a wort boiling time of 0–360 min. This research seeks to discover the kinetics of degradation of iso-alpha-acids as a function of temperature and pH value. The aim of this study was to investigate the free energy of activation and the reaction rate constant (k) of the degradation of iso-alpha-acids. A purified iso-alpha-acids extract in different pH buffered aqueous solution was used. The determination of the reactions order was computed by a program compiled in Matlab R2007a language: first order. We developed a compact 10-min HPLC method for the analysis of the concentration of total iso-alpha-acids, isocohumulone, isohumulone, and isoadhumulone. It was found that increasing the pH value decreases the free energy of activation. A typical plot of the natural logarithm of the iso-alpha-acids concentration in percentage versus time indicates that this reaction follows an Arrhenius equation. The Arrhenius equation demonstrates that the reaction energies of degradation of total iso-alpha-acids, isocohumulone, isohumulone, and isoadhumulone are nearly identical. The acyl side chain at the C2 atom of the iso-alpha-acids has no effect on splitting the isohexanoyl side chain at the C4 atom during the boiling process. Raising pH values from 4.5 to 5.5 and from 5.5 to 6.5 decreased the reaction energy by approximately 20 kJ/mol. We developed a kinetic model for the irreversible consecutive reaction; iso-alpha-acids are isomerized from alpha-acids by the application of heat in solution and, at the same time, iso-alpha-acids are degraded to humulinic acids and other compounds. This kinetic model fit the experimental data and allows the determination of all relevant reaction constants. This kinetic model is useful in calculating the precise point of time to add hops into the boiling kettle.

Mekonnen Melaku Gebremariam received his B.S. degree in chemistry from Debub University, Ethiopia. He began employment with the Ethiopian Ministry of Education in July 2000 as a chemistry teacher in the South Nations and Nationality People Region. He terminated his contract agreement with the Ministry of Education after four years. He next was employed as a chemist in the Federal Micro and

Small Enterprises Development Authority. After 18 months with this company, he terminated the contract agreement and joined Addis Ababa University for further studies. He graduated from Addis Ababa University, Ethiopia, in 2007 with an M.S. degree (with great distinction) in food engineering. Immediately after graduation he was employed as a lecturer and researcher by Hawassa University, Ethiopia. After about two-and-a-half years of work at Hawassa University, he went to Germany for his Ph.D. studies with the support of his employer, Hawassa University. Currently he is pursuing his doctoral studies at the Technical University of Munich, Germany.

146. Dry hopping—The history and its current importance. Presenter: Christina Schönberger, Barth Innovations, Nuremberg, Germany. Co-author(s): Andreas Gahr, Hopfenveredlung, St. Johann, Germany.

Due to the success of U.S. craft brewers dry hopping is a very popular hopping method. With this success its importance in European countries is once again growing. This presentation is a review that covers the history of dry hopping, its cultural roots, and traditional techniques. It also covers its current importance in the brewing industry in different countries and various current methods that are applied. The second part covers the scientific knowledge on dry hopping in regard to hop utilization and sensory and microbiological effects.

Christina Schönberger studied brewing and beverage technology at the Technische Universität München-Weihenstephan, Germany (1995–1999), graduating as an engineer in 1999. After working as a brewing intern in 2000 at Suntory, Japan, she pursued doctoral thesis work at the Chair of Brewing Technology I on "Sensory and Analytical Characterisation of Non-volatile Taste Compounds in Bottom Fermented Beers," with which she graduated summa cum laude in December 2003. For her doctoral thesis she received the Dr. Nienaber Award in 2005. After working for the German Brewers Association for one year as a consultant for technical and governmental issues, she joined the Barth Haas Group in 2005 as manager of technical sales. Within this role she is also responsible for the guidance of research projects and authors hop related professional articles. Christina currently holds the role of International Director on the ASBC Board of Directors.

147. HBC 369—A new flavor hop variety. Presenter: Gene Probasco John I. Haas, Inc., Yakima, WA, USA. Co-author(s): Jason Perrault, Select Botanicals Group, Toppenish, WA, USA; Scott Varnum, John I. Haas Inc., Yakima, WA, USA.

HBC 369 is a new flavor hop variety developed and released by the Hop Breeding Company LLC (HBC). Brewers who have brewed with HBC 369 often describe the flavor of their beers using the general descriptors of tropical fruit, citrus, and floral, while other descriptors include fruity, herbal, spicy, and earthy. The presence of this complex and varied aroma profile has also been confirmed in the hop by data collected from HBC sensory panels. HBC 369 has an attractive pedigree that includes the well-known flavor variety Simcoe YCR 14 as the mother and a father that is 50% Nugget. HBC 369 is 50% YCR 14, 25% Nugget, and the remaining 25% is Tomahawk, Brewers Gold, Early Green, and some unknown variety. HBC 369 combines a very complex hop aroma with a relatively high alpha-acids content and low cohumulone content to serve a dual purpose as both a bittering and flavoring hop. This combination ultimately provides clean bitterness and aroma profiles that cannot be accomplished with other hop varieties. The agronomic characteristics of this powdery mildew resistant variety are excellent, and production is rapidly increasing to meet the rising brewery demand for this new flavor hop. A name for HBC 369 will be selected and announced in the near future.

Gene Probasco received an undergraduate degree in biology from Central Washington University and a M.S. degree in plant pathology from Washington State University. After graduation, he spent six years at Washington State University, where he conducted research on hop breeding and diseases of hops. After joining John I. Haas, Inc., he started the first private hop breeding program in the United States and has since patented and released a number of new hop varieties into the U.S. hop industry, several of which constitute major varieties in the current industry. In addition to hop breeding, he has conducted agronomic research for the hop industry and more recently has been conducting research on use of hop products for non-brewing purposes, for which he also has several patents. For the past 20 years he has been a vice president for John I. Haas, Inc., where he has the additional responsibility for hop production on the company-owned hop farms and serving as a liaison between brewing customers and hop producers.

148. Hop and hop substances—Induction, reduction, or suppression of gushing? Presenter: Antonie Herrmann, Hochschule Weihenstephan-Triesdorf, Institut für Lebensmitteltechnologie, Freising, Germany. Co-author(s): Jean Titze, National University of Ireland, University College Cork, School of Food and Nutritional Science, Cork, Ireland; Christina Schönberger, Barth-Haas Group, Barth Innovations, Joh. Barth und Sohn, Nuremberg, Germany; Sylvie M. Deckers and Guy Derdelinckx, K.U.Leuven, Department of Microbial and Molecular Systems, MBS, LFoRCe, Heverlee, Belgium; Vladimír Ilberg, Hochschule Weihenstephan-Triesdorf, Fakultät Gartenbau und Lebensmitteltechnologie, Freising, Germany.

Gushing refers to the spontaneous overfoaming of carbonated beverages directly after opening a bottle or can. As gushing is still a problem for the brewing and beverage industries, it is of worldwide interest in the technological and scientific fields. After comprehensive research on raw materials over many years, no single substance has been identified. It is generally agreed, that hops have gushing suppressing properties in beer. A suitable hop technology in the brewing process can lead to a distinct gushing reduction. Practical industrial experience has proven that stronger hopping (e.g., pilsner beer) provides a significant reduction in the gushing potential of beer. While developing a new test method to determine the gushing potential of malt, hop could be used. Current gushing tests (according to MEBAK) still use the overfoaming amount of a test-specific carbonated malt extract to determine the gushing potential of malt. Unfortunately, overfoaming amount can fluctuate, so a precise quantitative comparison of malt samples in terms of gushing is difficult. The new concept quantifies the gushing potential of malt more precisely by applying the modified Carlsberg test. As in this method a "zero point" where gushing is neutralized by hops, the problem of fluctuating overfoaming amounts does not exist anymore. In this context the following question is raised: which specific substances in hops are responsible for gushing reduction or suppression? Phenolic acids, like sinapic acid, ferulic acid, cinnamic acid, and vanillic acid, as well as constituent parts of the essential hop oils, were examined. It turned out that some hop substances exist that can cause gushing. There are two possible reasons. 1) Hydrogen bond interaction between phenolic acid molecules occurs intensively. This results in larger molecule interconnections where CO_2 can be released. 2) According to the multi-functional groups CO_2 can directly interact via hydrogen bonds with phenolic acids. The gushing positive effect of phenolic acids could be compensated for by the addition of constituent parts of essential hop oils, where the different monoterpenes myrcene, guaiene, ocimene, and limonene with

palmitic acid suppressed gushing, while pinene and linalool with palmitic acid have only a gushing reducing effect. The high fugacity of constituent parts of essential hop oils requires measurement in a closed system. With a DSC (differential scanning calorimeter) it could be shown that gushing substances form mixing units while essential hop oil is present. The formed mixing units lead to gushing reduction or suppression. A further indication of mixing unit formation could be observed by applying a shock test. It appears that the mixing units, which suppressed gushing before, could be destroyed by abrupt mechanical agitation; afterward gushing occurred again. The gushing suppression effect of the mixing units was not reversible after several days.

Antonie Herrmann studied food technology at the University of Applied Science Weihenstephan-Triesdorf from 2006 until 2010. Before 2006 she apprenticed as a chemical-technical assistant. In 2010 she wrote her diploma thesis about the stability of the FMD virus at the Inertvet International GmbH in Cologne and started working in 2011 as an assistant in quality control at the same company. Since August 2011 she has been working as a scientific assistant at the University of Applied Science Weihenstephan-Triedorf, focusing her research on protein, colloidal, and particle analyses.

149. Identification of hop cultivars using high resolution melt curve analysis. Presenter: William Deutschman, Westminster College, Salt Lake City, UT, USA. Co-author(s): Julie Kilpatrick and Brian Avery, Westminster College, Salt Lake City, UT, USA.

It is important for hop breeders, growers, and brewers to be able to accurately distinguish hop varieties. Here, we report the use of high resolution melting (HRM) of DNA fragments to identify and differentiate DNA microsatellite sequences from various hop strains. HRM is a PCR based technique that allows for the identification and differentiation of closely related DNA sequences that may differ in sequence by as little as a single base. HRM monitors the fluorescence of a dye that binds specifically to double stranded DNA and therefore can precisely measure the fraction of a sample that is annealed at any given temperature. HRM analysis is then based on the melting temperature profile of a PCR product, which depends both on the length and specific DNA sequence of that product. Thus, different products that are identical or nearly identical in length and cannot be distinguished by gel-based analyses can be easily discriminated using HRM. We performed HRM on 10 commercial hop cultivars and 3 native hop samples from northern Utah. Multiple primer pairs were used to amplify different microsatellite regions in order to assess the discriminatory power of each microsatellite region. Our results show that the HRM technique can differentiate between microsatellite alleles that were previously scored as being identical using gel-based detection methods. Thus, HRM shows great promise as a fast technique with stronger resolving power than traditional methods for identification or verification of hop cultivars. The goal of our work is to take advantage of the benefits of HRM to establish a broader DNA based scheme for cultivar identification that will be of use to hop breeders, growers, and brewers.

William Deutschman earned his Ph.D. degree in chemistry in 2001 at the Institute of Molecular Biology at the University of Oregon. From 2001 to 2006, he taught biochemistry at Plattsburgh State University in Plattsburgh, NY. In 2006, he moved to Westminster College in Salt Lake City, where he currently teaches chemistry, biochemistry, and brewing science, while also pursuing research projects with undergraduate students in the areas of brewing and fermentation science.

Malt and Grains

150. Gamma-Aminobutyric acid (GABA)—A practical indicator for the detection of heterogeneities during malting? Presenter: Philip Wietstock, Technical University, Berlin, Germany. Co-author(s): Christian Mueller, Technical University, Berlin, Germany; Maik Kleinwaechter and Dirk Selmar, Technical University, Braunschweig, Germany; Frank-Jürgen Methner, Technical University, Berlin, Germany.

Nowadays, very large malt batches are processed, which frequently leads to heterogeneities within the grain beds. As a result beta-glucanase activities, among others, vary within the batches, thus high beta-glucan concentrations remain unhydrolyzed in parts of the batches and can lead to lautering and filtration problems during the brewing process. The kernels' enzyme activities mainly depend on their physiological status. Accordingly, the metabolic status of the seeds corresponds to a reliable marker for detecting heterogeneities in the grain beds and for predicting potential processing problems. Up to now, the Calcofluor method according to Carlsberg has been the only standardized method to determine the kernels' homogeneity; however, its results are not very precise. In this study, malting trials under differing conditions were carried out to assess if gamma-aminobutyric acid (GABA) can be used as a reliable marker for determining the physiological status of the kernels. Malting trials from different barley varieties were conducted under differing conditions (steeping degree, steeping, germination temperature, and anoxia) and using different scales (pilot and industrial trials). The malts produced were evaluated by standard malt analyses. Additionally, the content of GABA was analyzed in samples taken during malting using HPLC and compared with germination control parameters (chitted kernels, acrospire length). The study demonstrates that the accumulation of GABA in malt kernels is influenced by many factors such as anoxia and long wet periods; however, relevant parameters such as higher steeping degree and raised temperatures during steeping and germination were shown to increase GABA formation to a higher extend. Further outcomes of this study suggest that short periods of anoxia during malting do not harm the kernel's physiological status; the kernels survived, and germination resumed. The heterogeneities in large malting batches were examined in trials using an industrial malting plant. Varying temperatures and O_2/CO_2 ratios could be detected in the high grain beds of three different steeping systems. Without aeration of the grain, oxygen consumption was very fast, especially in the later steeping phases (dry and wet). Anoxia again did not lead to a dying of the kernels but to a delayed growth of rootlets and acrospires. Nevertheless, the formation of the most important enzymes alpha- and beta-amylase and beta-glucanase was slower at the beginning of the germination, but no markable lack of enzymatic activities could be found at the end of germination. During kilning the formed GABA was decomposed faster in the lower layers of the grain bed. The outcome of this research provides the brewing and malting industry with new results concerning the use of the stress metabolite GABA as an indicator for evaluating the physiological status of germinating barley.

Philip Wietstock is a scientific assistant at the Technische Universität Berlin, Germany. After graduating from his biotechnology studies with a diploma in engineering from the Technische Universität Berlin (2009), he worked for one year as an intern at the Department of Food Science and Technology at Oregon State University, Corvallis, OR. In 2011, he transferred to his present position, where he is working on his dissertation which focuses on the investigation of the influence of hops on oxidative beer stability.

151. 5% > extract and more $ for brewers—Hulless barley malt a dramatic difference. Presenter: Brian Rossnagel, University of Saskatchewan, Canada. Co-author(s): William Legge, Agriculture & Agri-Food Canada, Canada; Michael Edney, Canadian Grain Commission, Canada; Aaron Beattie and Graham Scoles, University of Saskatchewan, Canada.

Hulless barley development has been a significant segment of western Canadian barley breeding and research programs at the Crop Development Centre (CDC), University of Saskatchewan; the Agriculture and Agri-Food Canada, Brandon Research Centre; and the Grain Research Lab, Canadian Grain Commission for more than three decades. Since the CDC's release of the first hulless Canadian variety Scout (a feed variety) in 1982, program emphasis has shifted to hulless barley for food and malting/brewing. The high performing, agronomically superior variety CDC McGwire (released in 1999) set the stage for and provided the baseline for further hulless malting barley variety development. CDC ExPlus and Taylor, with improved malting quality, were released in 2009 specifically as hulless malting varieties. These have been followed by CDC Clear (released in 2012), which demonstrates agronomic improvement versus the agronomic standard CDC McGwire and malting quality advantages versus CDC ExPlus and Taylor, having lower grain protein, lower malt beta-glucan, and increased malt enzyme activity. Malting and brewing research with other hulless malting prototypes and these varieties consistently demonstrates a dramatic improvement in malt extract. Even a 50% replacement of hulled malt can provide effective filtration results in a >2% extract increase. Combined with less spent grain and lower freight cost for barley and malt, these factors should catch the attention of cost-conscious brewers.

Brian Rossnagel received his Ph.D. degree from the University of Manitoba in 1978 and is currently Professor Emeritus, Crop Development Centre (CDC), University of Saskatchewan. He was a barley and oat breeder at the CDC from 1977 to 2011 and has been the lead scientist in the release of more than 90 varieties of barley and oat.

152. Characteristics of ascorbate peroxidase in malt. Presenter: Makoto Kanauchi, Miyagi University, Sendai, Japan. Co-author(s): Charles Bamforth, University of California, Davis, CA, USA.

Oxidation promotes off-flavors and haze in beer, as well as impacting parameters such as wort separation. Increasingly attention has been drawn to the extent to which oxidative reactions occur in the brewhouse. Several oxidase and peroxidase enzymes may play a role in promoting such reactions. Thiol oxidase and oxalate oxidase levels increase during germination. Thiol oxidase produces disulfide (-s-s-) bonds in proteins that result in reduced lautering rates. Both oxidases produce hydrogen peroxide in the reactions they catalyze, and this reactive species can be the precursor of the even more reactive hydroxyl. Barley develops a number of peroxidases capable of consuming peroxide by reaction with polyphenols in the mash; however, some argue that it would be desirable to preserve such molecules in the beer where they can function as antioxidants. Our attention has therefore turned to an enzyme not hitherto studied in barley, namely ascorbate peroxidase. Levels of this enzyme were highest at 5 days of germination, coinciding with the highest level of ascorbic acid. Furthermore, enzyme activity during germination was higher when the grain was sprouted under an atmosphere of 15% CO_2. The optimum pH of the enzyme was 5.5, and 40% of the activity survived 30 min at 50°C, although the enzyme would not survive at mash conversion temperatures. The enzyme, a monomer with a molecular weight of 25,000, is inhibited by iron, mercury, and copper.

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 worked in Charles 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 at the Department of Food Management, Miyagi University. He has also been a lecturer in enzymology and alcoholic beverages (mainly spirits and wine) at the Tokyo University of Agriculture since 2005.

153. Developing an NIRS method for assessing black point in single kernels of malting barley. Presenter: Glen Fox, University of Queensland, Toowoomba, Australia. Co-author(s): Loraine Watson, University of Queensland, Toowoomba, Australia; Alison Kelly, DEEDI, Toowoomba, Australia; Cheng Dao Li, DAFWA, Perth, Australia; Wendy Lawson, DEEDI, Warwick, Australia.

Malting barley grain buyers purchase grain that meets a number of physical and chemical specifications, such as grain size and protein content, respectively. These traits are measured using objective testing. However, there are grain traits that are measured using subjective (visual) assessment. Black point (BP), also called black tip or germ-end staining, is one of these subjective traits. BP is a brown/black discoloration over the germ (embryo) of the grain. In Australia, less then 5% of any malting barley load can have BP to reach domestic malting specifications. For some export markets, 0% is the standard. Historically, BP has been associated with fungal infection and in some cases with unusual environmental conditions. Even a slight level of BP is viewed as a defect in malting barley. In our study, we have used near infrared spectroscopy (NIRS) to ascertain key wavelengths to assess BP with a view to develop an objective single kernel assessment system. Single kernels plus and minus BP, as well as individual husks removed from BP plus and minus kernels, were scanned using a Foss NIRSystems 6500 (400–2,498 nm) at 2 nm increment (WinISI V1.5). The spectral data showed significant changes in wavelengths around 1,868–1,888 nm that are associated with C=O and NH bonds. These chemicals bonds are associated with fiber (cellulose and lignin) and protein in the single kernels and single husk scans. There was an increase in other chemical regions in the BP plus single kernels, for example proteins, which would be associated with biological activity of the germ producing a chemical response to the induced stress (fungal attack or physiological stress). The results showed the potential to use NIRS technology to assess for BP in single kernels. However, at this stage there is one major issue to overcome and that is the orientation of the kernel as it is seen by the NIR instrument. The instrument must see the germ to be able to collect spectra associated with BP. While this issue presents a challenge in engineering, the opportunity to assess single kernels that could be segregated from a bulk sample would improve the subjective assessment and provide samples to understand the effect of BP on quality post-harvest, in storage, and on malting and brewing quality.

Glen Fox joined the University of Queensland, Queensland Alliance for Agriculture and Food Innovation, in 2010 after 25 years of conducting research projects with the Queensland government. His areas of research are in cereal quality, specifically barley, wheat, sorghum, and maize. He has a vast amount of knowledge in value-added cereals, in particular barley, malt, and beer quality. He has been a project leader in numerous national grains projects for barley. His main re-

search area has been near-infrared spectroscopy (NIRS), with calibration development in cereals (wheat, barley, triticale, sorghum, and maize), peanuts, soybeans, and animal feed. Currently, Glen is researching use of NIRS on single kernels of grain, as well as hyperspectral imaging of single kernels. He has more than 150 publications, including book chapters, journal articles, and conference papers. He has also supervised many post-graduate students in Australia and overseas. Glen is on a number of technical committees, including the Institute of Brewing & Distilling Asia-Pacific Analytical Methods sub-committee and has been on a number of specific subcommittees for the American Society of Brewing Chemists. In 2011, he was made an adjunct associate professor at Stellenbosch University in South Africa for his contribution to the science of NIRS in cereal and grains.

154. Fermentability of Canadian two row malting barley varieties: Wort turbidity, density, and sugar content as measures of fermentation potential. Presenter: Chris Bourque, Dalhousie University, Halifax, NS, Canada. Co-author(s): Alex Speers Dalhousie University, Halifax, NS, Canada.

There has been little research conducted on the relative fermentability of Canadian barley malt varieties, despite their putative high enzyme content, which suggests high fermentation potential. The primary goal of this study was to investigate and compare the fermentation performance of malt produced from 11 Canadian two-row barley varieties grown during the 2008 and 2009 crop seasons. Thus, 22 samples were analyzed. Common malting varieties tested included Harrington (a de facto "gold standard"), AC Metcalfe, CDC Copeland, and CDC Kendall, while feed varieties CDC Dolly and CDC Bold provided a negative fermentability control. Less common malting varieties, CDC Helgason and McLeod, were also tested. As well, three experimental varieties (TR251, TR306, and BM9752D-17) were included in this study due to their varied display of enzymatic activity; of chief interest was the amylase thermal stability exhibited by each, and its effect on attenuation. Fermentation was carried out using a miniaturized fermentation assay consisting of 33 test tubes, each with 15 mL of wort, allowing triplicate measurement at 0, 1, 6, 22, 26, 30, 46, 50, 54, 70, 74, and 78 hr throughout fermentation. Wort was pitched to an initial concentration of 1.5×10^7 cells/mL using SMA yeast, and test tubes submerged in a water bath at a constant temperature of 21°C. Apparent extract (AE) and absorbance were measured using a digital density meter and spectrophotometer (600 nm), respectively, and samples were collected for carbohydrate and ethanol HPLC analysis at each time interval. Wort attenuation, including AE and calculated real extract (RE), carbohydrate consumption, and ethanol production, were modeled using the logistic equation. Global *F* tests were performed between each variety and the standard Harrington, and parameters of RE and fermentable carbohydrates were analyzed using two-way analysis of variance and step-wise multiple linear regression. Results indicate that all but the feed varieties fermented well, achieving low final attenuation and exhibiting similar fermentation characteristics. Despite only minor performance differences among the top fermentors, it was found that between crop seasons both AC Metcalfe and CDC Copeland fermented as well or better than Harrington, as measured by their respective apparent degree of fermentation (ADF). Harrington displayed substantial performance variation between seasons, reaching an ADF of 0.88 in 2007 and only 0.83 in 2008. BM9752D-17 fermented consistently between years, displaying enhanced fermentation to that of Harrington in 2008. HPLC and kinetic analysis of sugar consumption throughout fermentation confirmed that fermentable carbohydrates are consumed in an orderly and overlapping manner. The rate of fermentable sugar consumption (glucose, maltose, maltotriose, and fructose) was successfully modeled with the logistic equation. It was found that initial glucose levels positively affected the rate of sugar consumption at the midpoint of the fermentations. Two-way ANOVA of all logistic parameters for RE and fermentable carbohydrates revealed the majority of fermentation variation resulted from inherent differences between varieties rather than seasonal variation.

Chris Bourque received a B.S. degree with first-class honors in human kinetics and health science from St. Francis Xavier University, Antigonish, NS, Canada. He pursued further education in the Department of Microbiology and Immunology before starting work on an M.S. degree in brewing science at Dalhousie University, Halifax, NS, Canada, in January 2010. He expects to defend his dissertation in May 2012.

155. Improvement of beer flavor stability through the LOX-less barley approach. Presenter: Junhong Yu, Tsingtao Brewery Co., Ltd., Qingdao, China. Co-author(s): Jianjun Dong, Shuxia Huang, Shuli Huang, Jia Liu, Zongming Chang, Yuhong Tian, and Junhuang Hao, Tsingtao Brewery Co., Ltd., Qingdao, China.

A set of pilot scale (1,000 L) brewing trials was conducted in the R&D Center of Tsingtao Brewery Co., Ltd. to investigate the effect of a LOX-less barley malt on the flavor stability of beer. The results clearly indicated the LOX-less variety has less nonenal potential (T2NP) than the control, although their LOX activities in malt were both relatively low. It was found that the beer brewed from the LOX-less barley malt contained much lower concentrations of *trans*-2-nonenal (T2N) and gamma-nonalactone, especially after the (forced or natural) aging of the beer, than those brewed under the same conditions from the barley variety with normal LOX activity. While the sensory panel indicated similar results in the general flavor profile, the freshness score of beer brewed from the test malt was higher than those from the control, especially after forced aging. Another exciting finding was that the trial beer had much better foam stability based on NIBEM value, by almost 30 sec. These results indicate that the use of LOX-less barley malt may be beneficial to beer flavor and foam stability. The LOX-less barley variety used was PolarStar, a new malting barley variety developed by the joint breeding program of Sapporo, Prairie Malt Limited, and the University of Saskatchewan. Metcalfe barley malt was the control for normal LOX activity.

Junhong Yu is the deputy director of the R&D Center of Tsingtao Brewery Co., Ltd. Junhong is responsible for research and development in new applied brewing technology, as well as technical support for almost 60 breweries of the Tsingtao Brewery Co., Ltd. across China. She joined Tsingtao in 1997 after she received her master's degree in biological engineering from Wuxi University of Light Industry (now known as Jiangnan University). She received her Ph.D. degree in microbiology from Ocean University of China in 2001. Her research interests focus on the biochemistry and chemistry of beer quality, especially foam stability, haze stability, malt LOX, and starch-degrading enzymes. In her position with the company, she was sent to Brewing Research International (BRi) for training for three months in 2007. She was an oral presenter at the 2007 ASBC Annual Meeting.

156. Limitations to predicting malt quality by using malt friability analysis during breeding of malting barley. Presenter: Ramón Huerta, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Texcoco, México. Co-author(s): Mauro Zamora, Salomón Solano, and Martha López, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Texcoco, México.

Malt friability analysis is used to measure the endosperm modification of barley during malting. For its correlation with malting quality factors, the use of this analysis for advanced genotype evaluation in a malting barley breeding program has been discussed and proposed as a useful tool. The aim of this study was to evaluate the usefulness of malt friability analysis to predict malt quality during malting barley breeding and determining the minimum value for Mexican genotype discrimination. The evaluated genotypes from two winter crops (2008/2009 and 2009/2010, Roque, Guanajuato, México) were the advanced breeding lines (>F8) M171, M173, M174, M175, and M10542 and malting barley varieties Adabella, Alina, Armida, Esmeralda, and Esperanza. As expected, malt friability was associated with extract fine grind ($r = 0.631$), total protein ($r = –0.812$), diastatic power ($r = –0.506$), and Kolbach index ($r = 0.522$), but in the individual assessment of Esmeralda, Esperanza, M171, and M10542 showed no correlation with extract fine grind (EMF) and Kolbach index (KI). Moreover, malt friability was not associated with extract differences in any of the genotypes tested. It was noted that malt friability ranged from 30 to 85% throughout the evaluated material, mainly due to changes in total protein, and Alina, Armida, M175, and M173 showed the greatest variation. It was not possible to set the minimum value of malt friability for the selection considering observed values of EMF. Significant changes in friability (>20%) and EMF (>0.98%) occurred with protein variation higher than 1.40%, and a minor change in protein did not affect endosperm modification parameters. Therefore, malt friability analysis is a useful tool only to predict malt quality in one genotype when protein variation does not exceed set limits according to its modification parameters, but not for genotypes discriminated during malting barley breeding when significant protein changes are common among filial generations.

Ramón Huerta received food engineering and master of science degrees from Universidad Autónoma Chapingo, México State, México. He began employment at Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) in 2008, as a researcher of malt quality in the national barley breeding program of INIFAP. This program, since 1957, has developed Mexican malting barley varieties for summer and winter crops like Esmeralda and Esperanza.

157. Research on malting technology of hulless barley used for brewing hulless barley beer. Presenter: Guangtian Zhou, School of Food and Bio-engineering, Shandong Institute of Light Industry, Jinan, China. Co-author(s): Song Wang, and Naibin Zhang, School of Food and Bio-engineering, Shandong Institute of Light Industry, Jinan, China; Zhihong Xu, Process and Equipment Engineering, Pöyry Shandong Engineering Consulting Co., Ltd. Jinan, China.

Hulless barley (*Hordeum vulgare* subsp. *vulgare*) is a particular grain crop produced in Tibet, China. It has a wealth of dietary fiber, and every 100 g of barley flour contains: 0.32 mg of VB1, 0.21 mg of VB2, 3.6 mg of Vpp, and 0.25 mg of VE. It is also rich in mineral elements, such as calcium, phosphorus, iron, copper, zinc, and selenium. These materials play a vital role in promoting human health. Research found that hulless barley contains the highest beta-glucan in barley and wheat crops with an average content of 6.57%. A good variety of hulless barley, No. 25, is even running at 8.6%. Beta-glucan is a polysaccharide that has special physiological functions. It can significantly improve cardiovascular function and also has a good curative effect in the treatment of diabetes. However, beta-glucan has a high viscosity. If hulless barley is used to brew beer, wort separation will be extremely difficult, and the stability of the finished beer will also be affected. Therefore, the purpose of this study was to obtain high quality hulless barley malt through the improvement of the malting process. A high-quality hulless barley variety, Zangqing No. 85, and normal barley were used as a test and control, respectively. The results showed that during malting, under the same conditions, hulless barley hydrated quickly, developed rootlets easily, and germinated vigorously and malting loss was relatively large. Meanwhile, the content of beta-glucan in hulless barley was changed too. Further research has studied the effect of different germination parameters on beta-glucan content, such as temperature, time, and the amount of gibberellin (GA3) added. We optimized the germination parameters of hulless barley by the corresponding surface methodology. Results showed that under the precondition of minimum beta-glucan content (3.1 g/L, in comparison the content of beta-glucan in Australian barley Baudin malt is 2.0 g/L) the best germination conditions were as follows: germination temperature at 16.6°C, germination time of 101.5 hr, and GA3 addition at 0.085 mg/L. The beta-glucan content was greatly reduced in this process. With 30% of the proportion of the hulless barley malt as adjunct, beta-glucan content of the wort was 272.8 mg/L, alpha-amino nitrogen was 260.7 mg/L, viscosity of the wort was 1.573 mPa·sec, and pH value was 6.42. Thus, the separation of wort becomes easier and so is brewing performance. Hulless barley beer is designed so the beer is brewed with top-fermenting yeast, somewhat similar to wheat beer. The finished hulless barley beer has a unique taste and harmonious flavor. The beer also has a lot of advantages in terms of health benefits, due to the higher content of beta-glucan.

Guangtian Zhou received his B.S. degree in bioengineering from Shandong Institute of Light Industry, Jinan, China, in 1982. He was then employed with the Jinan Beer Group as a brewer. Guangtian studied in Doemens Akademie, Munich, Germany, from August 1987 until November 1988. After graduation, he became chief brewer of the Jinan Beer Group. Since July 1994, Guangtian has functioned as professor, tutor of M.S. degree students, and the director of the China-Germany Beer Technology Center in the School of Food and Bioengineering, Shandong Institute of Light Industry, teaching and researching beer production. At present, he serves as an editor of China Brewing and a council member of Shandong Society for Microbiology.

158. The relationship between barley starch structure and the sugar profile of wort. Presenter: Shang Chu, University of Queensland, Brisbane, Australia. Co-author(s): Jovin Hasjim, University of Queensland, Brisbane, Australia; Kevin Redd and Evan Evans, University of Tasmania, Hobart, Australia; Glen Fox, University of Queensland, Brisbane, Australia; Robert Gilbert, University of Queensland, Brisbane, Australia.

During the barley mashing process, starch molecules are hydrolyzed to smaller sugars and dextrin by degrading enzymes (either naturally occurring in the malt or commercial exogenous enzymes). It is well known that the sugar profile of wort plays an important role in the fermentation process. There are several controlling factors in the production of fermentable sugars during mashing. Starch structure may well be one of these factors, as different starch structural features lead to different enzymatic degradation rates, and thus affect the fermentability of wort. In order to study the role of starch structure in the production of fermentable sugars in wort, 10 different varieties of both barley malts and un-malted barley grains were used to undergo the mashing step. A number of commercial exogenous enzymes were added for un-malted samples. Twenty portions of wort were then collected for sugar profile analysis using high-performance liquid chromatog-

raphy. To characterize starch structure, the branched and debranched size distributions of starch molecules from the 20 barley malts and grains were obtained using size-exclusion chromatography. Detailed chain length distributions of the samples were obtained through fluorophore-assisted carbohydrate electrophoresis. The structural characteristics of starch crystalline in the samples were described using small and wide angle X-ray scattering. Logistic regression was applied to analyze the relationship between barley starch structure and fermentability of wort. The results indicated a significant correlation between structural characteristics of barley starch and the sugar profile of wort. By increasing the understanding of the role of starch structure in beer brewing, we can provide brewers and breeders with improved methods to select more suitable barley for the beer they wish to produce.

Shang Chu received a B.S. degree in biology and a B.Eng. degree in water resources from Wuhan University in Wuhan, China. He then received an M.S. degree in biotechnology from the University of Queensland, Brisbane, Australia. He is currently enrolled in his Ph.D. program under the supervision of Robert G. Gilbert since 2010 in the Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, the University of Queensland. He is a member of the Nutrition Society of Australia.

159. Varietal effect of teff (*Eragrostis tef*) on the dimethyl sulfide (DMS) content and enzyme activities of teff malt. Presenter: Mekonnen Gebremariam, Institute of Brewing and Beverage Technology, Center of Life and Food Sciences, TUM, Weihenstephan-Freising, Germany. Co-author(s): Martin Zarnkow and Thomas Becker, Institute of Brewing and Beverage Technology, Center of Life and Food Sciences, TUM, Weihenstephan-Freising, Germany.

Variations in cereal, growing region, year of cultivation, cultivation practices, and malting conditions have considerable influence on the enzyme activities and DMS level of malt. This research was aimed at studying the influence of using different teff (*Eragrostis tef*) varieties on the DMS content and enzyme activities of the final malt. Five teff varieties (Kuncho [DZ-Cr-387], Ivory, Brown, Dessie, and Sirgaynia) obtained from Ethiopia and North America were investigated as possible raw materials for the production of gluten free malt. Portions of the samples were used for analysis of thousand corn weight, gelatinization temperature, and germination energy. The remaining portions were steeped for 5 hr on the first day and 4 hr on the second day at 24°C, and germinated for 4 days at 24°C in a temperature controlled chamber with 95% relative humidity. Kilning was for 18 hr at 30°C, 1 hr at 60°C followed by 3 hr at 65°C. Thousand corn weight, germination energy, gelatinization temperature, and malting losses due to rootlets ranged between 0.27 and 0.28 g, 96 and 100%, 69 and 73°C, and 1.95 and 5.49%, respectively. Teff variety Kuncho had the highest malting loss (5.49%), while Brown had the lowest (1.95%). There was a significant increase in amylolytic enzyme activities throughout the germination process, but some of the amylolytic enzyme activities decreased during the kilning process. The moisture contents, DMS levels, and alpha-amylase, beta-amylase, and limit dextrinase activities of the malts ranged from 3.04 to 3.8%, 2.2 to 4.1 mg/kg, 14 to 68 U/g, 10 to 440 U/g, and 375 to 1,072 U/kg, respectively. The enzyme activities were markedly ($P < 0.05$) influenced by the type of teff cultivar. The alpha-amylase activities of all teff varieties increased in the first few hours of kilning but started to decrease in the later stages. However, the limit dextrinase and beta-amylase activities of all samples decreased throughout the kilning process. At the end of kilning, there was 7–50% higher alpha-amylase activity in the final malts than in the green malts, whereas the more temperature sensitive beta-amylase and limit dextrinase activities were about 8–53% and 5–17%, respectively, less than in the green malts. The highest increase in alpha-amylase activity (50%) during the kilning process was recorded for teff variety Sirgaynia, whereas the lowest increase (7%) was for Ivory. The highest loss in beta-amylase (53%) and limit dextrinase (17%) activities were for teff varieties Dessie and Kuncho, respectively, and the lowest were for Ivory and Brown, respectively. In this study, teff variety Dz-Cr-387 had the highest enzyme activities compared with the other cultivars studied. This variety had the best malting characteristics and brewing potential, with alpha-amylase, beta-amylase, and limit dextrinase activities of 68 U/g, 440 U/g, and 1,072 U/kg, respectively. In general, it can be concluded that the use of different teff varieties yields malts with significantly different malt quality attributes.

Mekonnen Melaku Gebremariam received his B.S. degree in chemistry from Debub University, Ethiopia. He began employment with the Ethiopian Ministry of Education in July 2000 as a chemistry teacher in the South Nations and Nationality People Region. He terminated his contract agreement with the Ministry of Education after four years. He next was employed as a chemist in the Federal Micro and Small Enterprises Development Authority. After 18 months with this company, he terminated the contract agreement and joined Addis Ababa University for further studies. He graduated from Addis Ababa University, Ethiopia, in 2007 with an M.S. degree (with great distinction) in food engineering. Immediately after graduation he was employed as a lecturer and researcher by Hawassa University, Ethiopia. After about two-and-a-half years of work at Hawassa University, he went to Germany for his Ph.D. studies with the support of his employer, Hawassa University. Currently he is pursuing his doctoral studies at the Technical University of Munich, Germany.

160. Wort amino acid composition of new Canadian malt barley varieties and their relationship with grain protein. Presenter: Aaron MacLeod, Canadian Grain Commission, Winnipeg, MB, Canada. Co-author(s): John O'Donovan and Kelly Turkington, Agriculture and Agri-Food Canada, Lacombe, AB, Canada; Michael Edney, Canadian Grain Commission, Winnipeg, MB, Canada.

The fermentability of malt wort is dependent on providing an adequate supply of the amino acids necessary for yeast metabolism. In addition to supplying the required amounts of total free amino nitrogen, the relative concentrations of individual amino acids must also be considered given their differences in absorption and use by the yeast. It is unclear how amino acid composition of wort is affected by factors such as cultivar, nitrogen application, and growing environment; factors that are all known to have significant effects on grain protein levels. In the present study, five Canadian malting barley varieties (AC Metcalfe, Major, CDC Meredith, Bentley, and Merit 57) were grown with different nitrogen application rates (0, 30, 60, 90, and 120 kg/ha) producing grain with a range of protein levels. The amino acid composition of the barley and resulting malt worts was determined using ultra performance liquid chromatography (UPLC). The proportions of glutamic acid, proline, and phenylalanine in barley grain were positively correlated with protein content, whereas the levels of other amino acids showed a negative correlation with protein content. This relationship was consistent among all varieties. While total levels of free amino nitrogen in wort generally increased with barley protein, the proportions of most essential

amino acids such as histidine, lysine, and leucine, which can not be synthesized by yeast, were negatively correlated with grain protein. One notable exception was arginine, for which concentrations increased. Variety also had a significant effect on the amino acid profile in the grain and wort. Levels of amino acids in the barley grain of low protein varieties such as Bentley, CDC Meredith, and Merit 57 were consistent with their protein content. However, this relationship was not maintained in the resulting worts, likely as a result of protease activity in the mash. Different brewing practices require malts with various protein, amino acid, and enzyme levels depending on gravity and levels of adjunct used. A better understanding of the relationship between these factors will aid in the development of new varieties to meet the needs of the modern brewer.

Aaron MacLeod is a chemist in the Applied Barley Research unit of the Canadian Grain Commission Grain Research Laboratory. The unit provides quality assurance for malting barley grown in western Canada and conducts research on factors affecting malting barley quality and quality measurement methods. Aaron earned a B.S. degree in chemistry from the University of Western Ontario. An active ASBC member since 2008, Aaron has participated in the collaborative study of numerous new methods and is currently serving on the ASBC Technical Committee.

Microbiology

161. Adaptation of *Lactobacillus brevis* to beer—Role of metal trace elements and membrane lipids. Presenter: Patrick Preissler, Technische Universität München, Freising, Germany. Co-author(s): Jürgen Behr and Rudi Vogel, Technische Universität München, Freising, Germany.

Manganese is an essential metal for lactic acid bacteria, which is part of many enzymes, often replacing (redox) functions of iron in enzymes of other bacteria. As bacteria lack compartmentation, metal ion homeostasis is maintained primarily by regulation of metal cation flux across the cell membrane. It has been shown that hop compounds induce efflux of manganese, which is part of bacterial stress response and adaptation to iso-alpha-acids. On the other hand, the permeability of the membrane is adjusted through its membrane lipid composition, which may influence (manganese) transporter effectiveness and also intrusion of weak acids and other antibiotic compounds. As a result, a decrease in membrane fluidity could contribute to hop tolerance and beer-spoiling ability. In this study, we have investigated the influence of initial intracellular manganese levels and incubation in lager beer on the effects of metal trace elements and the composition of fatty acids in the cell membrane. Cells of beer-spoiling *Lactobacillus brevis* TMW 1.313 exhibited reduction of manganese and decreasing zinc concentrations after adaptation to the beer environment. At the same time, intracellular calcium, iron, and magnesium levels were increased. The analysis of cytoplasmic fatty acids composition showed that adaptation to beer after 5 days of incubation resulted in a reduction of the amounts of saturated fatty acids 12:0 and 16:0 3OH, whereas those of the two cyclic fatty acids (17:0 and 19:0), as well as the saturated fatty acid 18:0 and the unsaturated 20:2 ,were increased in the cell membrane. In conclusion, both tuning of the membrane composition and balanced metal ion content contributed to improved survival of *L. brevis* in beer by reduction of hop sensitivity and concomitant acid stress. This may indicate that functionality of metal ion transporters is modulated in such a membrane and both traits are connected.

Patrick Preissler was born in 1981 in Erfurt, the regional capital of Thuringia. He studied nutrition science at Friedrich-Schiller-Universität in Jena. In 2011 he finished his Ph.D. thesis on mechanisms of hop tolerance in beer-spoiling Lactobacillus brevis at Technische Universität München under the supervision of Rudi F. Vogel at the Chair of Technische Mikrobiologie in Weihenstephan.

162. Assessment of airborne microorganisms in a craft brewery. Presenter: Amanda (Mandy) Miller, Colorado State University, Fort Collins, CO, USA. Co-author(s): Marisa Bunning, Martha Stone, Doreene Hyatt, and James ZumBrunnen, Colorado State University, Fort Collins, CO, USA.

Pathogenic bacteria have little chance of surviving in beer due to intrinsic antimicrobial hurdles; however, there are other microorganisms capable of persisting in the environment and causing undesirable changes that spoil beer. The quality of all food products including beer is affected not only by the integrity of raw materials and cleanliness of the equipment and packaging materials, but also by the purity of the environmental air surrounding the processing area. Bottling and canning lines in breweries often are considered non-closed production equipment and have the ability to become contaminated from outside sources, including the environment. The purpose of this project was to examine the environmental microbial air quality within various areas of a craft brewery with emphasis on potential beer spoiling bacteria. Air samples were collected inside and outside the brewery to establish a baseline of data, identify areas of concern, and examine the effects of seasonality. Areas of concern then were sampled more often based on the risk of product contamination. The air was sampled 307 times over 22 months using an automated impaction sieve sampler pulling 80 L of air, and samples were plated both aerobically and anaerobically. Aerobic results were used to evaluate general cleanliness, while anaerobic testing was included to assess the prevalence of beer spoiling organisms. The canning line within the brewery was identified as a specific area of concern and was predicted to be contaminated with beer spoiling bacteria an average 75% of the time. Seasonality impacted aerobic microorganism levels, with fivefold increases observed in the spring. The results of this study revealed that testing for airborne microorganisms is highly recommended in the craft brewing industry. Critical areas in the brewery, such as the bottling and canning lines, should be routinely tested for airborne microorganisms to prevent final product contamination.

Amanda (Mandy) Miller received an M.S. degree in food science, with an emphasis on food safety, from Colorado State University in Fort Collins, CO, in 2011. In 1999, she began working at New Belgium Brewing Company in the Quality Assurance Department. After 10 years working as a quality assurance analyst, she now holds the position of beverage safety and sanitation specialist within the Risk Management and Environmental Affairs Department. She has been a member of ASBC for more than 10 years and a member of MBAA for more than 5 years.

164. Classification, identification, and detection of beer spoiling microorganisms—A review. Presenter: Mathias Hutzler, Forschungszentrum Weihenstephan, TU Muenchen, Freising, Germany. Co-author(s): Robert Riedl, Jennifer Koob, and Fritz Jacob, Forschungszentrum Weihenstephan, TU Muenchen, Freising, Germany.

Beer spoiling microorganisms are categorized according to taxonomic, phylogenetic, physiological, and technological aspects. From a technological point of view they are categorized as obligate beer spoilers, potential beer spoilers, and indirect beer spoilers. Classification according to beer spoilage potential and occurrence depends on the selective, antimicrobial parameters of individual beer types. When considering many novel beer types with differing antimicrobial parameters,

this classification becomes more difficult. This review summarizes the recent classification of and knowledge about beer spoiling microorganisms according to their taxonomic and technological characteristics. It should also include information about the novel beer spoiling species *Lactobacillus paucivorans*, *Lactobacillus acetotolerans*, *Lactobacillus backi*, *Lactobacillus rossiae*, *Pectinatus haikarae*, and *Megasphaera paucivorans* that have been described in recent years. Cultivation methods, molecular biological and chemotaxonomic identification, and detection methods for beer spoiling bacteria and yeasts are overviewed. Additionally a case study shows the current possibilities to detect, identify, and classify a beer spoiling microbe in modern brewing microbiology. In summary this study highlights recent knowledge about beer spoiling microorganisms and the different ways to cultivate, detect, and identify them.

Mathias Hutzler was born in 1978 in Regensburg, Germany. In 2004, graduated in food technology and food biotechnology, Technische Universitaet Muenchen- Weihenstephan, Germany. From 2004 to 2009, scientific assistant and doctoral degree at Chair for Brewing Technology, Technische Universitaet Muenchen-Weihenstephan. Since 2004, division manager microbiology at the Research Center Weihenstephan for Brewing and Food Quality, Technische Universitaet Muenchen-Weihenstephan. Main research fields include beer-spoiling bacteria and yeasts, molecular biological detection and identification methods, brewing yeast strain differentiation, hygienic monitoring, and biofilm characterization.

165. Differentiation of *Lactobacillus brevis* strains along their beer-spoiling potential using MALDI-TOF MS. Presenter: Carola Kern, Technische Universität München, Freising, Germany. Co-author(s): Patrick Preissler, Rudi Vogel, and Jürgen Behr, Technische Universität München, Freising, Germany.

Lactobacillus brevis is one of the most frequently encountered bacteria in beer-spoilage incidents. The species *L. brevis* comprises strains with the ability to grow in low hopped wheat beer, as well as strains that can grow in lager beer or even highly hopped pilsner beer. Accordingly, differentiation and classification of *L. brevis* based on their beer-spoiling ability improves quality control in breweries. Matrix assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) has been shown as a powerful and rapid tool for species and subspecies differentiation of bacterial isolates. In principle, MALDI-TOF MS of microbial samples is based on the generation of fingerprints of biomarker molecules, where low molecular weight proteins form a distinctive peak pattern, reflecting protein expression in the cell. The aim of this work was to elucidate whether differences in the proteome of two ecotypes of *L. brevis* are sufficient to differentiate *L. brevis* below-species ecotypes by MALDI-TOF MS and with special emphasis on their beer-spoiling ability. *L. brevis* strains were characterized according to their tolerance to iso-alpha-acids and their growth in wheat, lager, and pilsner beers. Two groups of *L. brevis* strains, each representing a distinct ecotype, were formed, among which one group showed the ability to grow in pilsner beer or lager beer and generally had a tolerance to iso-alpha-acids above 10 μM. The other group was only able to grow in low hopped wheat beer and exhibited an iso-alpha-acids tolerance below 10 μM. The two groups were analyzed using MALDI-TOF MS. Strain level identification and cluster analysis of the data were performed. Strain level identification was achieved in 85% of a total of more than 200 samples. Misidentification occurred only among strains belonging to the same ecotype, referring to a strong or weak ability to spoil beer. Cluster analysis split strains into three subgroups: one closely related group of strains that were, with the exception of a single strain, able to grow in lager beer, and two groups of strains, which were only able to grow in wheat beer. Taken together, MALDI-TOF MS allowed strain level classification of *L. brevis* in about 85% of all samples analyzed and furthermore showed potential to discriminate strains and group them according to ecotypes based on their ability to grow in beer.

Carola C. Kern was born in 1984 in Austria. She obtained her master's degree in nutritional sciences from the University of Vienna in 2010 and is currently a Ph.D. student at the Technical University of Munich, where she's working on the identification and differentiation of microbial contaminants in beverages by MALDI-TOF mass spectrometry at the chair of Technische Mikrobiologie under the supervision of Rudi F. Vogel.

166. Effect of plasmid loss on the beer-spoiling phenotype of *Pediococcus claussenii* ATCC BAA-344T. Presenter: Barry Ziola, University of Saskatchewan, Saskatoon, SK, Canada. Co-author(s): Emily Ewen and Vanessa Pittet, University of Saskatchewan, Saskatoon, SK, Canada.

Pediococcus claussenii is a species known to spoil beer. The *P. claussenii* ATCC BAA-344T (Pc344) genome was recently sequenced; however, many of the genes that permit this organism to proliferate amid the harsh conditions in beer have yet to be identified. It has been determined that Pc344 carries eight plasmids, six of which have coding capacity and two of which are cryptic. One of these plasmids has previously been shown to harbor *horA*, a well-established beer-spoilage associated gene involved in hops resistance. To further examine the role that plasmids may play in the ability of Pc344 to spoil beer, we obtained a collection of isolates with altered plasmid profiles and put these isolates through a series of phenotypic analyses designed to mimic the harsh conditions present in beer. Plasmid-minus Pc344 variants were generated either by repeatedly culturing in a non-beer medium or by incubating in a non-beer medium with a sub-lethal concentration of the antimicrobial compound novobiocin. Single colonies were selected by spreading onto MRS agar plates and then screened for an altered plasmid profile using multiplex polymerase chain reactions with primer sets designed to specific regions of each of the six plasmids with coding capacity. The plasmid-minus isolates obtained were then subjected to comparative phenotypic analyses, including monitoring growth in the presence of varying levels of hops using gradient agar plates, as well as relative growth rates in beer. Variability in beer-growth rate was noted among the plasmid-minus variants compared to the typical growth pattern of the parental Pc344 isolate possessing a complete set of plasmids. Variability of growth was also observed when analyzing for hops resistance. The correlation between plasmid elimination and altered phenotype confirms that the methodology used here is an appropriate starting point for investigating the role that plasmid genes play in the beer-spoiling capability of Pc344. Being able to attribute an organism's beer-spoiling capacity in part to the presence of specific plasmids provides a clear direction for a more refined search of beer-spoilage associated genes.

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

167. Effectiveness of a new automatic cell viability counter in comparison to established methods. Presenter: Thomas Kunz, Technische Universität Berlin, Department of Biotechnology, Chair of Brewing Sciences, Berlin, Germany. Co-author(s): Cecilia Cruz Palma and Frank-Jürgen Methner, Technische Universität Berlin, Department of Biotechnology, Chair of Brewing Sciences, Berlin, Germany.

To assure high fermentation quality it is necessary to get as much information as possible on the actual yeast condition. To achieve uniform fermentation two major factors have to be taken into account: yeast cell concentration and its viability. Since it is not possible to attain 100% viable cells, the relationship between dead and living yeast cells is a very important factor needed to adjust the pitching rate. Furthermore information about the influence of different fermentation parameters such as extract composition, temperature, pressure, etc. can be gathered when monitoring the yeast cells during fermentation. Several methods have been developed to determine and analyze cell number and viability using a minimum amount of effort. The aim of this study was to compare a new fluorescence microscope detection system, an automatic cell viability counter (Cellometer M10 - PeQlab), with the established Nucleocounter (Chemometec) using the fluorescent dye propidium iodide in the chamber and the traditional Thoma counting chamber using methylene blue. The different cell counting systems were evaluated according to the following parameters to support brewers in an attempt to find the best qualified method for yeast management, e.g., accuracy, variance of distribution, handling, expenditure of time, costs. All three methods are suitable for determining the total yeast cell concentration and the viability. Viability determination using fluorescence dyes is easier to deal with as it penetrates the cell instantly and stays unchanged. Methylene blue stained cells increase with the incubation time leading to a distortion in detected viability. The Nucleocounter and Cellometer present advantages against the Thoma chamber in ease of handling and fast measurement times. The new Cellometer demonstrates high sensitivity and accuracy when performing a visual examination. Additionally, the best detection of dead cells and viability was demonstrated. Overall it can be said that the new Cellometer is a qualified, accurate method for yeast counting and viability determination. It has the big advantage that the parameters for each yeast strain can be specifically optimized leading to more accurate detection. This makes it possible to analyze yeast strains with small shapes such as *Ludwigii* species and yeast mixtures in more detail. The high investment cost disadvantage, in comparison to the Nucleocounter, is compensated for by the extreme lower cost of each measurement. Furthermore only one measurement per sample is needed for determining both the total cell concentration and the viability.

After qualifying as a certified technician in preservation engineering (1991–1993), Thomas Kunz completed his basic studies in chemistry at the University of Applied Sciences, Isny (1994–1995), and his basic studies in food chemistry at Wuppertal University (1995–1998), before studying food technology at the University of Applied Sciences, Trier (1998–2002). After graduating, he worked as a chartered engineer in the area of ESR spectroscopy at the Institute of Bio Physics at Saarland University (2002–2004). Since January 2005, he has been employed as a Ph.D. student at the Research Institute of Brewing Sciences, Berlin Institute of Technology (Technische Universität Berlin). His main research focus lies in analyzing radical reaction mechanisms in beer and other beverages using ESR spectroscopy.

168. Exploration of matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) as a fast identification tool for beer spoilage bacteria. Presenter: Anneleen Wieme, University College Ghent, Faculty of Applied Bioscience Engineering, Laboratory of Brewing and Biochemistry, Ghent, Belgium. Co-author(s): Anita Van Landschoot, University College Ghent, Faculty of Applied Bioscience Engineering, Laboratory of Brewing and Biochemistry, Ghent, Belgium; Peter Vandamme, Ghent University, Faculty of Science, Laboratory of Microbiology, Ghent, Belgium.

Beer is a beverage with good microbiological stability because it contains almost no oxygen and nutrients for bacterial growth. In addition, low pH, high CO_2 content, and the presence of ethanol and antibacterial hop compounds ensure microbial stability. Nevertheless, beer spoilage induced by bacteria is a common problem in the brewing industry and these spoilage bacteria typically cause visible turbidity, acidity, and off-flavors. Currently, these bacteria are detected with culture-dependent methods using selective media or with faster identification methods such as DNA typing, ribotyping, and other PCR-based techniques. These approaches are notoriously laborious, expensive, time-consuming, and, moreover, lack specificity and sensitivity. The present study aims to develop a quick, specific, and inexpensive method to detect and identify beer spoilage bacteria in the brewing industry. To achieve this, an extensive database comprising MALDI-TOF MS profiles of more than 260 established and accurately identified contaminants and beer spoilage strains was built. In addition to these strains, strains of the same species originating from other niches, besides spoiled beer, were also included in order to encompass the phenotypic diversity of the spoilage species. Among others, strains of *Lactobacillus brevis* (29), *L. lindneri* (3), "*L. brevisimilis*" (1), *L. buchneri* (5), *L. coryniformis* (1), *L. plantarum* (8), *L. parabuchneri* (15), *L. paracollinoides* (2), *L. perolens* (10), *Pediococcus damnosus* (9), *Pediococcus inopinatus* (10), *Pectinatus cerevisiiphilus* (1), *Pectinatus frisingensis* (2), *Selenomonas lacticifex* (1), *Megasphaera cerevisiae* (2), and *Zymophilus raffinosivorans* (2) were included in the database. The resulting set of profiles (±6,500 good quality profiles) allowed the assignment of reproducible species-specific biomarker peaks for all spoilage species. All strains were not only cultured under species-specific conditions (type medium, growth temperature, oxygen requirements, growth time), but also on selective and non-selective media. Different media were used to enable the exclusion of medium-associated peaks from species-specific biomarker peaks. Consequently, identification of novel beer spoilage isolates can be easily and rapidly performed. Nevertheless, the final aim of this research was to detect and identify these bacteria in a spoiled sample with minimal, time-consuming culture steps. Financial support was provided by the Research Fund of the University College Ghent.

Anneleen Wieme graduated in 2009 as a master in industrial sciences biochemistry at the University College Ghent. Currently, she is working at the University College Ghent, and in association with Ghent University she is performing her Ph.D. studies at the Laboratory of Microbiology at the Faculty of Sciences. In the future the results of her Ph.D. thesis, "Exploration of MALDI-TOF MS as a Fast Identification Tool for Beer Spoilage Bacteria," will help the brewing industry in quickly identifying and controlling bacterial beer spoilage.

169. Fast and reliable identification and differentiation of beverage spoiling yeasts by MALDI-TOF MS. Presenter: Julia Usbeck, Technische Universität München, Freising, Germany. Co-author(s): Carola Kern, Rudi Vogel, and Jürgen Behr, Technische Universität München, Freising, Germany.

The growth of beverage spoiling yeasts in beverages during the production process or upon packaging leads to defects such as formation of estery off-flavors, hazes, and turbidity, which result in economic and image losses. Therefore, species-specific biochemical and DNA-based identification methods have been developed that are widely applied in microbiological quality control. However, these methods are generally time-consuming and laborious. Matrix assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) could deliver discriminative peptide mass fingerprints within minutes and could thus be a rapid and reliable tool for the identification and differentiation of beverage spoiling yeasts. Up to now, routine analysis of yeasts by MALDI-TOF MS has been impaired by low reproducibility of data acquisition. Furthermore, the effects of differences in the physiological state of the organism on the reliability of the identification method are still unknown. It was therefore the aim of this study to optimize sample preparation, as well as the MALDI-TOF MS parameterization, by using three strains of yeasts commonly associated with spoilage incidents belonging to the species *Saccharomyces cerevisiae* var. *diastaticus*, *Wickerhamomyces anomalus*, and *Debaryomyces hansenii*. Especially the influences of varying physiological conditions like respiration or fermentation and different nutrients on protein mass signatures were analyzed. Routine identification for yeast samples was established by optimized sample preparation and MALDI-TOF MS settings. Environmental or physiologic parameters including the availability of oxygen, different nutrients, yeast cell density, or growth phase revealed small differences in the peptide mass fingerprint. The status of a spoilage yeast performing fermentation or respiration could be precisely differentiated along these small differences (biomarkers) in the mass spectrum. Still a core of mass peaks remained constant under all tested conditions enabling reliable identification. Yeast cell concentration did not affect the spectra distinctly and an influence of available nutrients could not be measured in each case. Significant differences caused by specific culture or environmental conditions can be connected to their respective origins.

Julia C. Usbeck was born in 1984 in Wuppertal, North Rhine-Westphalia, Germany. In 2009 she finished her studies in food chemistry at the Westfälische Wilhelms-Universität, Münster, followed by a mandatory practical year to accomplish the second state examination. Currently she is working on her Ph.D. thesis on the ability to detect beverage spoiling yeasts using MALDI-TOF mass spectrometry at Technische Universität München under the supervision of Rudi F. Vogel at the Chair of Technische Mikrobiologie in Weihenstephan.

170. Gene expression measurement by real time PCR, relevant for the synthesis and the degradation of acetate esters and 4-vinylguaiacol, in top fermenting yeast. Presenter: Hubertus Schneiderbanger, Research Center Weihenstephan for Brewing and Food Quality, Freising, Germany. Co-author(s): Mathias Hutzler and Fritz Jacob, Research Center Weihenstephan for Brewing and Food Quality, Freising, Germany.

Gene expression measurement by PCR is a well-known instrument to get a view into yeast and microorganism cells. So far research has been focused on bottom fermenting yeast. In this study the gene expression responsible for aroma compound synthesis of top fermenting wheat beer yeast (yeast strain TUM 68) was analyzed. The most important aroma compounds, especially for wheat beer (isoamyl acetate, ethyl acetate, 4-vinylguaiacol, and higher alcohols) were synthesized and degraded by different enzymes, which are encoded in the genetic structure of the yeast cells. It is therefore possible to measure messenger-RNA, which encodes the buildup plan for enzymes, using real-time PCR like it has been done for bottom fermenting yeasts in the past. In this study a method was introduced for measuring gene expression levels in top fermenting wheat beer yeasts of the genes *ATF1*, *ATF2*, *IAH1*, and *PAD1*. The genes *ATF1* and *ATF2* encode alcoholtransferases of *Saccharomyces cerevisiae*, which are responsible for the synthesis of ethyl acetate and isoamyl acetate. These esters are degraded by an *IAH1*-encoded esterase. The *PAD1* gene encodes the yeast enzyme phenylacrylic acid decarboxylase, which has previously been associated with the synthesis of 4-vinylguaiacol. The aim of this work was to further the monitoring of the enzymatic synthesis and degradation including the flavor production during main fermentation. Therefore it is possible to gain knowledge of the preferential synthesis and degradation times of these important aroma compounds under realistic conditions. The fermentation trials, which have been done, have been varied using harvested yeast, propagation yeast, and yeast under pressure conditions. It was shown that top fermenting yeast mainly synthesizes enzymes for acetate-ester production between 12 and 24 hr after the beginning of fermentation (depending on the type of yeast used). The expression of *IAH1* genes increased mainly at the end of fermentation. *PAD1* genes were present at relatively low levels in all trials, which were done with yeast strain TUM 68. This method enables measurement of genes in order to predict the behavior of yeast cells. For the future it could therefore be possible to predict yeast behavior in a technological way in order to achieve the best aroma for this type of beer.

Hubertus Schneiderbanger was born in 1982 in Scheßlitz, Germany. In 2003 he started studying at the TU Munich and graduated in 2008 as an engineer (Dipl.-Ing.) for brewing science and beverage technology. After graduation he worked at the Lehrstuhl fuer Technologie der Brauerei II (2008–2009). Since 2009 he has been working as a consulting engineer and a Ph.D. student at the Research Center Weihenstephan for Brewing and Food Quality. His research focuses on the aroma profile of wheat beer.

171. Identification of bacterial contaminants in beverages by MALDI-TOF MS. Presenter: Carola Kern, Technische Universität München, Freising, Germany. Co-author(s): Julia Usbeck, Rudi Vogel, and Jürgen Behr Technische Universität München, Freising, Germany.

The growth of microbial contaminants in industrially produced beverages can cause turbidity, haze, and off-flavors resulting in quality loss and often rendering the product undrinkable. Therefore, rapid and reliable identification and differentiation of spoilage bacteria is crucial in the beverage industry to ensure efficient quality control. As traditional methods for bacterial identification are usually very laborious and time-consuming, there is a high demand for alternative methods. In this work we present matrix assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) based on the generation of peptide mass fingerprints, which form a distinctive protein peak pattern, as a rapid, reliable, and powerful tool for the identification of spoilage bacteria in beverages. Three strains belonging to the species *Lactobacillus brevis*, *Pediococcus damnosus*, and *Leuconostoc mesenteroides* were used to optimize sample preparation and MALDI-TOF MS settings to ensure resulting spectra were of the best achievable quality. Since MALDI-TOF MS requires a culturing step, and routine quality control procedures differ vastly in practice, growth conditions such as culturing time and availability of oxygen and nutrients were varied to assess

their influence on the acquired protein peak pattern. Additionally, about 50 strains, belonging to the families *Lactobacillaceae*, *Leuconostocaceae*, and *Acetobacteraceae*, which are frequently encountered in spoilage incidents, were used for the establishment of a reference spectra database upon optimization of sample preparation. Data processing was performed using ClinProTools 2.2 and MALDI Biotyper 3.0. Routine identification of bacterial samples was successfully implemented. Among the tested parameters, neither culturing time nor availability of oxygen or nutrients impaired identification of the bacterial isolates on the species level, yielding only slight differences in spectra. Closer examination of these differences showed that more than 90% of all spectra could be correctly assigned to the medium used for culturing, whereas no such correlation could be established for culturing time and oxygen availability. Taken together, MALDI-TOF MS allowed differentiation on the species level regardless of the culture conditions used. The application of specific environmental conditions resulted in variations in spectra, which were sufficient to be detected and reliably assigned.

Carola C. Kern was born in 1984 in Austria. She obtained her master's degree in nutritional sciences from the University of Vienna in 2010 and is currently a Ph.D. student at the Technical University of Munich, where she's working on the identification and differentiation of microbial contaminants in beverages by MALDI-TOF mass spectrometry at the chair of Technische Mikrobiologie under the supervision of Rudi F. Vogel.

172. Investigation of beer-spoilage ability of *Dekkera/Brettanomyces* yeasts and development of multiplex PCR method for beer-spoilage yeasts. Presenter: Satoshi Shimotsu, Asahi Breweries, Ltd., Research & Development Laboratories for Brewing, Ibaraki, Japan. Co-author(s): Shizuka Asano, Kazumaru Iijima, Koji Suzuki, Hiromi Yamagishi, and Masayuki Aizawa, Asahi Breweries, Ltd., Research & Development Laboratories for Brewing, Ibaraki, Japan.

In the brewing industry, microbiological control of wild yeasts is very important to produce high quality beers. Among the wild yeasts, *Saccharomyces cerevisiae*, including *S. cerevisiae* var. *diastaticus*, *Dekkera anomala*, and *D. bruxellensis*, have been reported to cause turbidity and off-flavors in beer. *Brettanomyces custersianus* and *B. nanus*, which are closely related to the established beer-spoilage *Dekkera* species, have been isolated from beers, but their beer-spoilage ability remains poorly characterized. In this study, we therefore investigated the beer-spoilage ability of *Brettanomyces* yeasts and developed a rapid and simple method to identify beer-spoilage yeasts. To evaluate beer-spoilage ability, the strains of *B. custersianus*, *B. nanus*, and *B. naardenensis* were inoculated in pilsner-type beers. The inoculated beers were incubated at room temperature and examined regularly for visible growth for up to three months. Sediment was observed in all beers inoculated with *B. custersianus* strains. On the other hand, it was found that *B. nanus* and *B. naardenensis* strains were unable to grow in beer. These results indicate that *B. custersianus* should be treated as a beer-spoilage yeast in addition to the established beer-spoilage species *S. cerevisiae*, *D. anomala*, and *D. bruxellensis*. Subsequently, we developed a multiplex PCR method for detecting and identifying beer-spoilage yeasts, *S. cerevisiae*, and three beer-spoilage *Dekkera/Brettanomyces* species by designing PCR primers and optimizing PCR conditions. PCR primers were designed in the 26S rDNA region to amplify the different sizes of PCR product from each target yeast to make it possible to identify individual target species simultaneously. Specificity, reactivity, and sensitivity of the designed primer pairs were evaluated by conducting multiplex PCR. It was found that amplicons were obtained from only target species, while no false positive reactions were detected for other non-target species tested. Reactivity was investigated using several strains of *S. cerevisiae*, *D. bruxellensis*, *D. anomala*, and *B. custersianus*, and it was verified that all of the tested strains could be detected. Sensitivity was examined by comparing the detection limit of spoilage wild yeasts. Because the detection limits of the multiplex primer mix were identical to those of the universal primer pairs with 10^3 cells/tube detection limits, the sensitivity of this method was sufficient for detection. These results indicate that the developed multiplex PCR method has high specificity and reactivity. Taken together, the developed multiplex PCR method is considered an effective tool to detect beer-spoilage yeast, contributing to microbiological quality assurance in breweries.

In 2008, Satoshi Shimotsu received a master's degree in food hygienics from Kyushu University, Japan, where he majored in biofilm of food-poisoning bacteria. He joined Asahi Breweries, Ltd. in April 2008. He is now engaged in microbiological quality assurance in breweries and alcoholic beverages.

173. Methods for induction, separation, and identification of haploid strains of industrial brewer's yeast. Presenter: Weina Xu, Jiangnan University, Wuxi, China.

Yeast is the soul of beer fermentation. During fermentation, yeast produces numerous beer flavor compounds, including esters, acetaldehyde, and higher alcohols. At the same time, some other characteristics of brewer's yeast, such as cell age, fermentation rate, and flocculation, are also important in the brewing industry. Brewer's yeast has a decisive influence on beer fermentation. Most industrial brewer's yeasts are lager yeasts and are generally natural genetic hybrids, heterothallic polyploidies, or aneuploidies. Their intricate ploidies present a great challenge to genetic studies and strain improvement. Haploid breeding is an effective method to overcome these difficulties. In this study, we established a systematic method to induce, separate, and identify haploid strains of industrial brewer's yeast. Firstly, efficient sporulation medium was selected to induce sporulation of industrial brewer's yeast strain G-03. Then spores were isolated from vegetative cells and formed colonies on YPD plates. Flow cytometry was employed to determine the ploidy types of the pre-judged haploid candidates. The genotypes were analyzed by PCR for the MAT locus and mating test. Using this protocol, 26 yeast strains were obtained by spore isolation. Four that were pre-judged as haploid candidates were finally confirmed as haploid by flow cytometric analysis, two of them were MATa, and others were MATalpha. This protocol proved practical for breeding haploid strains of other industrial brewer's yeasts as well.

Weina Xu received a B.E. degree in bioengineering from Dalian University and is currently a doctoral student in fermentation engineering at Jiangnan University. She has studied microbiology for several years, and now is devoting herself to the breeding and improving of brewer's yeasts.

174. Optimizing hops gradient plates for assessing bacterial beer-spoilage potential. Presenter: Barry Ziola, University of Saskatchewan, Saskatoon, SK, Canada. Co-author(s): Barry Bushell and Vanessa Pittet, University of Saskatchewan, Saskatoon, SK, Canada.

Important to the brewing industry is the ability to determine if bacteria present in post-filtered beer are capable of growing in (i.e., spoiling) the product. A major factor for bacterial growth in beer is hop resistance, which we have previously

shown can be assessed with hop extract (iso-alpha-acids) infused agar (HGA) plates with a bitterness gradient ranging from 0 to 29 BU. To make hop agar gradient plates more applicable to a brewery setting, we assessed various parameters, including the steepness of the BU gradient, effect of the presence of ethanol, and stability of hop extract over time. HGA plating consists of pouring a dual layer agar plate such that the bottom layer containing the maximum BU concentration forms a 10.5 degree wedge of hop-infused agar, which is then covered by a top layer of hop-free agar. Diffusion from the bottom agar layer creates a hops gradient at the agar surface. Lines of bacterial cultures are stamped upon the surface, and the distance of growth indicates the BU level at which growth is inhibited. The effect of ethanol on hop resistance can be assessed by including ethanol in both agar layers. Gradient plates containing four different ranges of hop concentrations were tested (0–29 BU, 1×; 0–58 BU, 2×; 0–87 BU, 3×; 0–116 BU, 4×). In addition, to assess hop stability, two samples of the same commercial hop preparation separated by 5 years in storage at 4°C were tested. In this study, 26 isolates (22 *Lactobacillus* and 4 *Pediococcus*) were assessed. Of these, 10 grew completely through the 1× gradient plates, and hop resistance could not be scored. With 2× hop-gradient plates only four isolates could not be scored. Ultimately, all four could be scored (grew only partially along the hop gradient) using a 4× plate. With 2× plates containing 5% ethanol, three isolates showed increased hop resistance—two of which we had previously described as having ethanol-enhanced hop resistance. This increased hop resistance in the presence of ethanol was verified for one of the three isolates by growth trials in liquid medium. Lastly, no difference in antimicrobial efficacy of the two hop extracts was found. Based on these results, the use of 2× hop gradient agar plates (0–58 BU) is recommended for routinely assessing bacterial hop resistance, with highly hop-resistant isolates requiring a 4× gradient plate (0–116 BU). As well, properly stored hop extracts can be used over extended periods of time in hop gradient plates.

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

176. Quantitative real-time PCR analysis of putative beer-spoilage associated genes in *Pediococcus claussenii* and *Lactobacillus brevis.* Presenter: Jordyn Bergsveinson, University of Saskatchewan, Saskatoon, SK, Canada. Co-author(s): Vanessa Pittet and Barry Ziola, University of Saskatchewan, Saskatoon, SK, Canada.

Although the unique chemical and physical composition of beer provides an incredibly inhospitable environment for bacterial growth, lactic acid bacteria (LAB) frequently survive in and spoil beer. The presence of contaminating bacteria therefore poses a great threat to the brew quality and economic success of a brewery. *Pediococcus* and *Lactobacillus* are common genera of LAB isolated from spoiled beer; however, not all isolates of a given species in either genus can grow in beer. This indicates there is genetic specialization in beer-spoiling organisms such as *Pediococcus claussenii* ATCC BAA-344T (Pc344NR; non-ropy) and *Lactobacillus brevis* BSO 464 (BSO 464), both of which are capable of flourishing in a beer environment. Despite the evident genetic adaptations of beer-spoilage LAB, very few genes have been found to correlate with the beer-spoiling ability of an organism. To investigate the role of several putative beer-spoilage related genes within each organism in relation to growth in beer, quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was utilized. RNA was extracted from both Pc344NR and BSO 464 cultures at mid-logarithmic growth in both beer and non-beer environments and then converted to cDNA and subjected to qRT-PCR analysis. In order to accurately assess differential gene expression in the beer environment, appropriate internal control genes are needed to normalize the experimental setup for sample variation and experimental error. As such, expression stability (in both growth conditions) of 12 candidate normalization genes was performed and analyzed with the application geNorm. Subsequently, cDNA samples were analyzed to determine the expression levels of the putative beer-spoilage related genes *hitA*, *horA*, *horB*, *horC*, and *bsrA*. Using this methodology, we now have a better understanding of the role these genes play during growth in beer for our two LAB isolates. It is anticipated that this work will be extended to include qRT-PCR analysis of possible beer-spoilage related genes in a broad range of LAB with the intent to definitively identify genes that can serve as genetic markers for assessing bacterial beer-spoilage potential.

Jordyn Bergsveinson received a B.S. degree (with honors) in microbiology and immunology in 2011 from the University of Saskatchewan. She is currently working toward completing an M.S. degree in health sciences through the College of Medicine at the University of Saskatchewan, under the supervision of Barry Ziola. Her research topic and area of interest concerns the genetic and molecular analysis of beer-spoiling microorganisms.

177. The application of antifungal protein (AFP) from *Aspergillus giganteus* to the malting process and its effect on malt and corresponding beer. Presenter: Deliang Wang, China National Institute of Food and Fermentation Industries, China. Co-author(s): Yue Yuan, Division of Food Sciences, Xinjiang Agricultural University, China; Zhiping Lin and Jiafeng Chao, Technical Research Center of Beijing Yanjing Brewery Group Co. Ltd., China.

Filamentous fungi infections in malting barley can have severe effects on malt quality and fermentation performance of brewing yeast, such as reduction of barley germination rate, induction of premature yeast flocculation (PYF), beer gushing, and off-flavors. In addition, most filamentous fungi, especially *Fusarium*, can produce a series of mycotoxin in barley (deoxynivalenol [DON], nivalenol, T-2, HT-2, diacetoxyscirpenol, zearalenone, aflatoxins, ochratoxin A, and fumonisins), and they may have a great negative influence on malt quality and food safety. In this study, the antifungal protein (AFP) was isolated from the fermentation supernate of *Aspergillus giganteus* identified by a series of experiments, such as morphologic observation and molecular biology. Its application and the influence of AFP on malting were analyzed on laboratory and pilot scales. And, its parameters from corresponding beer production were also tested. Results showed that AFP can effectively and significantly inhibit the growth of most kinds of filamentous fungi on barley and reduce both the residues of mycotoxin and corresponding gushing potential; however, in comparison to the control, the data also showed that its application has no negative effects on fermentation performance during beer production.

Deliang Wang is the director of the Brewing Engineering Technology Department (BETD), a subdivision of the China National Research Institute of Food & Fermentation Industries (CNRIFFI). Since Deliang graduated, he has been acting as an applied scientist presiding

over the technical consultants and cooperative projects with many large Chinese breweries, such as Anheuser-Busch InBev, Yanjing brewery (top 8 in the world), Kingstar brewery (no. 12 in the world), Zhujiang brewery (no. 15 in the world), Kingway brewery, Yinmai brewery, etc. The beer volume produced by these breweries is more than one-third of the total Chinese beer production each year. Besides domestic enterprises, Deliang is also responsible for cooperation with international partners, such as DSM food limited, ICT-Czech Republic, VLB-Berlin, etc. Deliang has also published many articles at home and abroad, and some articles have been published in the Journal of the American Society of Brewing Chemists. Deliang is a member of both ASBC and MBAA.

178. The spoilage of microbrewery beer from *Bacillus* species isolated from pelletized hops. Presenter: Nathan Traw, Mother's Brewing Company, Springfield, MO, USA.

The food and beverage industries are affected by the growing population of microbes that are resistant to current antimicrobial chemicals. Specifically, the brewing industry is seeing the emergence of new beer spoiling organisms that were previously not a concern. *Bacillus* species are used as an organic pesticide in the United States by hop growers. This experiment seeks to correlate the spoilage of microbrewery beer by *Bacillus* species and its use as an organic pesticide by the hop industry. Spoiled beer from a recently opened microbrewery was plated on Schwarz differential agar (SDA). *Bacillus* was isolated and confirmed by traditional microbiology methods that included Gram staining, endospore staining, and enzyme tests. The microbrewery's hops were then analyzed. Hops were supplied by the microbrewery and were commercial pelletized hops purchased from a brewer's supply store. The hops were in a vacuum sealed bag and pelletized. The hops used by this particular microbrewery were not purchased as organic. Hops were aseptically sampled and placed in sterile nutrient broth. After 24 hr of incubation, 400 µL of the nutrient broth was plated on SDA. *Bacillus* was confirmed by traditional microbiology methods as the sole isolate from the hops. The *Bacillus* culture isolated from the hop sample was then incubated in microbrewery beer. After 48 hr of incubation, the beer was observed microscopically for *Bacillus*. The presence of live *Bacillus* cells was confirmed. This confirms that a beer-resistant strain of *Bacillus* introduced by commercially prepared hops is capable of spoiling microbrewery beer by unknown mechanisms. Future studies on this topic include beer stability, dissolved oxygen concentrations, and genetic sequencing.

Nathan Traw received his B.S. degree in biology-microbiology and biotechnology from Missouri State University in May 2011. During his time at MSU, Nathan spent nearly two years as a student researcher studying the transcription of genes in macrophages in response to bacterial stimuli. Nathan is now the quality assurance and control lead for Mother's Brewing Company in Springfield, MO. He is currently preparing for the 2012 IBD Diploma in Brewing examinations.

179. Using PCR in the brewery routine makes you see microbiology from a new angle. Presenter: Gudrun Vogeser, PIKA Weihenstephan GmbH, Pfaffenhofen, Germany.

Microbiological analyses are well established in breweries' routine control, but mostly are not relevant to make a decision—the results either come out too late or they are too indifferent to be of practical use, in the worst case they are both. Fast detection methods including polymerase chain reaction (PCR) are continuously being devised. With improvements in instrumentation and sample handling for PCR analysis, this method is spreading into laboratories for daily production control. The basic thought behind use of PCR is to gain better and earlier knowledge about microbial process contamination due to the considerably lower detection limits of PCR compared with conventional enrichment methods. Beginners in using PCR in microbiology keep their focus on the gain of time due to the shorter duration of pre-enrichment. This study shows applications of PCR and results from routine brewery analyses comparing traditional enrichment methods with results from PCR. Based on case studies it is demonstrated that not only the detection limit factor is important—especially for troubleshooting the precision and specificity of this method is unbeatable. Some contaminations were only detected due to PCR, remaining invisible with the traditional enrichment methods. A major benefit can be drawn from PCR results if sampling within the process line is rethought and adjusted to the new method; fewer samples taken at other time points in the process line resulted in the most effective output.

Gudrun Vogeser received a diploma in microbiology from Eberhard Karls University in Tuebingen, Germany. Her first job was at the Technical University of Munich, Germany, where she finished her Ph.D. thesis in 1992. She was then employed as a scientist at the Chair of Brewing Technology at TU Munich-Weihenstephan, where she examined the use of molecular biology methods, mainly PCR, to detect and analyze beer spoiling microorganisms. In 2000 she founded PIKA Weihenstephan, Pfaffenhofen, Germany, a company that specializes in brewery and beverage microbiology, where she is working as managing partner. Gudrun is a founding member and since 2009 chair of the European Brewery Convention (EBC) Microbiology Sub-committee and an ASBC member.

Nutrition/Health

180. Arabinoxylans and fructans in the malting and brewing process. Presenter: Moritz Krahl, Radeberger Gruppe, Frankfurt am Main, Germany. Co-author(s): Werner Back, TU München, Freising, Germany.

Nowadays our nutrition is mainly based on only three cereals: wheat, rice, and corn. They contribute over 75% to the world's starch production. At the same time diseases caused by wrong or unbalanced diets are becoming a severe problem in Western countries. In this regard the enrichment of soluble dietary fiber in the malting process with the objective of providing their health-beneficial effects to the consumer is a possible approach to better nutrition. Arabinoxylan (AX) and fructan, two health-beneficial dietary fiber fractions have been measured during the entire malting and brewing process. The total AX in barley, wheat, rye, triticale, oats, and spelt wheat did not change during the malting process. Water-extractable arabinoxylans (WEAX) increased during malting, and the influence of the germination parameters time, temperature, and moisture content was determined for each of the cereals. The malting process is influenced by the quality of the raw material and by several process parameters (e.g., moisture, temperature, and time); in addition changes in dietary fiber content depend on the same variables. To reduce the necessary number of trials for the evaluation of optimal conditions we used software for the design of experiments. This software supports several different statistical approaches like various factorial designs or response surface methods (RSM). With RSM the interactive effects of various process conditions are modeled empirically. WEAX increased during germination in all malted cereals. Kilning had no effect on the amount of WEAX in most cereals. Only in rye did WEAX content increased by 30% during kilning. At the same time the viscosity of Congress wort decreased. The probable explanation for this phenomenon is a

cleaving of diferulic acid-bridges by the heat of the kilning process. These cross-linkages have been found only in WEAX of rye. Using optimum malting parameters WEAX can increase up to threefold the level found in unmalted kernels. Malting did not affect the fructan content of most cereals, so no losses of this dietary fiber fraction were observed. In spelt wheat the fructans increased during standard malting, and the final level was 67% higher than in the raw material. During the brewing process only mashing had an influence on AX content. During lautering, wort boiling, fermentation, and storage no changes were observed. Fructans were not influenced by mashing, wort boiling, and lautering. During fermentation and storage more than 90% of the fructans found in cooled wort were fermented by the yeast. The experiments showed that malting is an effective way to enrich WEAX in cereals and fructans in spelt wheat. In the brewing process the final WEAX concentrations depend on the raw materials and not on the mashing parameters.

Moritz Krahl was born in Schwetzingen, Germany. After passing the German Abitur (A levels) in 2000, he began studying brewing and beverage technology at Technische Universität München in Weihenstephan, Germany. In 2004 he graduated with a B.S. degree and in 2005 with a Dipl.-Ing. (graduate engineer) degree. From 2005 to 2010 Moritz worked on his Ph.D. on "Functional Beverages Based on Malted Cereals and Pseudocereals" at the Institute for Brewing and Beverage Technology in Weihenstephan. From 2010 to 2011 he worked as head engineer for plant and process optimization for MEG. In October 2011 Moritz joined the Radeberger Group with key responsibility in product and process development for new beverages.

181. Development of 0.00% alcohol beer, focusing on the characteristic bitterness and body of regular beer. Presenter: Takayuki Kosugi, Value Creation Department, Sapporo Breweries Ltd., Yaizu, Japan. Co-author(s): Tsutomu Yamaki, Yoichi Kozaki, Kiyoshi Takoi, Toru Shioi, and Junji Watari, Value Creation Department, Sapporo Breweries Ltd., Yaizu, Japan.

In Japan, the 0.00% alcohol beer market has grown in the last two years, in contrast to the shrinking alcoholic beer market. This is presumably due to the health habits and negative views toward alcohol among average Japanese consumers. The market is expected to show further growth, so major Japanese breweries are making efforts to develop technologies and products in this category. Almost all of these products have focused on drinkability, low bitterness, and light body to appeal to light consumers of beer. As a result of our market research, however, we found that approximately 30% of consumers of non-alcoholic beer are regular consumers, and they were not satisfied with the flavor and quality of these products. Therefore, we have developed our product with the aim of achieving the bitterness and body of regular beers. We investigated the correlations among bitterness, flavor, and stability in non-alcoholic beer and found that gushing volume is related to the volume of bitter ingredients. In this presentation, we describe the knowledge obtained from the development of our 0.00% alcohol beer product and our additional efforts to achieve more authentic beer flavor and enhanced quality.

Takayuki Kosugi graduated from Kobe University with a master's degree in biological and environmental science in 2001. He began working for the quality control and research section in Kizakura Breweries Ltd. He then joined the Product & Technology Development Center of Sapporo Breweries Ltd. in 2005. He has been engaged in new product development.

184. OSHA and proposed diacetyl limits in the workplace—What effects can we expect on the brewing industry? Presenter: David Radzanowski, Radzan Associates, Madison WI, USA.

Two years ago, OSHA, in conjunction with NIOSH and CDC, invited members of industries that handle or produce diacetyl during their production processes to gather together and attempt to define a reasonable safe exposure limit for workers exposed to the chemical in the workplace. This was in reaction to the "popcorn lung" that was detected among workers in the snack food and baking industries. A few died, and many were disabled. The disease was attributed to diacetyl exposure. After the meetings, the consensus seemed to be that the limit should be somewhere around 1 ppm/day of exposure. This seemed to exempt the brewing industry from concern since we have not found diacetyl content in brewing products above 200 ppb. OSHA recently published a proposed action limit of 5 ppb, which caught all participants by surprise. While the brewing industry, at least at first, would not be a "target industry," we must be concerned and attempt to have these limits reviewed. We will discuss the reasons for concerns.

David Radzanowski began his brewing career in 1962, joining the Duquesne Brewing Company of Pittsburgh, PA, after studying chemical engineering at Carnegie Tech (now Carnegie Mellon University). After graduating with the 1970 Siebel Institute Diploma Class, David was named the associate supervising master brewer, sharing production responsibilities with the vice president of production. With the closing of the Duquesne brewery, David joined the Joseph Huber Brewing Company of Monroe, WI, in 1973 as director of QC and assistant master brewer, eventually becoming master brewer and vice president of production. In 1992, David joined the Siebel Institute of Technology as vice president of educational services, becoming president of the institute in 1998, serving until 2000. In 2000, David and his colleagues joined Alltech Inc., resulting in the formation of the Alltech Institute of Brewing and Distilling (AIBD) in conjunction with Herriot-Watt University. With Alltech, David had a dual role as technical manager for Asia-Pacific, covering brewing, ethanol production, and distilling, and as administrator of educational services of AIBD. He is now president of Radzan Associates, offering services to the brewing, distilling, and ethanol industries.

185. Silicon in lager beers and its balance during the brewing process. Presenter: Pavel Dostalek, Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology, Prague, Czech Republic. Co-author(s): Rudolf Cejnar, Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology, Prague, Czech Republic; Oto Mestek, Department of Analytical Chemistry, Institute of Chemical Technology, Prague, Czech Republic.

Silicon is an important essential trace element. The recommended daily intake is about 10–25 mg/L. Silicon deficiency is mostly associated with losses of connective tissue components, such as glycosaminoglycanes, collagen, and elastin. The most readily absorbable form of silicon is orthosilicic acid. Foods derived from plants rather than animals provide the highest sources of dietary silicon, because certain plants, especially cereals, are silicon accumulators. In particular, high levels of bioavailable silicon are found in beer, which is made from barley malt, from which orthosilicic acid is released into the beer. Inductively coupled plasma mass spectrometry (ICP-MS) was used for determination of silicon in lager beers from the Czech market and in brewing semiproducts. The goal was to establish silicon concentrations in Czech lager beers and to find out which individual processes are the most significant in terms of silicon concentration in beer. Silicon concentration in Czech lager beers ranged from 16.3 to 113.0 mg/L, and it was shown that the concentration depends primarily on two factors. First, the silicon content of beer rises with the original

wort concentration, and second, during decoction mashing silicon is leached much more than in the case of infusion mashing.

Pavel Dostálek was born in 1963. He studied as a graduate engineer at the Faculty of Food and Biochemical Technology of the Institute of Chemical Technology Prague, Czech Republic (1985). He holds a Ph.D. degree in fermentation chemistry and technology from the same institute (1991). In 1987 he was an assistant scientist in food technology. In 1990 he became an assistant professor for brewing science, and in 1993 he stayed at the Dublin City University. In 1996 he finished post-graduate courses on food technology at Hebrew University, Agricultural Faculty, Rehovot, Israel, and in 1997 became a lecturer in the Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology Prague. He has been an associate professor in biotechnology since 2007.

186. The glycemic index—Chance or threat for the beverage industry? Presenter: Moritz Krahl, Radeberger Gruppe, Frankfurt am Main, Germany.

Jenkins introduced the concept of the glycemic index (GI) in the 1970s. In the following years diseases caused by poor or unbalanced diets have developed into severe problems in the Western world. The growing number of patients affected by diabetes mellitus type II especially seems to be directly related to the amount and type of carbohydrates consumed in the daily diet. The GI classifies carbohydrates with regard to their individual resorption time from a consumed food. The resulting postprandial glucose levels are compared to those measured after consumption of a reference food, notably glucose or white bread. The GI of glucose is set at 100, the GI of sucrose is set at 66, and the GI of fructose is set at only 20. In this paper the concept of the IG and the fundamentals of carbohydrate metabolism are explained. Measured GI values for different types of beverages, both alcoholic and non-alcoholic, are correlated with the amounts of sugar present in these beverages. In recent years consumers and non-governmental organizations have become increasingly aware of the possibly negative health impact of high GI beverages. In consequence the industry needs to focus on low calorie and low GI products as an alternative to traditional beverages containing high GI carbohydrates. Sweeteners and low GI carbohydrates can be used in this regard. The advantages of different alternative sweeteners like steviol glycosides, polyols, erythritol, isomaltulose, and trehalose are compared and discussed. As all these sweeteners offer attributes different from sucrose and glucose, product formulations need to be adapted. By combining two or more different sweeteners and using their synergistic effects, taste profiles close to sucrose sweetened beverages can be guaranteed. In conclusion it can be stated that alternative low calorie and low GI sweeteners offer an alternative to glucose and sucrose based sweetening of beverages. For beverage producers it is of major importance to focus on synergistic effects, changes in flavor expression and stability, and differences regarding mouthfeel and consumer acceptance during product development.

Moritz Krahl was born in Schwetzingen, Germany. After passing the German Abitur (A levels) in 2000, he began studying brewing and beverage technology at Technische Universität München in Weihenstephan, Germany. In 2004 he graduated with a B.S. degree and in 2005 with a Dipl.-Ing. (graduate engineer) degree. From 2005 to 2010 Moritz worked on his Ph.D. on "Functional Beverages Based on Malted Cereals and Pseudocereals" at the Institute for Brewing and Beverage Technology in Weihenstephan. From 2010 to 2011 he worked as head engineer for plant and process optimization for MEG. In October 2011 Moritz joined the Radeberger Group with key responsibility in product and process development for new beverages.

Packaging (Bottles, Draft, Cans)

187. Development of barrier materials for bio-based beverage packages. Presenter: Ali Harlin, VTT Technical Research Centre of Finland, Finland. Co-author(s): Thomas Gädda, Mika Vähä-Nissi, and Annika Wilhelmson, VTT Technical Research Centre of Finland, Finland.

Bio-based materials, like polylactic acid (PLA) and lately bio-based PET, are increasingly present in beverage applications due to environmental sustainability requirements of consumers and the demands of increased green image by brand owners. Simultaneously retailers and society have an increasing interest in finding solutions for waste management. Bio-based PET is finding its way into carbonated soft drinks. PLA is still used for water bottles because of material limited barrier properties. In order to apply the polyesters in the future for even more demanding applications improved barrier properties are required. VTT has actively developed bio-based barrier solutions. Novel barrier polymers such as polyglycolic acid (PGA) has shown exceptionally good oxygen barrier properties. Thin layer barriers like atomic layer deposition (ALD) oxygen barrier properties have been previously demonstrated with PLA. This work reports novel barrier results for development materials based on their potential for future beverage packaging. Suitability for brewery products will be discussed.

Ali Harlin is a research professor for bio-based materials and heads the Industrial Biomaterials spearhead program in VTT, the Technical Research Centre of Finland, targeting industrial applications for materials produced using renewable raw materials to generate new, sustainable value chains and to reduce our dependency on oil and the carbon footprint of consumption. The spearhead program focuses on the development of materials and production technologies based on fibers and nano-cellulose, as well as biomass-based monomers and polymers. The aim is to integrate these new value chains into existing bio-refineries. Ali previously worked in industries ranging from petro-chemistry to forest industry and in several academic positions at different universities.

188. Draught beer equipment and microbiology—Investigations to avoid microbiological contamination. Presenter: Johannes Tippmann, TU München, Wissenschaftszentrum Weihenstephan, Lehrstuhl für Brau- und Getränketechnologie, Freising, Germany. Co-author(s): Simon Henke, Jens Voigt, and Karl Sommer, TU München, Wissenschaftszentrum Weihenstephan, Lehrstuhl für Verfahrenstechnik Disperser Systeme, Freising, Germany; Thomas Becker, TU München, Wissenschaftszentrum Weihenstephan, Lehrstuhl für Brau- und Getränketechnologie, Freising, Germany.

In the brewery, cleaning and sanitation are one of the most important issues in producing a perfect beer. In contrast, in many draught systems sufficient cleaning is not carried out, and the hygiene situation is more than insufficient. As a consequence, the quality of the beer is destroyed during the last meters, just before the consumer enjoys it. In many cases poor quality standards are the result of two main factors: lack of knowledge and disbelief, although the challenges are nearly the same as in the brewery. For this reason, a number of extensive and complex investigations have been done in the last few years to eliminate the remaining ambiguities surrounding this issue. The investigations were done with a focus on the microbiological situation in the draught system, which depends on the following factors: the influence of daily tap cleaning, the influence of regular cleaning of the coupler, the influence of correct and regular cleaning of the draught lines including critical components like pumps or flow meters, the age of the

equipment, and the correct construction of a draught system. The results show scientifically that the experiences and recommendations given in many rules and standards are correct and significant. Periodic cleaning of the tap and the coupler prevent retrograde contamination via the open areas of a draught system. These are the two main critical points at which microorganisms enter the draught system. In unavoidable cases, a microorganism can still enter the system; in such cases, it must be ensured that growth of a biofilm is inhibited by performing regular and sufficient cleaning. In addition to prevention through cleaning, prevention can be enabled through correct construction of draught systems using the latest hygienic design criteria. The abovementioned conditions are necessary qualifications for the customer to receive the same high quality beer in a pub or restaurant as the beer has in the brewery. This presentation, which is partially a review (issue of investigations of tap hygiene), describes the failure, investigations, results, and solutions for the issues mentioned above.

Johannes Tippmann graduated from university in 2004 as a diploma engineer for brewing sciences and beverage technology. In 2005 he started his Ph.D. thesis with Karl Sommer at the Lehrstuhl für Verfahrenstechnik Disperser Systeme, TU München, on solids handling in the brewhouse. He collected wide experience with the procedures in beer production during his studies, conducting student research projects and his diploma thesis on this topic. In 2012 he changed his affiliation and is now working for the Lehrstuhl für Brau- und Getränketechnologie, TU München. He is group leader for the work group Brewhouse Processing and Dispense Systems. Since 2000 he has worked as a student research assistant with dispensing systems and has collected much experience in this subject area. Since 2006, he has been responsible for research issues in dispense systems. He is also a member of the Dispensing Systems Technical Committees of the government association for the food and catering industry (BGN) and of the DIN German Institute for Standardization. He is working for the MEBEK dispense group and has published a number of papers.

190. IBD Master Brewer Module 5 project: The construction and implementation of a packaging quality laboratory for a large craft. Presenter: Gregory Deuhs, Craft Brewers Alliance, USA.

The construction and implementation of a packaging quality laboratory for a large craft brewery. The Redhook Ale Brewery located in Woodinville, WA, is a large craft brewer producing 175,000 bbl of beer per year. The brewery is part of a larger corporation, Craft Brewers Alliance, which operates four breweries within the United States. The brewery does not have a formal packaging quality program or the dedicated laboratory and equipment to conduct meaningful packaging quality analysis. The current packaging quality program consists of a Zahm & Nagel caustic-based, bottle headspace analyzer, a bottle cap crimp gauge, and a scale to determine fill heights. This project encompasses the design, construction, equipment, and staff support to successfully support packaging operations for bottle and draught product. This project also incorporates quality check procedures used elsewhere in the company and the training of two new laboratory technicians dedicated to packaging quality. The end project result is a fully functional laboratory that checks headspace dissolved oxygen, bottle quality, cap, code dates, secondary packaging quality, extensive bottle defect detection, draught beer quality, and micro integrity. The craft beer industry in the United States is a very competitive market. Gone are the days of placing a six pack of beer on the shelf and having it sell itself because it is different from the mainstream American beers. Craft beer today must meet or exceed the customer's expectations, compete favorably with competitors' beers, and match industry standards. A recall of beer for any reason is extremely costly in both reputation and lost sales.

Gregory Deuhs received an MBA from Cardinal Stritch University in Milwaukee, WI. He is a graduate of the UC-Davis Master Brewer and of the Villanova University Six Sigma Black Belt Certification programs. Greg started his brewing career as the head brewer at the James Page Brewing Company in Minneapolis, MN. From there, he served the industry as an assistant brewmaster at The Stroh Brewery Company, brewing team leader at the Miller Brewing Company, and director of operations at the Craft Brew Alliance. His current role is brewmaster for Pabst Brewing Company, Milwaukee, WI. Greg is a diploma member of the Institute of Brewing & Distilling (IBD) and conducted this project in conjunction with the IBD Master Brewer Examination Module 5 requirement.

191. Improvement on the oxidative beer flavor stability using active packaging material—Advantages or disadvantages in comparison to SO_2-addition. Presenter: Victoria Schiwek, Technische Universität Berlin, Department of Biotechnology, Chair of Brewing Sciences, Berlin, Germany. Coauthor(s): Thomas Kunz, Constanze Ruff, and Frank-Jürgen Methner, Technische Universität Berlin, Department of Biotechnology, Chair of Brewing Sciences, Berlin, Germany.

The beer off-flavor caused by oxidation has been attributed to the formation of specific aging compounds (e.g., aldehydes), through several reaction mechanisms. Although the mechanisms differ in their reactive components, they all involve species formed from molecular oxygen, highlighting the importance of avoiding oxygen entry in the brewing process and the final beer by using suitable packaging material. The aim of this study was to figure out the influence of SO_2 addition as an antioxidant before filling in comparison to the use of different crown liner materials with O_2 scavenger properties and the effect of a combination of both on oxidative flavor stability. Recent studies have illustrated that the application of EPR spectroscopy in combination with the determination of specific aging compounds by GC-MS, such as 3-/2-methyl butanal as an oxygen indicator, SO_2 determination, and sensory analysis are useful tools to demonstrate the influencing factors on consumption of natural antioxidants and oxidative flavor stability over the course of shelf life. The described technique combination is predetermined for the evaluation of SO_2 addition in the brewing process or active packaging materials and their impact on oxidative beer stability. For the investigation it was necessary to use the same beer matrix and the same filling station with different crown caps to get an objective comparison with significant results. A part of the beer was separated in a different tank to add 6 mg of SO_2/L before filling at the same filling station. As expected, the beer with SO_2 addition and oxygen scavenger in the crown cap exhibited higher flavor stability (as measured by all measurement techniques) during storage in comparison to the non-scavenger controls or without SO_2 addition. But, the influence on oxidative beer stability of each O_2 scavenger material was very different. A remarkable and, for the reaction mechanism, important point is the comparable increase in oxidative stability with SO_2 addition or O_2 scavenger. It also leads to a different positive effect on the formation of specific aging compounds during storage. In summary, the highest oxidative stability was observed with the best crown liner and SO_2 addition. On the other hand the best O_2 scavenger was able to compensate for more than 6 mg of SO_2 addition per liter during storage so the beer had a higher oxidative stability after 4 months of storage, suggesting it acts directly after filling and during storage. The worst

but cheapest O_2 scavenger showed an effect only after longer storage through the inhibition of oxygen diffusion through the crown cap. All together this research work offers brewers useful knowledge about the best procedure for increasing oxidative beer stability through SO_2 addition and/or active packaging material.

Victoria Schiwek studied pharmaceutical and chemical engineering at the Beuth University of Applied Sciences from 2002 to 2007. The topic of her diploma thesis was "Optimized Analytical Methods for the Determination of SO_2 in Beer and Malt." Since 2010 she has been working as vice head of laboratory for the Chair of Brewing Science at TU Berlin.

192. LineMET—Efficiency analysis tool in bottling plants. Presenter: Stefan Flad, Chair of Food Packaging Technologie, TUM, Freising, Germany. Co-author(s): Tobias Voigt, Chair of Food Packaging Technologie, TUM, Freising, Germany.

Problem bottling is a very cost-intensive part of beer and liquids production. One problem is that the efficiency of bottling plants isn't high enough. To increase the efficiency of the plant, operators need to know the critical points of their plant. In addition they need to measure the influence of different parameters on efficiency. For this, the LVT (Chair of Food Packaging Technology) of TUM (Technische Universität München) and partners started the research project LineMET, which was funded by the Federal Ministry of Education and Research. LineMET should generate an efficiency analysis tool that helps the operator to find critical points and influences of different parameters automatically using operating data. Based on the research presented at the 2007 MBAA Convention by A. Kather, LineMET had the following goals: development of an integrated efficiency analysis tool (demonstrator application); extension of the component library (LineMod) for model base diagnosis; extension of the LineMod system boundary to rate influences from logistics and intralogistics to the plant; extension of the diagnosis functions to complex patterns; and development of a tool to generate line models from the component library methods. The project was divided in four parts. One was a database that contains data points according to the Weihenstephan Standards. This information is enough for key performance indicator (KPI) calculation and line diagnosis. The most important part from a scientific point of view was the model based diagnosis, where LineMod, the model library, was extended. New components were built, like split and merge for splitting/merging object flows. With this library nearly all kinds of filling plants could be modeled. The extension of the models to analyze minor speed of bottling plants was also a result. Another part was the development of ATLAS. ATLAS is a graphic tool to build plant models. Users only need to rebuild their line with drag and drop out of the component library and to set some parameter, like death time of conveyors. The last part is visualization. LineMET gives examples of how to visualize the diagnostic results and to calculate article related KPIs. It is important to investigate influences of bottle type or product, for example on the efficiency of the whole plant. LineMET had two big results. 1) Model based diagnosis can now be used for bottling plants. The determination of reasons for filler downtime is now possible, with a rate over 85%, and all types of bottling plants can be modeled. 2) A demonstrator application was implemented in a commercial tool. It calculates article related KPIs based on real plant data. The only requirement is that the plant provides data according to the Weihenstephan Standards.

Stefan Flad, born in 1982 in Freising, received the university entrance diploma in 2002 at the Dom-Gymnasium in Freising. After that he studied from 2003 until 2008 at the Technical University of Munich, Garching. In 2008 he graduated as an engineer (Dipl.-Ing.) for mechatronics and information techniques. Focus areas were microtechnics and control engineering. In late 2008 he started as a Ph.D. student at the Chair of Food Packaging Technology, Technical University of Munich. He was project coordinator of the LineMET project. He works on the member committee of the Weihenstephan Standards for data acquisition and teaches process control engineering at TUM.

193. New data on bisphenol A (BPA) concentrations in canned beers. Presenter: Leif Garbe, TU Berlin/VLB Berlin, Germany. Co-author(s): Xu-Liang Cao, Bureau of Chemical Safety – Health Canada, Ottawa, ON, Canada; Julie Zech, VLB Berlin/TU Berlin, Germany.

Bisphenol A (BPA) is a chemical agent that exists in many everyday products. In the food industry BPA reaction products are usually introduced as inner can coatings to prevent contact between food and metal surfaces. The presence of unpolymerized BPA monomers is considered the main problem when BPA polymers are used in immediate contact with food. Monomers originate from uncompleted polymerization, mechanical decomposition, or cleavage by chemical hydrolysis. BPA migrates from coating to packed product and contaminates the foodstuff. The consumption of products from BPA containing packages is the main source of consumer exposure to BPA. BPA uptake is suspected to have several negative effects on human health, thus consumers are alarmed and are demanding information. In 2011 more than 500 surveys on BPA were published; still there are no reliable long term studies that prove or disprove adverse health effects of low dose BPA exposure. BPA has been traced in almost every canned food and beverage, as well as in canned beer. Up to now the analytical data have been rare and uncertain. In the present work, the analysis of BPA in canned beer was re-evaluated using several analytical procedures. Varying sample preparation procedures were combined with sensitive GC-MS and LC-MS instrumentation. A stable isotope dilution assay using lab made D4-BPA and commercial D16-BPA stable isotope labeled BPA was employed to enable accurate trace level quantification from the complex beer matrix. In cooperation with the Health Canada Bureau of Chemical Safety in Ottawa (Ontario) analytical data were validated in an inter-laboratory test. The survey demonstrates updated data on BPA in canned beer. It indicates that BPA concentration is very low (approximately 50–400 ng/L), much lower than in most other canned foodstuffs. Obviously, the beer matrix and processing techniques do not favor BPA migration into the product. Even BPA exposure through beer consumption is very low and presumably safe (the U.S. court required the U.S. FDA to decide on a BPA ban by March 31, 2012). Independent of the decision, precise control of BPA levels in foodstuffs and especially in beer seems necessary since BPA will be a ubiquitous chemical for many years even if it is banned today.

Leif-Alexander Garbe is professor for biochemical and technical analysis at the Berlin Institute of Technology (TUB). Additionally, he chairs the Department for Special Analyses at the Research and Teaching Institute for Brewing in Berlin (VLB). Leif graduated in 1996 from TUB with a diploma in chemistry. Then he worked as a researcher and teacher at VLB and TUB. He supervised biotechnology and brewing students and performed several research projects in brewing and life sciences. He finished his Ph.D. thesis in April 2002 on the "Metabolism of Hydroxy-Fatty Acids in Yeasts," and his habilitation thesis in 2009 on "The Biochemistry of Oxidized Lipids: Analytical Characterization of Bioactive Metabolites" at TUB. Today Leif's research interests focus on mass spectrometry, NMR, trace analysis, biotransformation, isotope dilution technique, and Maillard reaction of peptides/proteins.

Sensory

196. A university course on fermentation science in a global society with a study abroad flavor. Presenter: Casey Raymond, Department of Chemistry, SUNY Oswego, Oswego, NY, USA. Co-author(s): Jeffery Schneider, Department of Chemistry, SUNY Oswego, Oswego, NY, USA.

Over the past seven years we have developed and taught a course that explores the interdisciplinary impact of fermentation and distillation science in a global society. The overall goals of this course are for the students to gain an understanding of the scientific principles involved in fermentation and to develop an appreciation of the impact of fermentation on the global society. We discuss how the science of fermentation is connected with history, culture, art, and other facets of a global society and how students develop an understanding of these connections throughout the course. This presentation will address the development of this course as well as student experiences during the course's study abroad component in Belgium, the Czech Republic, the Netherlands, and Scotland. During the course we discuss how water quality impacts beer production and how different styles of beer resulted from the combination of available ingredients and the water quality present. A couple of examples that we discuss are the development of the dry stout style in Dublin and the extra special bitter/English pale ale style in Burton-on-Trent. To get students thinking about using their senses when consuming food and fermented products, we usually have a blind tasting in class. Past examples include: 1) Coke with high fructose corn syrup, Coke with cane sugar, and Diet Coke; and 2) tonic water, club soda, and flavored carbonated water. While we are aboard, we ask students to use their sensory skills to discuss locally produced fermented products and to compare different products. While we are in Belgium, we tour the Rodenbach and Cantillon breweries and then ask students to write about the similarities and differences they observe in production methods and the flavors of the resulting beers. After visiting multiple distilleries in Scotland, we ask students to use their notes from each visit and tasting to write about the similarities and differences between the whiskies. Whatever the venue, we always ask students to write about their experiences with the local culture, which have included food, drink, museum visits, and transportation.

Casey Raymond received a B.S. degree in chemistry from Michigan State University in 1991, a Ph.D. degree in chemistry from Colorado State University in 1996, and a post-doctoral fellowship at Northwestern University. He was an assistant professor at Kent State University prior to his position at the State University of New York, College at Oswego. Casey has worked with Jeffery Schneider to develop new courses at SUNY Oswego related to fermentation science, targeting both science and non-science majors. This is currently leading toward an interdisciplinary minor at the campus after consultation with InBev-AB personnel in Baldwinsville, NY. Both professors have research projects, involving undergraduate students, related to fermentation science.

197. Acceptance of off-flavors in beer by common consumers. Presenter: Moritz Krahl, Radeberger Gruppe, Frankfurt, Germany. Co-author(s): Stefan Hanke, Bitburger Braugruppe, Bitburg, Germany.

In recent years the global beer market has experienced a substantial consolidation in market share, and simultaneously a rather standardized type of beer emerged. Common off-flavors like diacetyl, dimethyl sulfide, and stale flavors, as well as microbial infected beers, have become rare due to technological improvements, as well as to high quality standards set by global brewing companies. On the other hand due to globalization and a prolonged distribution chain beer faces a certain amount of aging before it reaches the consumer. This work shows the results obtained by a preference tasting, including several off-flavors (diacetyl, dimethyl sulfide) and forced aged beer, as well as linalool. Linalool was included as a flavor in the tasting trial because it is known as an indicator substance for late hopped premium beers. Additionally concentrations of linalool are sub-threshold in the standardized beers mentioned above. In the trial each volunteer was presented a set of two beer samples. One was a traditional commercially available Bavarian style lager; the other was the same beer spiked with a specific pure flavor or forced aged, respectively. Tasters were asked to state which beer they preferred. The results show that fresh beer samples were not significantly preferred by consumers. Addition of off-flavors resulted in a significantly lower preference for the beer samples. Also the addition of linalool resulted in decreased preference. In conclusion this work shows, that consumers seem to be used to aged beer. However a differing flavor profile resulted in a lower preference.

Moritz Krahl was born in Schwetzingen, Germany. After passing the German Abitur (A levels) in 2000, he began studying brewing and beverage technology at Technische Universität München in Weihenstephan, Germany. In 2004 he graduated with a B.S. degree and in 2005 with a Dipl.-Ing. (graduate engineer) degree. From 2005 to 2010 Moritz worked on his Ph.D. on "Functional Beverages Based on Malted Cereals and Pseudocereals" at the Institute for Brewing and Beverage Technology in Weihenstephan. Form 2010 to 2011 he worked as head engineer for plant and process optimization for MEG. In October 2011 Moritz joined the Radeberger Group with key responsibility in product and process development for new beverages.

200. Good sensory techniques for training a beer panel. Presenter: Mona Wolf, The Wolf Group, Cincinnati, OH, USA. Co-author(s): Jill Rolfes, The Wolf Group, Cincinnati, OH, USA.

A panel that provides sound, quality data is one that clearly understands its objectives and purpose for creation. In the initial selection phase, screening for various tasting acuities is paramount to understanding the panel's strengths as well as limitations. Clearly acknowledging how different tasting techniques affect the palate and preparing uniform samples ensures panelists better understand the attributes being profiled. Continual and ongoing practice sessions with real time feedback foster confidence within the panel. "Feedback" references panelist analysis with report generation that clearly brings to light key attributes that need reintroduction for future practice and eventual mastery. The development of a scaling system depends on proper attribute references and acute lexicon development specifically tailored to the innate nuances of beer. These references provide a way for all panelists to "be on the same page," as it is understood all palates and olfactory abilities are not created the same—much in the same way the development of proper lexicon terminology is critical for the panel to "think" about the product as a cohesive unit starting from the same point of reference. Proper lexicon development ensures single flavors are not simply piled into one group when they are in fact separate attributes. Moderators must be aware of fatigue within the panel. Allowing sufficient break time between sampling sessions and setting concrete limits to the number of samples tested are favorable ways to avoid this most common and often overlooked pitfall.

Mona Baker Wolf, founder and president of The Wolf Group, encompasses more than 30 years of knowledge, expertise, and practice

in sensory evaluation. Since 1988, Mona has established herself and The Wolf Group as a respected partner in the consumer research industry. Mona is also a consultant, speaker, and trainer in the application of state-of-the-art sensory techniques. Mona is the president and founder of WolfSENSORY, Inc. a private sensory-consulting firm with clients across the United States and Canada. In addition, she is the founder and president of WolfSERVICES, Inc., which is a contract testing lab specializing in quality control and quality assurance testing, combining traditional analytical and microbial techniques with sensory profiling. Mona holds a B.S. degree in food science from Purdue University, and an MBA from the Rochester Institute of Technology. She serves as chair for the ASTM Sub-committee E18.05 for Sensory Applications – General and is past chair of ASTM Sub-committee E18.08 on Sensory Evaluations by Consumers. She is the coeditor of ASTM Manual 26 on Sensory Techniques. She is currently chair-elect for the main committee of ASTM E-18 on Sensory Evaluation. Mona has been a professional member of the Institute of Food Technology and a member of the SED Division since 1972.

201. How accelerated aging can help to assess the physiological state of yeast in bottle-refermentation beers. Presenter: Caroline Scholtes, Université Catholique de Louvain, Earth and Life Institute (ELIM), Louvain-la-Neuve, Belgium. Co-author(s): Etienne Bodart, Florence Peeters, Laurent Melotte, and Sonia Collin, Université Catholique de Louvain, Earth and Life Institute (ELIM), Louvain-la-Neuve, Belgium.

The evolution of short chain fatty acids, esters, and fusel alcohols was investigated using various aging procedures. In unrefermented beers, a 5 day treatment at 40°C or 3 days at 60°C led to an evolution similar to what 1 year at 20°C would have induced. These assays also were representative of two refermented beers in which the yeast was in particularly good shape, as shown by the relative stability of its esters. Yet, for global sensorial analyses, both accelerated aging procedures were unable to mimic other defects, like Madeira and phenolic off-flavors. In refermented beers where yeast was autolyzed (decrease in fruity esters), accelerated aging strongly overestimated the C5, C6, C8, and C10 carboxylic acids, as well as beta-phenylethanol. In that case, released yeast glucosidases were suspected to hydrolyze glucosyl esters and glycosides available in the medium. When *Brettanomyces* strains were present in the bottle, heat also strongly promoted the levels of isovaleric, hexanoic, and octanoic acids. In these Belgian-style beers, accelerated aging could help to assess the physiological state of yeast in the bottle.

Graduating in 2007 as a bio-engineer from Catholic University of Louvain (Belgium), Caroline Scholtes completed her education in 2008 with a master's degree in brewing science. In 2010, she started a Ph.D. program at the same university. Her research focuses on aging of Belgian special beers with regard to raw materials, brewing process, and storage conditions and correlates this to modification of organoleptic profile, especially Madeira off-flavor and volatile phenols. She is also a teaching assistant in beer chemistry at the same university.

202. Improving and controlling hop flavor in dry hopped bottom fermented beers by the use of activated carbon. Presenter: Andreas Brandl, Doemens Academy GmbH, Gräfelfing, Germany. Co-author(s): Christina Schönberger, Joh. Barth und Sohn, Nürnberg, Germany; Urs Wellhoener, Boston Beer Company, Breinigsville, PA, USA.

Beer flavor is very complex, being derived from components that arise from a number of sources. In bottom fermented beers the sulfur-containing components from yeast metabolism, such as hydrogen sulfide and sulfur dioxide, make a significant contribution to beer flavor, whereas in top fermented beers often the fruity and estery substances are dominant. Especially in freshly filled beers hydrogen sulfide can exceed the very low flavor threshold (5 µg/L) leading to an unpleasant off-flavor. During beer storage sulfur flavor will be degraded due to oxidative processes. Thus the sulfury notes need to be controlled in fresh beers and especially in dry-hopped beers, as the fruity hop aroma interferes with the sulfury components, which leads to an unbalanced sensory impression. An easy and pragmatic way to control hydrogen sulfide is by adding a special activated carbon as a filtering aid during filtration or lagering. Tests with non–dry-hopped beers with the addition of activated carbon showed a significant reduction in hydrogen sulfide leading to improved acceptance in sensory evaluation. In a pilot scale (5 hL) we adopted a commercial dry-hopped beer recipe and filter dry-hopped bottom fermented beers with and without activated carbon to determine the flavor impact on the final product. The beers were controlled by sensory evaluation, and the hydrogen sulfide level was measured, as well as the concentration of hop aroma substances. The aim was to emphasize the characteristic hop derived spicy and fruity notes coming from the dry-hopping resulting in a more balanced beer even in fresh conditions.

Andreas Brandl studied brewing and beverage technology at TU Munich-Weihenstephan. From 2001 to 2005 he worked on his Ph.D. thesis on the implementation of PCR-based methods in brewery quality assurance. In 2005 he began employment as a project engineer for aseptic filling lines at Krones AG, and in 2007 he changed to the Bitburg Brewery Group as head of the brewery pilot plant. In Bitburg he was responsible for the organization and documentation of brewing technology trials. Since 2010 he has been working for the Doemens Academy as head of the microbiology lab and consultant in brewing technology.

203. Influence of beer CO_2 content on its drinkability. Presenter: Petr Kosin, Budejovicky Budvar, n.p., Ceske Budejovice, Czech Republic. Co-author(s): Jan Savel and Adam Broz, Budejovicky Budvar, n.p., Ceske Budejovice, Czech Republic.

Drinkability is an attribute that reflects how easily a beer can be drunk in large amounts. It doesn't have to correlate with sensorial preferences and is often described by its opposite, the ability to saturation. Drinkability is generally attributed to the matrix of beer, specifically to the content of polyphenols, acids, or higher alcohols. The CO_2 content has rarely been discussed with beer drinkability, although its connection with the ability of drink to saturation is known from the soft drink industry. After drinking CO_2 promotes blood circulation in tongue and production of saliva, which is accompanied by faster loss of thirst. CO_2 is then rapidly liberated from the beer and partly fills the digestion system with gas and partly is absorbed by the gastric wall, which both cause a feeling of saturation. The CO_2 content of beer in a glass is influenced not only by the original beer CO_2 content from production, but also by the way in which the beer is dispensed into the glass. If beer is dispensed so violently that the majority is converted to foam, the CO_2 content can fall by half of its original concentration. If beer is dispensed so gently that no foam is created, the CO_2 content does not change significantly. The drinkability of beer dispensed with high CO_2 lost or no CO_2 lost was compared by two tests. In the first test, called "free selection test," drinkers freely chose a beer to drink, and the results were expressed as total consumption of beer over time. In the second test, called "test of consumption velocity," all drinkers alternatively drank samples with high or low CO_2 content, and the results were expressed as the speed of consumption of individ-

ual samples. Both tests indicated that CO_2 content could influence beer drinkability.

Petr Kosin received engineering (M.S. equivalent, 2006) and Ph.D. (2012) degrees in brewing and malting at the Institute of Chemical Technology Prague, Czech Republic. He worked on both of his theses, "Application of Modern Methods for Yeast Activity Control in Brewery" and "Consumer Perception of Beer Qualitative Characteristics," at Budweiser Budvar, N.C. in Ceske Budejovice. He has been working in research and development at Budweiser Budvar, N.C. since his graduation. He has been a member of the EBC Brewing Science Group since 2011.

204. Re-inventing the wheel: The intimate sensory links between beer balance, flavor strength, and drinkability. Presenter: Alex G. Barlow, ALL BEER, Sheffield, UK.

Organoleptic evaluation of beer flavor is the ultimate test of its acceptance to the brewer and target consumers. Descriptive techniques have largely centered around the flavor wheel first characterized by Mailgaard in the 1970. While researching the *ALL BEER Guide* book, Alex Barlow reinvented the flavor wheel format and descriptions in the format of a balance containing three groups of sensory characters depicting aroma, taste, and mouthfeel. This *ALL BEER Flavour* notepad was used to evaluate the flavors of approximately 1,000 beers spanning a broad range of families and styles from many different nations. Sixty-five data points were characterized for each beer and consolidated into a database to analyze and search beers by flavor characteristics. Preliminary analysis determined patterns of flavor characteristics that define certain styles of beer and that the most pleasant and drinkable beers are those that exhibit higher degrees of relative balance, based upon the split of flavor characteristics into left side (e.g., fruity, sweet, and full-bodied) versus right side (e.g., roasted, bitter, and astringent). Furthermore certain beer styles have a greater tendency to balance, irrespective of brewer or % ABV, while others are characterized by a favoring of the left or right side. The findings indicate that not all great or renowned beers are balanced; however, the most drinkable, session-able beers tend to be, even if they are intensely flavored. The data illustrate flavor patterns, intensity, and degrees of balance created by different beers styles, whether old or new. By combining characteristics from left and right sides a total flavor score was created that clearly indicates the overall intensity of each beer's flavor. Evaluation of total flavor scores from a wide range of beers with different % ABV and style led developers to create a 1–7 index, termed the ALL BEER Flavour intensity (ABFi) rating that has been used to provide concise descriptors for use by brewers, retailers, and consumers to aid clear communication of beer flavor characteristics and attributes, irrespective of beer style, format, or background. The flavor notepad has also been successfully used as a tool to design beer flavor, relative strength, balance, and suitability for different pack formats with effective results by several brewers in the United Kingdom, America, and Canada. These tools have been used to effectively communicate flavor information via functioning website and print media. Further research on the data continues at Nottingham University Brewing School.

Alex Barlow was brought up in Chester, northwestern England, and in Zambia, Africa. His 25 years of experience in brewing and passion for beer started as a bar and cellarman in his local pub, before studying for two medical sciences degrees. He learned brewing skills at Bass' U.K. breweries, becoming the youngest qualified masterbrewer in 1991, before moving to police beer quality in the pubs and clubs of Yorkshire, Lincolnshire, and northeastern England. He became the first Englishman to manage a Czech brewery, with Staropramen in Prague, and developed two new beer brands while gaining a taste for European beer styles. Alex is an independent brewing and flavor consultant, presenter, and sommelier. He regularly judges international beer competitions and presents beer experience events in the United Kingdom, Europe, and North America to consumer audiences and beer retailers. He is author of the ALL BEER Guide, winner of four international awards, and has contributed to publications such as The Times, Arena, and Imbibe. Alex provides independent beer evaluations and food pairings for www.allbeerfinder.com and continues to research beer sensory projects with the Nottingham University Brewing School. Alex's mission is to change consumer perceptions about beer, assisting flavor discovery and choice. He believes beer's superior spectrum of flavors makes it an ideal partner for food and takes a practical approach to winning new audiences for beer and food pairings.

205. Sensory and chemical differences between naturally and artificially carbonated beer. Presenter: Eric Allain, Appalachian State University, Boone, NC, USA. Co-author(s): Ben Hogue, Seth Cohen, Brett Taubman, and Shea Tuberty, Appalachian State University, Boone, NC, USA.

The importance of an appropriate level of carbonation to the sensory perception of beer is well known. Carbonation can be produced in beer naturally where dissolved carbon dioxide is produced by yeast or artificially in which external carbon dioxide gas is applied to the beer at a pressure sufficient to achieve the desired level of dissolved carbon dioxide. While both methods can be used to achieve a beer with identical dissolved carbon dioxide concentration, some claim that natural carbonation produces a beer that is perceptibly different than an artificially carbonated beer. We investigated this claim by brewing a number of batches of beer in which, after fermentation, half of the beer was artificially carbonated while the other half was naturally carbonated to the same level of dissolved carbon dioxide. Sensory evaluation and difference testing was performed using a triangle test to determine the ability of tasters to discriminate between artificially carbonated and naturally carbonated beer samples. A possible cause for any sensory difference between naturally carbonated and artificially carbonated beer could arise during the fermentation process that occurs during natural carbonation. This possibility was investigated by analyzing the beers using gas chromatography/mass spectrometry to identify any differences between naturally and artificially carbonated beers.

Eric Allain graduated from Illinois Benedictine College with a B.S. degree in biochemistry in 1990. Eric attended graduate school at the University of Illinois in Champaign-Urbana, where he received a Ph.D. degree in biochemistry in 1997. His thesis work involved development of an enzymatic synthesis scheme for compounds important to the pharmaceutical industry. From 1997 through 1999, Eric worked for Nalco Chemical Company, where he studied and developed enzyme applications for water treatment. In 1999 he joined Novozymes, where he worked to develop new enzymes for the fuel alcohol industry. Significant accomplishments include the development of lab-scale methods to study enzyme behavior in fuel alcohol fermentations, development of engineered enzymes that are up to 10 times more efficient in the breakdown of starch, and development of a mathematical model describing how systems of enzymes work together to catalyze the conversion of starch to glucose. In 2005 Eric joined the faculty of Appalachian State University as a professor of biochemistry. Here, he is part of a growing team focusing on brewing and fermentation science research. Eric also serves as a board member for The Ivory Tower Brewery, a full-scale education- and research-based brewery at Appalachian State University in Boone, NC.

206. Sensory comprehensive evaluation on beer in China supermarket. Presenter: Chunfeng Liu, Key Laboratory of

Industrial Biotechnology, Ministry of Education, Jiangnan University, China. Co-author(s): Jianjun Dong, R & D Center, Tsingtao Brewery Co. Ltd., Qingdao, China; Xiangsheng Yin, Cargill Malt, Wayzata, MN, USA; Qi Li, Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University, China.

In order to build an effective evaluation method for beer harmony characteristic tasting, 60 ordinary people and 10 beer evaluation experts were invited to judge beer taste using a 5-point Likert-like scale of suitability. The method of fuzzy mathematics comprehensive evaluation was applied in the research, and the mouthfeel features of 14 types of beers were determined by the membership grade, which includes three steps: 1) first, identifying the system of beer taste compatibility comprehensive assessment indicators, boundary of indicators, and grade of comments; 2) second, evaluating a single indicator of beer harmony taste, confirming the fuzzy power weight vector, *a*, and establishing the model of fuzzy comprehensive evaluation (FCE); and 3) third, analyzing the result of beer harmony taste character. Moreover, descriptive analysis, hedonic test, and the whole harmony characteristics evaluation of beer samples were conducted, and the results processed by the date processing software SPSS13.0. ANOVA results of descriptive analysis showed significant differences ($P < 0.05$) among the beer samples in this study. The results also showed that beer samples 7 and 2 possessed the best and worst harmony characteristics, respectively. Therefore, FCE can be used for beer harmony characteristic tasting as a optional method.

Chunfeng Liu received an master of engineering degree in fermentation engineering from Jiangnan University in Wuxi, China. She began employment with Jiangnan University in 2008 as a teacher in the Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangsu Province, China.

207. Sensory perceptions of people liking or disliking beer. Presenter: Hiroko Kanauchi, Miyagi University, Sendai, Japan. Co-author(s): Makoto Kanauchi, Yoshie Abe, and Akira Morita, Miyagi University, Sendai, Japan; Charles Bamforth, University of California Davis, Davis, CA, USA.

The consumption of beer has decreased since 1994 in Japan for several reasons. One is that low malt beer is less expensive than conventionally consumed beer. Furthermore, alcoholic beverages other than beer are consumed by Japanese people in their 20s, who apparently dislike the bitter beer taste. A questionnaire and sensory evaluation of a model product were conducted with 83 panelists. Panelists in each group were asked to discriminate a bitter taste either of a bitter solution in caffeine or deionized water. The model beers were prepared using a non-alcohol beer-like beverage with added hop oil and lactic acid. The evaluation was conducted using a five-point rating scale method. Results show that the panelists could be grouped in six groups according to their like or dislike of beer, beer drinking frequency, etc. According to the sensory evaluation, the group liking beer tended not to discriminate bitter taste in solution, but the group disliking beer tended to discriminate the taste in the solution. Bitter taste is an important factor for the evaluation in the group drinking more than 350 mL of beer per week. However, in the group drinking less than 350 mL of beer per week, sweet taste is an important factor. Panelists who frequently drink beer were insensitive to bitterness, but bitter taste was the most important factor for beer taste. Furthermore, the panelists infrequently drinking beer were sensitive to bitterness, and sweet taste was an important factor for them.

Hiroko Kamiya-Kanauchi graduated from Chuo University in 1995 (bachelor of law) and from the Tokyo University of Agriculture in 1998 (bachelor of brewing science). She received an M.A. degree from the Tokyo University of Agriculture in 1998. Her master's thesis examined alcoholic beverage taste, especially bitter phenols in wine. Subsequently, she was employed at Ito-Yokado, a supermarket chain store in Japan (2000–2005) as a buyer and distributor. She merchandised alcoholic beverages for about 180 stores. Since 2008, she has been at the Department of Food Management, Miyagi University, studying career development.

208. Volatile phenols: Emergence of specific profiles among Belgian specialty beers. Presenter: Caroline Scholtes, Université Catholique de Louvain, Earth and Life Institute (ELIM), Louvain-la-Neuve, Belgium. Co-author(s): Masatatsu Shiba, Asahi Breweries Ltd., Ibaraki, Japan; Thomas Haube, Sabrina Nizet, and Sonia Collin, Université Catholique de Louvain, Earth and Life Institute (ELIM), Louvain-la-Neuve, Belgium.

Volatile phenols are responsible for spicy notes in a large range of beverages. Various flavor active phenolic compounds have been detected in beer. Among them, 4-vinylguaiacol and 4-vinylphenol are well known to release clove-like flavor in Belgian white beers made with unmalted wheat. In many other top fermented blond, amber, and brown beers, phenols also determine the overall flavor perception. In the present work, phenol-specific extracts of 14 Belgian special beers were investigated by gas chromatography-olfactometry (GC-O/AEDA methodology) and gas chromatography-mass spectrometry (GC-MS). Different profiles were highlighted according to the raw materials used (special malt, organic malt, wheat), yeast strain, or maturation vessel. 4-Vinylphenol, 4-vinylguaiacol, and vanillin were found well above their respective thresholds in beers produced with highly Pof+ yeast. On the other hand, a low ethyl/vinyl ratio was found in brands where *Brettanomyces* strains are used for secondary fermentation, bottle refermentation, or cask maturation. Finally, two original phenols emerged as interesting markers, allowing us to authenticate the use of torrefied/chocolate malts.

Graduating in 2007 as a bio-engineer from Catholic University of Louvain (Belgium), Caroline Scholtes completed her education in 2008 with a master's degree in brewing science. In 2010, she started a Ph.D. program at the same university. Her research focuses on aging of Belgian special beers with regard to raw materials, brewing process, and storage conditions and correlates this to modification of organoleptic profile, especially Madeira off-flavor and volatile phenols. She is also a teaching assistant in beer chemistry at the same university.

260. Kinetic characterization of beer aging and rapid prediction method for beer flavor stability. Presenter: Li Hong, Pearl River Brewery Co., Ltd. and South China University of Technology, Guangzhou, P. R. China, and China National Research Institute of Food and Fermentation Industries, Beijing, P. R. China. Co-author(s): Fang Guiquan and Li Huiping, Pearl River Brewery Co., Ltd., Guangzhou, P. R. China; Li Lin, South China University of Technology, Guangzhou, P. R. China; He Xi, Pearl River Brewery Co., Ltd., Guangzhou, P. R. China; Zhang Wujiu, China National Research Institute of Food and Fermentation Industries, Beijing, P. R. China.

TBA, concentration of staling substances, and aging intensity scores were analyzed at a certain interval during beer storage at 40, 50, and 60°C and at room temperature. Correlation analyses were conducted between TBA. as well as concentration of staling compounds and the aging intensity score, and the results show that the strongest correlation is between the TBA and aging intensity scores. The regression equations between TBA and beer aging intensity were acquired at different storage temperatures through regression analysis, and the

equation is one order linear equation and is statistically significant. The critical TBA above which ordinary consumers can perceive staling flavor is 0.486, as calculated according to the regression equation at room temperature. In this study the aging intensity score corresponding to the critical TBA was designated 2 (on a scale from 0 to 5). The kinetic characterization of beer staling at 40, 50, and 60°C and at room temperature conformed to the one order linear equation. It is seen from the staling kinetics that the rate of beer aging is expedited as the storage temperature increases. We can infer from the coefficients of aging kinetics that the rate of beer aging at 40, 50, and 60°C is accelerated by 14.2, 30.0, and 59.1 times, respectively, compared with actual storage conditions. In other words we can theorize that one-day of beer storage at 40, 50, and 60°C is equivalent to two weeks, one month, and two months of storage under normal conditions. Based on these findings, a method for predicting the time at which beer will begin to exhibit aging flavor is put forward.

Sustainability

210. Bag it up—Flexible vessels in brewing. Presenter: Troels Prahl, White Labs Inc., San Diego, CA, USA.

Flexible polymer based vessels are leading the market when it comes to food packaging. However, the brewing industry has only embraced these technologies in a very limited range of applications, mainly in dispense systems such as one-way kegs or film lined serving tanks. This study outlines the use of disposable flexible vessels in various steps of the brewing process, such as fermentation, maturation, yeast handling and propagation, and sampling, as well as beer packaging. Upfront challenges such as CO_2 permeability and off-flavor contribution from the plastics are overcome in modern film production and the increased consumer demand for sustainability and low environmental footprint is forcing the brewing industry to consider alternative technologies. Apart from reviewing existing solutions available in the marketplace this study reveals novel patent pending methodologies for incorporating flexible vessels in brewing operations of all sizes. Lab, pilot, and production scale trials showed great benefits of flexible vessels in critical operations such as yeast propagation and aseptic sampling. Furthermore microbrewery size batch fermentations were conducted with great results in terms of product quality, easy handling, and savings related to reduced tank costs, as well as reduction or even elimination of CIP chemicals. Film manufacturing trials led to optimization of material compositions and design but also showed important limitations to the technology when vessel volumes exceeded 50 hL. The latter limitation excludes the use of flexible vessels in large scale fermentations. However, it was shown that breweries of any size still can benefit from flex-vessel technology in processes such as yeast handling, sampling, research and development, and packaging/serving.

Troels Prahl received a B.S. degree in biotechnology from the University of Copenhagen, Denmark, specializing in fermentation science. Passionate to improve product and process quality within the brewing industry, he has dedicated the past 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. Besides consulting under his own business, Ferm, 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, U.K., from 2010 to 2011. During this period, the London microbrewery was not only established and lifted to a maximum capacity of 5,000 hL/year, but also won a silver medal at the Brewing Industry International Awards in 2010. In the summer of 2011 Troels moved back to the United States to work full time as a yeast application scientist at White Labs Inc. in San Diego, CA.

211. Chemical free sustainable cooling water treatment at a Texas brewery. Presenter: Philip Vella, VRTX Technologies, Schertz, TX, USA. Co-author(s): Peter Koestler, The Gambrinus Company, San Antonio, TX, USA.

VRTX Technologies implemented the use of non-chemical technology for the treatment of cooling water from evaporative condensers at a brewery that has three evaporative condensers with a total cooling capacity of 1,500 tons. The technology used is controlled hydrodynamic cavitation (CHC). CHC is one of the most innovative technologies employed today and is unlike all other non-chemical technologies currently available. CHC provides scale, corrosion, and microbiological control in addition to water conservation and reuse options. In order to minimize the volume of water being discharged to the sewer, most evaporative cooling systems use a chemical scale inhibitor to prevent calcium deposits from building on those heat transfer surfaces. Eventually the water becomes so concentrated that chemical addition is no longer effective. The water is purged to the sanitary sewer and ultimately ends up at a wastewater treatment plant. Established in 1909, the Spoetzl Brewery, Inc. in Shiner, TX, is Texas' oldest independent brewery. The original chemical water treatment system used four different chemicals to maintain proper operation of the system. Sulfuric acid was added to prevent calcium carbonate buildup. Chlorine and a non-oxidizing biocide were used to control microorganism growth and as a corrosion inhibitor to reduce corrosion rates. In conjunction with the chemical treatment, cycles of concentration (COC) were kept low with an average around 2. Despite these efforts, significant calcium carbonate deposits accumulated on and around the condenser tubes and inside the condensers. Based on the poor performance of chemical treatment it was replaced by a CHC system. There were five objectives to the study: 1) provide scale, corrosion, and microbiological control, 2) improve condenser operating efficiency over the existing condenser systems, 3) conserve water by minimizing condenser makeup discharge, 4) produce a reduced quantity of condenser bleed that possesses minimal pollution, and 5) implement environmental improvements and worker safety wherever possible. With the CHC system in operation it was determined that the daily makeup water declined from an average of 19,251 to 12,619 gal/day, representing a 34.5% reduction. Daily blowdown declined from an average of 8,417 to 1,657 gal/day, representing a 80.3% reduction. The average COC increased from 2.3 to 7.6 resulting in an annual water saving of over 2.0 million gal. An added benefit to the water savings from CHC is that the facility has removed chemical addition from its water treatment program, including acid, resulting in a safer and more environmentally friendly workplace. Also, since the blowdown is free from any added chemicals it may be able to be used for landscape irrigation or exempt for NPDES permits.

Phil Vella received his Ph.D. degree in chemistry from the University of New York at Albany. He did his post-doctoral work at Argonne National Laboratory in Illinois. Phil is currently the technical director for VRTX Technologies. He is responsible for providing technical support in the cooling water treatment area and directing research and applications development using controlled hydrodynamic cavitation (CHC) for wastewater, produced water, biofuels, remediation, drinking water treatment, and other environmental areas. Prior to joining VRTX, he was the manager of technology support for Carus Corporation. His technical responsibility was in oxidation chemistry, including the application of ozone, hydrogen peroxide, AOP process-

es, and chlorine dioxide, with emphasis on permanganates used in the municipal drinking and wastewater markets, industrial applications, and for the remediation of contaminated soil and groundwater. In addition, he was responsible for phosphate products used for corrosion control and sequestration in drinking water systems. In his 25 years in the water industry he has more than 60 publications and presentations worldwide, 4 patents, and has participated in numerous technology transfer seminars. He is a member of ASHRAE, AWT, RETA, AWWA, and WEF.

212. Customizing sustainability through PET. Presenter: Nigel Pritchard, Petainer, Peterborough, UK.

This presentation explores the consumption of the world's resources from two perspectives, highlighting that we can live and work in a sustainable way. It reviews the key drivers for change and discusses the part that innovation in PET packaging and the development of new products and systems can and should play in sustainable development. It uses the examples of the PET keg and refillable bottle systems, highlighting that success will only come with the support, co-operation, and involvement of the whole supply chain, including the consumer.

Nigel Pritchard is group chief executive of Petainer UK Holdings, which acquired Rexam plc's Petainer business in late 2009, backed by private equity companies Next Wave Partners, WHEB Partners, and KBC Private Equity. Petainer is a specialist plastics packaging technology business, an innovator in the design and manufacture of PET containers. Nigel has extensive experience with more than 20 years in the packaging industry, including holding senior positions in a number of sectors encompassing industrial plastics, custom molding, high-performance films, glass, and PET, one of the most exciting sectors within the packaging industry, with tremendous opportunity to shape the future and improve the sustainability and environmental performance of the segments it serves.

213. Data on energy and water use in breweries. Presenter: Gordon Jackson, Campden BRI, Nutfield, UK. Co-author(s): Conor Donoghue and Anastassia Johnson, Campden BRI, Nutfield, UK; Fons Pennartz, KWA Business Consultants, Amersfoort, Netherlands.

This paper presents the results of benchmarking of energy and water use at the main brewing and packaging process steps. This is based on recently collected data from a range of breweries around the world. Data is collected by questionnaires, and the results will be presented as mean values and ranges for key brewing and packaging steps. This will enable brewers to compare energy and water use at these key steps with similar operations in other breweries. It will therefore highlight the steps where efforts should be targeted to minimize usage and hence reduce costs. Most previous benchmarking studies have concentrated on total energy use (MJ/hL of beer) and total water use (Hl/hL); however, while this enables the brewer to compare performance overall it does not indicate which areas of the process should be concentrated on for improvements. Collecting data at the process level allows a like-for-like comparison of energy and water use and helps to target resources.

Gordon Jackson has worked at BRI (now Campden BRI) for more than 30 years. He has been involved with a range of different issues for breweries and has worked onsite in over a hundred breweries. He has been involved with environmental issues for breweries for many years, for example in projects to benchmark energy and water use. He has also been involved with carbon footprinting for breweries. He has given presentations on environmental issues at several key conferences.

214. Energy efficient hop kilning system with integrated hop oil recovery from the exhaust air. Presenter: Ruslan Hofmann, VLB Berlin e.v., Berlin, Germany. Co-author(s): Roland Folz, VLB Berlin e.v., Berlin, Germany.

After harvest green hops show water contents of 70–80%, which must be reduced to approximately 10%. During the hop kilning process fresh air is heated to approximately 65°C. The exhaust air has temperatures of 28–35°C and a relative humidity of up to 100%. In conventional hop kilning systems the air is released directly into the atmosphere. With the help of the company WOLF Anlagen-Technik GmbH & Co. KG a pilot heat exchange system was integrated into an industrial size hop kiln in the Hallertau region of Germany. The goal of the project was to decrease the consumption of fossil resources for heating and to recover volatile hop components from the exhaust air of the hop kiln. Via suction pipes the exhaust air may be conveyed to the heat exchanger. One pipe was equipped with a carbon filter to trap volatile hop components from the exhaust air of the hop kiln. The carbon filter material was analyzed using gas chromatography. The heat exchanging process generated condensate, which was analyzed in the same way. Hop oils were detected in the condensate as well as the filter material. At least 0.25 mg of hop oils per hour and m^3 of exhaust air could be recovered. The exhaust air had a mean temperature of 31.3°C. Post heat exchange the air left the HE unit with a mean temperature of 23.2°C. In the mean, fresh air was heated from 15.0 to 24.4°C. Heat recovery resulted in an efficiency of approx. 50%. These figures led to a calculated 38 kW/hr energy recovery or a reduced energy consumption of 20.8%. According to the current price level (0.8 euro/L fuel oil) a theoretical saving of approx. 200 euro/day the kiln is running was achieved.

Ruslan Hofmann received his Diplom-Ingenieur (comparable to master of engineering) degree in brewing technology from Technische Universität Berlin, Germany. Before studying he worked for the Berliner Bürgerbräu Brauerei in Berlin. Since 2008 he has been employed at the research and educational institute Versuchs- und Lehranstalt für Brauerei (VLB) in Berlin e.V., where he worked in the packaging laboratory for nearly two years and afterward joined the Department for Brewing & Beverage Science and Applications. Ruslan is responsible for research projects in the field of packaging, raw materials, and flavor stability.

215. Guidelines for efficient water use in the brewery and bottled beverage industries. Presenter: Steve Froggett, Froggett & Associates, LLC, USA. Co-author(s): Joseph Cotruvo, Joe Cotruvo & Associates, LLC, USA; Richard Canady, Risk Science Innovation and Application Center of Excellence, International Life Sciences Institute – Research Foundation, USA.

Water is a critical resource and raw material for breweries in particular and the beverage industry in general, as both rely on water for production and facility maintenance. This recognition has led many companies to launch water stewardship initiatives in an effort to drive conservation and efficient use of water at their facilities. Individual breweries globally have undertaken the complex task of reducing their water footprint by increasing water reuse for non-potable uses. For example, published case studies from Australia show significant reductions in water use through efficient and safe reuse at their facilities. In one brewery, recycled water is used for first rinse cleaning of storage vessels, boiler feed, and cooling tower makeup, while at another brewery water is reused to irrigate an adjacent golf course. Overall water costs were reduced in both cases. Both cases illustrate the potential for sustainable use and reuse of water within breweries, reduction of the facility's water footprint, and the challenges faced when developing a reuse system. Pragmatic

guidelines outlining management and engineering needs to enable the safe and efficient reuse of water in bottling facilities is lacking. To address this need, the Research Foundation of the International Life Science Institute has convened an expert panel to draft guidelines that will outline the key considerations a plant must make when deciding if reuse is possible and beneficial at their plant. The guidelines will then present a detailed "how-to" enabling facility managers and engineers to plan a safe reuse system based on a hazard analysis and critical control points management approach. In addition, the guidelines will address monitoring, evaluation, and the training necessary to maintain the daily operation of the reuse system within established safety parameters. The use of these guidelines is intended to enable brewers and bottlers to significantly reduce water consumption and wastewater discharge in a cost-effective manner, but to do so in ways that reliably produce safe water of assured quality that is appropriate for the intended use in various processes and product production. For this to occur, the guidelines must be internationally acceptable to producers, regulators, and consumers concerned with product integrity and protection of the environment. The guidelines committee is an international expert group with a wide range of experience, and the guidelines will be reviewed by a broad spectrum of stakeholders interested in the safe reuse of water, including perspectives on the perceptual or acceptability aspects of reusing water, prior to completion.

Steve Froggett received a Ph.D. degree in neuroscience and behavior from the University of Massachusetts, Amherst, in 2002 and has served as a visiting scientist and faculty at the University of Kathmandu Medical School, Nairobi College of Health Science, and Patan Academy of Health Sciences. Subsequently, Steve served as a diplomacy fellow and as a scientific advisor in the Foreign Agricultural Service of the U.S. Department of Agriculture, advising on a broad range of issues related to new and emerging technologies for food production and sustainability. Since 2010, Steve has consulted for the Research Foundation of the International Life Sciences Institute and manages the efficient and safe water reuse guideline development project.

216. Novel approaches to recycling of production waste from yeast propagation. Presenter: Neva Parker, White Labs, Inc., San Diego, CA, USA.

With an ever-growing demand for manufacturing in the beer industry, a large carbon footprint can be created from various processes. Since regional and craft brewing have displayed marked increases in prior years, demand for fermentation yeast through propagation has increased significantly. The yeast production process itself can create a high volume of both water waste and solid product waste from spent or discarded yeast slurries. Propagating yeast that is suitable for successful brewing also typically results in high energy demands. In an effort to achieve a minimal environmental impact, recapturing waste from the propagation process, as well as any spent product, is of critical importance. Using technology such as tangential filtration, laboratories are capable of recycling spent growth media from yeast propagations and producing what is essentially water, which can be carried back through processing. This obtained water can be used for applications ranging from cleaning to further production of growth medium. In addition, the yeast itself can be repurposed. Yeast that is slated to be discarded or even spent yeast from production or lab-scale fermentations can be treated to produce yeast nutrients that would, again, be returned to the cycle. To maintain a minimal amount of energy to execute the reprocessing, a few components need to be put into place in the plant. This presentation covers the specific methods used to repurpose both spent growth media and discarded yeast, while maintaining a minimal energy load, and broadening the manufacturer's sustainability within the global market.

Neva Parker has been with White Labs, Inc. since 2002. She earned her bachelor's degree in microbiology from Gonzaga University in Spokane, WA, and first became interested in the brewing industry while studying abroad in London. Neva currently manages laboratory operations for headquarters in San Diego and the R&D facility in Davis, CA. She is also responsible for developing the White Labs training and consulting program. She has presented at several workshops and conferences and published articles for brewing magazines. She is an active member of the American Society of Brewing Chemists and the Master Brewers Association of the Americas.

217. Optimizing brewing process heating energy management with modular on-demand boiler systems. Presenter: Jason Smith, Miura North America, Inc., USA.

This presentation illustrates how emerging modular on-demand steam boiler technologies are well suited to address the energy and environmental challenges facing the North American brewing industry. Breweries, both startups and well established, face increasing challenges of minimizing the economic and environmental impacts of the brewing process on their production while achieving a consistent high quality brew. Boilers account for nearly half of industrial energy consumption and represent one of the most energy intensive systems involved in the brewing industry. While not always the most visible component of the brewing process, the utility side of brewing can represent an economic hurdle on the front-end via capital outlay and an energy management challenge during production due to process load variability as brewing, pasteurization, and CIP processes ramp up and down. Two key concepts—modularity and on-demand response—represent innovative strategies for both minimizing the initial capital investment tied to utility systems while optimizing boiler performance with enhanced energy management capability more precisely matched to process requirements. Modularity enables a startup brewery to minimize the up-front investment in its utility by purchasing only the boiler capacity needed with the flexibility to increase steam capacity with additional modules as production increases. On-demand response out of the utility enables the brewery to precisely match the boiler output to the process requirements at any point in time, eliminating significant energy losses associated with boiler part-load and perpetual idling operation during process lulls. In the same way that tank-less/instantaneous water heaters are enabling increased energy efficiency in the residential sector, compact modular on-demand boilers are poised to support the same kind of transformation in the industrial sector. Moreover, given the large amount of energy consumed and the sharp minute-to-minute variations in process steam demands in the brewing industry, on-demand steam generation can play a significant role in increasing energy efficiency while reducing a brewery's carbon footprint.

Jason Smith has a background in architecture and engineering, with more than 15 years of experience with the design and construction of high-performance "green" buildings and more than 5 years of experience as a LEED Accredited Professional integrating sustainable design solutions into facilities that address energy efficiency and contribute to reducing their environmental impact. Jason is celebrating three years with Miura North America, directing energy and environmental initiatives with a focus on energy efficiency advocacy, education, and market transformation in the area of thermal energy systems. Jason currently chairs the Energy Efficiency Deployment Subcommittee of the Department of Energy's ITP Steam Systems Best Practices Steering Committee and is an active member of the following organizations devoted to energy efficiency and sustainability: ESC, ACEEE, ASE, IDEA, APPA, ASHE, AEE, and USGBC.

219. Reuse of brewery wastewater—Aerobic and anaerobic membrane bioreactors. Presenter: Bill Musiak, Pentair X-Flow, Rockford, IL, USA.

In today's world, water is a scarce and valuable resource. In an ideal brewery, 1 bbl of water consumed would yield 1 bbl of beer. In the challenge to approach this level of efficiency, a big target is to collect, treat, and reuse the wastewater from malting, brewing, and CIP. This presentation explains how biological wastewater treatment (both aerobic and anaerobic) coupled with ultrafiltration membranes can reduce the organics and solids from wastewater to produce very high quality effluent suitable for reuse in boilers, cooling towers, and general non-product contact applications. The social, ecological, and economic benefits are numerous, and the ability to have a sustainable process will be a major factor in the success of businesses. The technology is already in place in a large number of breweries, malt houses, and beverage manufacturing sites in Europe, Russia, and Asia with capacities varying between 10 and 200 m^3/hr of wastewater. Different case studies are presented. For example, at one of the most modern malt houses in the world, steeping water is reused five times before the water is discharged to the sewer. One of the largest brewers in the world has equipped several breweries with MBR technology in order to dramatically reduce water consumption from an average of 5 to 2 hL water of water/hL of beer. The treated effluent is used as process water in applications like boiler feed water, CIP, and bottling (rinsing, pasteurizing, and bottle washing). In addition, in some cases where high strength waste is treated, biogas can be produced and reused as a replacement fuel.

Bill Musiak has more than 15 years of experience selling and engineering membrane-based water, wastewater, and process systems. Much of this experience was gained at Ionics Inc., where Bill spent a number of years in the Build-Own-Operate group working on ultrapure water systems for the power generation industry. Bill is knowledgeable on many different types of membranes, including MF, UF, NF, BWRO, SWRO, EDR, and EDI. In his current role at X-Flow, Bill is responsible for both the capillary and tubular ultrafiltration membrane products in the municipal and industrial markets. Bill has a B.S. degree in mechanical engineering and an M.S. degree in environmental engineering, both from Worcester Polytechnic Institute, and is currently pursuing his MBA.

220. Sustainability for Anheuser-Busch. Presenter: Gene Bocis, Anheuser-Busch, Inc., St. Louis, MO, USA.

While "being green" may be a trend these days, Anheuser-Busch and its brands have a long history of environmental stewardship. It all started with Adolphus Busch, our founder, in the late 1800s when he started recycling spent grains as cattle feed. Adolphus constantly looked for ways to become more efficient, which translates into lower environmental impacts. Anheuser-Busch is the largest user of bio-energy recovery systems that convert wastewater from the brewing process into a renewable fuel. This system provides up to 15% of the fuel needs for the 10 breweries where it is installed. That's enough renewable fuel to heat more than 25,000 homes. We focus first on using materials wisely to prevent the creation of waste. For example, we have reduced aluminum can weights by about 40% since the early 1970s. When we do create excess materials, our breweries recycle more than 99% of what is generated, nearly 3.5 billion pounds of materials a year. We've been recognized for our recycling and waste prevention leadership numerous times, including by the U.S. Environmental Protection Agency WasteWise Program as a Hall of Fame performer—something we're very proud of. Anheuser-Busch is the world's largest operator of bio-energy recovery systems, which convert excess nutrients from the brewing process into a renewable fuel. The company is also evaluating a variety of renewable energy technologies, including solar wind biomass, which involves the use of wood wastes as a fuel source and landfill gas—a Houston brewery is using the gas from natural decomposition of waste from a nearby landfill as a renewable fuel source. Between bio-energy recovery and landfill gas, the Houston brewery obtains more than 70% of its fuel needs from alternative sources. A better world is a place where adults enjoy our beers responsibly. We protect and preserve the environment by striving to brew and package our products efficiently, while also supporting local efforts through marketing and partnerships. We actively make a difference in the communities where we live and work. To summarize, we have a long and rich history of environmental stewardship and take great pride in our efforts. Our policies, programs, and performance targets are designed to promote responsible resource use and to reduce our environmental footprint.

Gene Bocis has spent more than 20 years with Anheuser-Busch after receiving his B.S. degree in electrical engineering technology from Northeastern University in 1992. He also received his master of science degree in management from Stevens Institute of Technology while working at the Newark Brewery early in his career. He has experience in packaging, brewing, maintenance, engineering, and utilities within Anheuser-Busch. His tenure also includes a three-year assignment on International Brewing Operations in China and India. He currently serves as the director of North American Zone Utilities Support for Anheuser-Busch and is responsible for breweries, can, bottle, malt, and rice plant utility operations in the United States and Canada.

221. Sustainable value creation with enzyme technology. Presenter: Sylvie Van Zandycke, DSM Food Specialties, South Bend, IN, USA. Co-author(s): Ron Duszanskyj, DSM Food Specialties, Delft, Netherlands; Marlos Fernandes, DSM Food Specialties, Sao Paulo, Brazil; Jeroen van Roon, DSM Food Specialties, Delft, Netherlands.

Sustainability will be a key differentiator and value driver over the coming decades for the brewing industry, where efficient use of raw material and energy go hand-in-hand with taking environmental responsibility. Exogenous enzymes are flexible tools for meeting the increasing demands for sustainable and cost-effective beer production. However, to make the correct business decisions, the sustainability and economic advantages of enzyme technology should be made explicit and quantifiable on a case-by-case basis. Based on internationally recognized IPCC GWP 100 and Eco-indicator 99 methodologies, a full beer life cycle analysis quantitatively illustrates how enzymes can lower the beer production carbon footprint through efficient use of raw materials, reduction of energy needs, reduction of water usage, and creation of opportunities to remove process steps in malting or brewing operations. The concept of life cycle analysis methodologies and results are presented and discussed. Subsequently, two quantitative examples are given on how to obtain simultaneous financial and environmental benefits. The first example demonstrates how replacing up to 100% of malt with unmalted barley and exogenous enzymes can reduce the carbon footprint of the beer from 17.9 to 14.3 kg CO_2 eq/hL of beer, a reduction of 20.1%. A real-life example of a commercial scale beer production with 100% barley is presented; the industrial process conditions and some recommendations are shared. Interestingly, independent physico-chemical and organoleptic analyses performed by the ICBD demonstrated that the quality parameters of the resulting beer were all in the range of a standard lager-type beer, with

normal head retention and flavor stability. The second example focuses on energy savings when beer is stabilized with enzyme technology, while simultaneously increasing maturation capacity and decreasing the carbon footprint of the beer. In the example discussed the carbon footprint was reduced by 6.1%, while maturation capacity increased by 50%. Rather then focusing on specific products, this lecture aims to demonstrate the advantages of enzyme technology in general with respect to reducing carbon footprint and costs.

Sylvie Van Zandycke studied biochemical engineering and fermentation at the Institute Meurice (Brussels, Belgium); she completed her degree in 1996. She then 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, and accepted a post with Lallemand as a 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, occupying her current position as support service manager for brewing enzymes in North America.

222. Techniques to reduce energy and water use in breweries. Presenter: Gordon Jackson, Campden BRI, Nutfield, UK. Co-author(s): Anastassia Johnson and Conor Donoghue, Campden BRI, Nutfield, UK.

This paper presents data from recent projects to collect information on techniques used by brewers to minimize consumption of energy and water. It highlights some case studies from breweries using data collected in questionnaires sent to breweries worldwide. This includes examples of initiatives in brewing, packaging, and logistics plus initiatives at the national level. It also presents data from studies onsite, for example, using thermal imaging to detect areas of heat loss. Finally it reviews some recent process engineering technologies and changes in process operations that have led to reductions in energy and water use. There is already a lot of published data on energy and water use in breweries to identify best practices; the examples presented in this paper are designed to assist brewers in moving toward achieving best practices.

Gordon Jackson has worked at BRI (now Campden BRI) for more than 30 years. He has been involved with a range of different issues for breweries and has worked onsite in over a hundred breweries. He has been involved with environmental issues for breweries for many years, for example in projects to benchmark energy and water use. He has also been involved with carbon footprinting for breweries. He has given presentations on environmental issues at several key conferences.

World Class Manufacturing

223. A new method for COD and COD peak alarm measurements in beer and soft drink plants. Presenter: Daniel Gore, Anton Paar, Graz, Austria. Co-author(s): Josef Bloder, Anton Paar, Graz, Austria.

All breweries and beverage manufacturers, regardless of size, rely on either private or public wastewater treatment centers and must adhere to local, state, and federal laws for effluent quality. Effluent COD (chemical oxygen demand) is not permitted to exceed specific limits, typically between 2,000 and 4,000 mg/L, and it is unfortunately not uncommon for higher level COD effluent to enter the wastewater stream due to equipment failure or human error. This paper explains how a new method for COD monitoring works and demonstrates how expensive fines or costs created by high COD levels can be avoided. Several options are currently available to measure COD, or BOD (biological oxygen demand), but they are relatively costly and rely on time-consuming measurement methods that create a very low measurement frequency. A new method, relying on density and/or sound velocity and conductivity, is used to continuously measure the wastewater stream and determine the COD in real time. Due to the system's inline location and continuous measurement, COD spikes are also monitored. As density and sound velocity are already a well-known and established method for measuring sugar and alcohol contents of final products in the beverage industry, they also correlate very well to COD. The reproducibility and accuracy of any COD measurement depend on variations in the composition of the wastewater. The various components found in brewery and beverage wastewater streams, namely the sugars maltose, glucose, and sucrose, as well as alcohol, acids, and alkalis, correlate very well with density and sound velocity and, therefore the COD value as well. Once additional acids and alkalis are added via CIP procedures, however, the density and sound velocity values are no longer reliable and require the addition of conductivity and/or pH to allow accurate measurement of all components. Sugars and alcohol have a very large effect on COD, and very small concentration changes have a huge impact on final COD. For example, an extract/sugar content change of 0.01°P is equivalent to 112 mg/L COD, and an alcohol content change of 0.01% m/m is equivalent to 209 mg/L COD. A total COD of 10,000 mg/L is actually less than 1°P! Depending on the needs of the brewery, a less advanced alarm only system, comprising sound velocity and conductivity, may be sufficient for avoiding large COD spikes. However, by combining density with sound velocity and conductivity, composition fluctuation errors are drastically reduced, and accuracy improves by a factor of five over sound velocity alone. A complete COD monitoring system is able to control more advanced measures such as wastewater release, addition of dilution water or chemicals, and shunt out-of-spec wastewater to a holding tank for further evaluation and blending.

Daniel Gore received his B.A. degree from the University of Maryland, College Park, including two years of study in Germany. After graduating in 1995 he returned to Germany and began an apprenticeship as a brewer and maltster at the Lammbrauerei Hilsenbeck. After successfully finishing his apprenticeship he worked in multiple breweries throughout Germany, including the Uerige Obergärige Hausbrauerei and Quenzer Bräu before moving back to the United States to assume the role of head brewer at the Long Trail Brewing Company. In 2006 he changed focus to work as a technical sales representative for Anton Paar, USA and continued to put his 12 years of practical brewing experience to good use serving the beverage industry. During this time Daniel was a member of MBAA and ISA and enjoyed working with local chapters in the Northeast. In 2010 he moved to Graz, Austria, to become Anton Paar GmbH's application specialist, supporting Anton Paar's existing applications in the beverage industry, as well as developing new beverage applications and technologies.

224. Hygienic membrane process design as an advantage in the brewing guild for secure beverage production—From the viewpoint of an equipment and plant manufacturer. Presenter: Jörg Zacharias, Krones AG, Neutraubling, Germany. Co-author(s): Dirk Scheu, Krones AG, Neutraubling, Germany.

Due to the increasing requirement for industrially produced beverages, aseptic processes are acquiring more and more rel-

evance. This is manifested in extended shelf lives and maximally natural products. Usually heat treatment is the common method of pasteurization. In terms of gentle product treatment and energy consumption, this is only second-best and has to be reduced. The authors map out what the requirements of a hygienic membrane process design for applications in the brewing industry have to be. Further they present solutions and discuss the problems involved in water production for beverages. As part of this, the following requirements for equipment and process have been specified. The following demands are the main characteristics for hygienic membrane processing technology. 1) Hygienically designed components up to an entire hygienic line, including piping, pumps, valves, connections, welding, and all its further components. 2) Hygienic construction (aseptic) of the complete product path on the past of water treatment (UF resp. RO)—filtrate side up to the filler. This is the critical path after the aseptic break-point. 3) This means easy-to-clean stainless steel construction of the plant with reference to the criteria of EHEDG and GMP for, for example, Ra values below 0.8, welding as well as materials. 4) On the product route, each unit has to be designed to hygienically designed criteria. 5) In accordance with this, hygienic connection of module junctions is essential, especially on the filtrate side of the modules. 6) This leads to stainless steel housings for the membrane assembly. 7) The possibility of sanitizing the membrane and module to suit the construction and materials used will be possible without excessive stress on the membranes or the material. Sanitization for this requirement means temperatures between 121 and 140°C to reach the requisite module sterilization conditions. 8) On-line sterility sensor technology enables on-line integrity to be monitored. This shows that in principle the requisite quality in terms of microbiological lethality can be achieved. The overall plant equipment and the principal hygienic plant design are a question and an aspect of planning the process. This entails some effort but can be solved in accordance with standard literature and design codes like EHEDG (European Hygienic Engineering & Design Group). In summary, the main themes for hygienic membrane technology are hygiene inside the modules and on-line sensor technology for integrity and acceptance of these techniques in the beverage industry. The remaining problems will be shone in more detail as exemplified by Krones AG's solutions.

Jörg Zacharias graduated in 1997 from Weihenstephan as an engineer in food science. In 2003 he finished his post-graduate studies with a doctoral degree from the Department of Fluid Mechanics and Process Automation at the Technical University of Munich-Weihenstephan. For more than five years he was an associate lecturer in food process technology at the Weihenstephan University of Applied Science. In 2005 he joined Krones AG in the Research and Development Division, where he was significantly involved in developing membrane filtration for beer clarification and fresh water treatment. He is an expert in the hygienic design of closed process designs for processing liquid foods. In addition, he is an expert in heat exchanger technology and the rheology of beverages.

225. Identifying critical control points (CCP) and optimizing process and laboratory instrumentation to the brewing process. Presenter: Daniel Gore, Anton Paar, Graz, Austria. Co-author(s): Keyvan Ghanaviztchi and Peter Brugger, Anton Paar, Graz, Austria.

Hazard analysis and critical control points (HACCP), a mainstay in the food industry, has been used in larger breweries for quite some time and is now a common component of many quality assurance program. Even smaller breweries, always willing to implement new ideas, see the advantages HACCP has to offer as part of a larger quality control program. HACCP is not a stand-alone program and must be combined with good manufacturing practices (GMP), standard operating procedures (SOP), and other measures to create a complete quality assurance program that meshes well with ISO 9001 and just-in-time (JIT) practices. This poster demonstrates the common critical control points in the brewery and how combined process and laboratory instrumentation are used to ensure customer and product safety and quality control while streamlining production. Traditional, periodic inspection and sample testing remain the standards with which all other measurements are compared, forming the base upon which all safety and quality testing stand, and are an absolute must in the brewery. They are, however, not as responsive to real-time, production needs and provide only a snapshot of production. From a public health and safety, quality control, and customer satisfaction point-of-view, static production snapshots alone are not enough. In-line process instrumentation, however, allows for continuous, live process control, augments laboratory testing, and helps fulfill many HACCP principles in a single step. The start of any HACCP program is a flow diagram of the entire brewing process and is specific to the needs of the individual brewery. After the safety and quality hazards have been analyzed, the critical control points are identified and appropriate instrumentation chosen and installed. Once the instrumentation is installed, the remaining HACCP principles follow logically and are straight forward to implement: establish critical limits and enter alarm limits into the instrument control; monitor the critical control points and make process corrections as needed and as they happen; establish corrective action to eliminate production errors at control points and follow through when it is required; keep simple and proper records to monitor quality and production trends; verify that all the criteria are met; and review the results to optimize quality and production. After completing the implementation of a HACCP program and collecting data, optimization begins to streamline production and trim costs. Known issues are fixed, and unknown issues may be identified. What may seem at first a daunting project is actually an organic process that grows with the needs and focus of the company.

Daniel Gore received his B.A. degree from the University of Maryland, College Park, including two years of study in Germany. After graduating in 1995 he returned to Germany and began an apprenticeship as a brewer and maltster at the Lammbrauerei Hilsenbeck. After successfully finishing his apprenticeship he worked in multiple breweries throughout Germany, including the Uerige Obergärige Hausbrauerei and Quenzer Bräu before moving back to the United States to assume the role of head brewer at the Long Trail Brewing Company. In 2006 he changed focus to work as a technical sales representative for Anton Paar, USA and continued to put his 12 years of practical brewing experience to good use serving the beverage industry. During this time Daniel was a member of MBAA and ISA and enjoyed working with local chapters in the Northeast. In 2010 he moved to Graz, Austria, to become Anton Paar GmbH's application specialist, supporting Anton Paar's existing applications in the beverage industry, as well as developing new beverage applications and technologies.

Yeast and Fermentation

227. A new method for estimating the premature yeast flocculation potential of malts using 180 mL scale fermentation. Presenter: Yasuhiro Muraoka, Frontier Laboratories of Value Creation, Sapporo Breweries Ltd., Yaizu, Japan. Co-author(s): Masahide Sato and Tatsuro Shigyo, Frontier Laboratories of Value Creation, Sapporo Breweries Ltd., Yaizu, Japan.

Premature yeast flocculation (PYF) refers to the phenomenon whereby yeasts flocculate prematurely during fermentation in the presence of high sugar concentrations. PYF is considered to be induced by so-called premature yeast flocculation inducing factors in malts, although the structure and the mechanism of how they cause PYF is unclear. It is very important for brewers to detect malts that cause PYF because it leads to low attenuation and results in an undesirable flavor in beer. In order to estimate the potential of malts regarding PYF (PYF potential), several methods have been developed in the past. However, these methods are time-consuming; they take 2 days to 1 week to complete the fermentation tests. In this report, we developed a new method for evaluating the PYF potential more effectively using 180 mL scale fermentation. In our method, the floating cells were measured by counting yeast cell numbers or by measuring the optical density of suspensions during fermentation. We prepared wort from malts having or not having PYF potential, performed fermentation, and compared the degree of flocculation. As a result, we were able to evaluate the PYF potential only after 24 hr of fermentation, which remarkably shortened the assay period. We expect our method to be useful for predicting the PYF potential of malts and enabling us to prevent PYF in brewing and to improve beer quality.

Yasuhiro Muraoka received an M.S. degree in biological sciences from the Nara Institute of Sciences and Technology. He joined the Hokkaido brewery of Sapporo Breweries Ltd. in 2005 as a brewing engineer. Since 2010, he has been working in Frontier Laboratories of Value Creation as a research brewer.

229. A technique to conclude the stage of fermentation from easy, accessible on-line measurements. Presenter: Martin Lutz, ProLeiT AG, Herzogenaurach, Germany.

Finding the right moment to stop fermentation and release a tank for cooling down or pumping over to a lagering tank is a common task in the fermentation cellar. Usually this moment is determined by regular gravity measurements as a trend in a standard fermentation diagram. Additionally the measurement of the VDK concentration gives the final assurance that the fermentation stage can be ended. Both measurements require the manual taking of the samples and then analysis by the brewer or in the laboratory, which means a lot of effort. In our work we investigated the possibility of concluding from easy accessible data the progress of the extract attenuation and so determining the current status of the fermentation. From a good start of the fermentation on the first day we measured the temperature and pressure on the tanks, which is standard in an automated fermentation cellar, and also looked at the activation of the cooling valves. With this we can deduct the amount of cooling energy needed related to the degree of fermentation. The system has to be adapted to local circumstances in the brewery and the influences of different wort types. The results achieved indicate that the different stages of the fermentation process can be distinguished. In particular, the end point of the main fermentation can be predicted with enough accuracy for the brewery to reduce the amount of sampling needed. Only one control analysis should be necessary to assure that the degree of attenuation is in a certain range and then to release the tank for switching over to the lagering phase. As soon as the system parameters are introduced and stable results are obtained there can also be a full automatic end to the fermentation period without additional sample taking. This reduces the workload in the cellar and provides significant advantages, for example, on weekends when nobody has to come to the brewery for this task. In the presentation real process values for a large brewery are shown, and conclusions from the experiment are explained.

Martin Lutz graduated as a brewmaster from Weihenstephan University in Munich. After several years of working in medium- and small-sized breweries, he joined ProLeiT AG in its business field of brewery automation. He has gained profound knowledge in the various aspects of this business and is connecting the technological requirements of the brewmaster with the possibilities and structures of modern process control systems.

231. An investigation of methylsulfonylmethane as a fermentation aid. Presenter: Eryn Bottens, Oregon State University, Corvallis, OR, USA. Co-author(s): Jeb Hollabaugh and Thomas Shellhammer, Oregon State University, Corvallis, OR, USA.

Fermentation time in the cellar directly affects potential brewery production as a whole. It is of practical interest to decrease the time needed where possible and increase efficiency. Decreased lag time in beer fermentations allows for higher production and efficiency in the brewery as well as decreased risk of contamination. This study examined the use of methylsulfonylmethane (MSM) as a nutrient supplement to stimulate yeast growth during fermentation. Small-scale (1 L), stirred fermentations were carried out at 15°C using a German lager yeast in a lightly hopped 11°P wort made from 70% pale 2-row malt and 30% high-glucose liquid adjunct. Two pitching rates were examined, 1×10^6 and 1×10^5 cell/mL °P, and four levels of MSM (0, 0.25, 0.5, and 075%, w/w). Fermentation gravity and viable cell counts were monitored throughout fermentation until the final attenuation was achieved (approximately 5 days). The application of 0.25% (w/w) MSM resulted in the shortest lag time in both adequately (1×10^6 cell/mL °P) and under-pitched (1×10^5 cell/mL °P) ferments. Additionally, this treatment reached stable, maximum cell counts and final gravity the quickest. Increased concentrations of MSM trended with higher terminal gravities and lower fermentability irrespective of pitching rate. Application of MSM to beer fermentations has the potential to decrease lag time and increase peak rate in beer fermentations.

Eryn Bottens is an undergraduate student seeking a degree in fermentation science from Oregon State University. Eryn works as a research brewery assistant in the brewing science lab and pilot plant. Eryn's assignment includes brewery production, analysis, and sensory work. Eryn is also involved in the Food and Fermentation Science Club in the role of brewmaster. Eryn has interned as a cellarman at Pelican Brewery and Pub in Pacific City, OR.

232. Application of near-infrared spectroscopy (NIRS) in the brewing industry for on-line determination of critical process parameters. Presenter: Lucas Vann, North Carolina State University, Raleigh, NC, USA. Co-author(s): Johnathon Layfield and John Sheppard, North Carolina State University, Raleigh, NC, USA.

Traditional analytical methods used in the analysis of fermentation media suffer from a number of limitations. These techniques are often expensive and time-consuming in part due to the chemicals involved and the amount of sample preparation required. In addition, analysis is not always done in-house, and results are obtained hours, even days, after the samples are initially taken. The search for more rapid and efficient methods has led to the development and application of near-infrared spectroscopy (NIRS) in the bioprocessing industry. NIRS offers a number of advantages over existing chemical methods: analysis is quick and passive so there are no destructive effects to the sample or waste products produced, and

sample preparation is not required. Analysis is also multivariate in that a single spectrum contains information about a number of analytes, and therefore several determinations can be made simultaneously. In addition, NIRS can be implemented in real time for maximum process monitoring and control capabilities. NIRS operates based on the principle that the atoms of molecules are in constant motion and vibrate at specific frequencies. Light frequencies that correspond to molecular vibrations are absorbed by the sample, and the resulting infrared spectrum comprises peaks of defined frequencies, band shapes, and heights that correlate to molecule concentrations present in the sample. To date, NIRS has been applied successfully in a variety of industrial processes: agricultural, food, chemical, and pharmaceutical, generally in the areas of raw material quality control, as well as intermediate and finished product testing. The present research explores its potential for on-line fermentation monitoring of cell number, specific gravity, sugar concentration, and alcohol concentration in a 300 L pilot-scale fermentor. Models were generated for each of these constituents, which overall exhibited favorable results. However, model predictions in dissimilar styles of beer did not exhibit satisfactory correlations suggesting that specific models would be required for each beer type. The findings support the possibility of incorporating NIRS into commercial brewing operations so that manufacturers can have a continuous "real time" assurance of quality through timely measurements of critical fermentation parameters. This would permit early fault detection and help to devise corrective actions to reduce the potential for lost batches while producing a more consistent end product.

Lucas Vann is a senior scientist in the Biomanufacturing Training and Education Center at North Carolina State University. He develops and teaches courses to NC State students, industry professionals, and FDA inspectors related to upstream biomanufacturing for the production of biopharmaceuticals and has extensive experience in the areas of fermentation, cell culture, process development, and automation. He has more than 10 years of upstream bioprocessing experience and is involved in industry-related bioprocess development projects at BTEC, where he provides strategic technical direction and guidance. He is currently pursuing a doctoral degree in bioprocessing at North Carolina State University, where he is conducting research specializing in bioprocess development and automation for process optimization. He holds both bachelor's and master's degrees in biosystems engineering from McGill University, where he helped design and develop a biosensor for fermentation process control.

233. Challenges in brewing higher alcohol *kvass.* Presenter: Alex Gertsman, Flottweg, Independence, KY, USA.

Kvass is a traditional Russian malt beverage, typically either non-alcoholic or slightly alcoholic (1–1.5%). Traditional homemade *kvass* uses rye bread as the primary raw material. In some recipes the rye bread is roasted, and its croutons are then actually used for making wort. After the wort is prepared and other ingredients, such as sugar, honey, raisins, and spices, are added depending on the recipe, the product is cooled off and undergoes a quick fermentation. In the old days of *kvass* making spontaneous fermentation was used and then replaced by use of baker's yeast. The fermentation process with baker's yeast usually would take place from 6 hr to 1–1.5 days, after which the product was cooled and was ready for consumption in about another day. *Kvass* brewers who wanted an extra kick from this great summer beverage and desired to have fewer off-flavors caused by baker's yeast strains, use of brewers' yeast became an alternative. Finding the right yeast became a challenge since the wort is not quite stable, being unhopped, and not suitable for the longer term fermentation that is carried out with beers. Naturally first choices were the English ale yeast strains, known for their shorter fermentation time and also of the same *Saccharomyces cerevisiae* species as baker's yeast. Differences include enrichment of brewer's yeast with essential minerals and B vitamins, but the real difference in strain characters is the ability of brewer's yeast to tolerate higher alcohol concentrations and its tendency to produce fewer off-flavors, but all at the expense of longer fermentation time than brewer's yeast. The big challenge is to obtain the desired attenuation while working with brewer's yeasts. It was empirically determined that some of the *Saccharomyces cerevisiae* strains could be pitched at temperatures of about 90–93°F as the wort being cooled underwent top fermentation at room temperature for 1.5–2 days; afterward green *kvass* was placed in a refrigerator and underwent further fermentation under cool temperatures for another day. The settled yeast was decanted, and another day of maturation in the refrigerator produced a very good flavored beverage with an alcohol content of 2.5–3% by volume.

Alexander Gertsman received a B.S. degree in chemical engineering from New Mexico State University. He has been working with centrifuges for 16 years, including employment with Alfa Laval and currently Flottweg, both in North America. Alexander has been responsible for brewery applications and sales for Flottweg in North America since 2005. He is also a profound crafter of homemade kvass, a Russian national malt beverage.

234. Construction of low acetaldehyde production brewing yeast with traditional mutagenesis strategy. Presenter: Jinjing Wang, Jiangnan University, Wuxi, China. Co-author(s): Qi Li, Jiangnan University, Wuxi, China.

Higher acetaldehyde concentration in beer is one of the main concerns of the current beer industry in China. Acetaldehyde is always synthesized during beer brewing by the metabolism of yeast. Here, using ethanol as the sole carbon source and 4-methylpyrazole as the selection marker, we constructed a new mutant strain with lower acetaldehyde production and improved ethanol tolerance via traditional mutagenesis strategy. European Brewery Convention tube fermentation tests comparing the fermentation broths of the mutant strain and the industrial brewing strain showed that the acetaldehyde concentration of the mutant strain was 81.67% lower, whereas its resistant staling value (RSV) was 1.0-fold higher. Alcohol dehydrogenase catalyzes the reaction of acetaldehyde formation from ethanol. Owing to the mutation of the alcohol dehydrogenase, the alcohol dehydrogenase activity of the mutant strain decreased to about 30% of the wild-type strain. In the meantime, the ethanol tolerance of the mutant strain increased by about 0.5–1% more than wild-type strain, which is very important to yeast strain, especially under high gravity or very high gravity fermentation conditions. The mutant strain constructed in this work could be applied to the beer industry directly due to its better performance in the brewing process.

Jinjing Wang received a Ph.D. degree in genetics from the Institute of Microbiology, Beijing, China. She spent a year and a half 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.

235. Control of sulfur volatile compound synthesis in lager beer production. Presenter: Jessica Herrera, Instituto de Biotecnología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Monterrey, México. Co-author(s): Luis Damas, Cuauhtémoc Moctezuma, Monterrey, México; Clara

Leal, Instituto de Biotecnología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Monterrey México; Juan Cabada, Cuauhtémoc Moctezuma, Monterrey, México; Luis Galan and Benito Pereyra, Instituto de Biotecnología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Monterrey, México.

The accumulation of sulfur volatile compounds (VSC), such as hydrogen sulfide, sulfur dioxide, mercaptans, and methyl thioacetate, negatively affects the aroma and flavor of beer. Most VSC are synthesized by brewing yeasts, and their production depends on wort composition and type of yeast strain. In this sense, the accumulation of some VSC has been related to levels of FAN and to specific amino acids present in wort. Moreover, using DNA microarray analysis, we identified different genes in two lager yeast strains whose expression impacted the biosynthesis of VSC. In this work we studied the effect of wort amino acid composition on the synthesis of VSC using two lager yeasts (C790 and C820). Fermentations were carried out with two worts: wort 1 had a FAN of 150 ppm, and wort 2 had a FAN of 200 ppm. Additionally, wort 1 was enriched with either 10 ppm Ser, 20 ppm Met, or a combination of both (10 ppm Ser plus 20 ppm Met). Genetic expression of genes implicated in the VSC production was evaluated by quantitative-PCR (qPCR) after 2 days of fermentation, and concentration of the VSC was measured by gas chromatography at the end of fermentation. Our results showed significant differences in the content of several amino acids between the worts. In particular, wort 2 had a 1.4 times higher concentration of Met, 12.0 times higher concentration of Ser, and 1.9 times higher concentration of Thr compared with wort 1. Irrespective of yeast strain used, the VSC concentration was higher in wort 1 than in wort 2; however, when wort 1 was supplemented with Ser plus Met, the VSC concentration was lower even than in high FAN content wort 2. Furthermore, the metabolic response of the two yeast strains was significantly different since the strain C790 produced less VSC than C820 when fermented in any of these worts. These results were correlated to those of qPCR, since the analysis of genetic expression of 18 VSC related genes showed that whereas strain C820 overexpressed CYS4, SER2, and MHT1 when fermented in wort 2 strain C790 kept all genes at the same expression level in the two worts. The contribution of these genes to sulfur compound production is discussed. Our results demonstrate that the accumulation of VSC in beer is mainly the consequence of the specific interaction of two factors: wort amino acid composition and yeast strain genetic background. Also, this work indicates that modification of the amino acid profile of wort can help to produce beers with desirable sensory properties.

Jessica Herrera received an M.S. degree in biochemical engineering from Instituto Tecnológico de Durango in Durango, México. Currently, she is a Ph.D. student in the biotechnology program of Universidad Autónoma de Nuevo León, México. Her thesis focuses on the study of the interaction between raw materials and the genetic response of yeast in the lager brewing process.

236. Determination of fermentor shear through empirical and theoretical methods. Presenter: Andrew MacIntosh, Dalhousie University, Halifax, NS, Canada. Co-author(s): Alexander McKinnon and Alex Speers, Dalhousie University, Halifax, NS, Canada.

The flocculation of brewing yeast cells within a fermentor is a well-documented phenomenon. It is understood to be influenced by several cell wall factors including zymolectin and hydrophobic interactions, as well as environmental conditions such as metal ions, ethanol, mannose, pH, and the shear forces within the fermentor. Since the late 1980s shear forces have been repeatedly shown to influence the rate and initiation of cell flocculation. However, within industrial fermentors, this parameter has been somewhat difficult to assess without dedicated instrumentation. As well, the current method to calculate shear within an industrial fermentor utilizes a theoretical approach from the 1960s that assumes the evolution rate of carbon dioxide (CO_2) and fermentor height are the only influences on shear and flocculation in the process. Until now, it was not possible to easily confirm these average shear rate calculations. Using colloidal aggregation theory we have measured the average shear rate within an industrial fermentor through observation of yeast flocculation behavior in wort samples subjected to various shear rates. Samples taken from industrial fermentors at ~1, 6, 22, 26, 30, 46, 50, 54, 70, 74, and 78 hr of fermentation were subjected to a range of shear conditions within a modified rheometer. At each sample time the rate of flocculation at the shear rate within the fermentor was used to calculate the orthokinetic capture coefficient of the yeast using a modified Smoluchowski equation. The shear rate at which the yeast floc reached an equilibrium size equivalent to that in the industrial fermentor was determined. Further testing within a modified rheometer was undertaken to confirm these findings. An empirically determined shear rate was found to vary from theoretical values by ~5 sec^{-1}. Therefore, while average shear determined theoretically using CO_2 evolution and height appears to yield a reasonable approximation, there are likely additional factors that influence fermentor shear, particularly near the beginning of fermentation. This novel empirical assessment technique gives researchers and industry a tool to study the shear within industrial fermentors.

Andrew J. MacIntosh has a Dip. Eng. degree from Saint Mary's University (Nova Scotia, Canada) and a B.Eng. degree in biological engineering from Dalhousie University (Nova Scotia, Canada). After working in industry for several years he took the opportunity to complete an M.A.Sc. degree in biological engineering and is now pursuing a doctorate in food science. He is near completion of the four-year "Engineering in Training" apprenticeship required to achieve the status of professional engineer. In addition to ASBC, Andrew is also a member of the American Society of Biological Engineers and regularly serves on the council of the Dalhousie Engineering Graduate Society. When not conducting research, Andrew is an avid home brewer. He has made many successful experimental brews and has had the odd (fermenting) catastrophe.

237. Differentiation of top- and bottom-fermenting brewing yeasts and insight into their metabolic status by MALDI-TOF MS. Presenter: Julia Usbeck, Technische Universität München, Freising, Germany. Co-author(s): Jürgen Behr and Rudi Vogel, Technische Universität München, Freising, Germany.

For the production of fermented beverages the most important industrially used yeast species belong to the genus *Saccharomyces*. Top-fermented ale-type beers are brewed with *S. cerevisiae*, while bottom-fermented lager beers, which are fermented at much lower temperatures, employ *S. pastorianus*. This yeast species is a genetic hybrid of *S. bayanus* and *S. cerevisiae*. Apart from technological parameters, each specific strain affects processing and the quality of the final product, e.g., flocculation behavior, temperature optima, fermentation speed and rate, the spectrum of secondary metabolites, and hence the aroma profile. These ecotypes are differentiated by time-consuming and laborious biochemical and DNA-based methods to enable a constant beverage quality and characteristics. However, their physiological differences must also be reflected in their enzymatic setting.

Matrix assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) offers a fast and easy method to differentiate yeasts along their peptide mass fingerprints. Therefore, we explored this method according to its differentiating potential for brewing yeasts and their metabolic status. Peptide mass fingerprints of top- and bottom-fermenting *Saccharomyces* strains were generated by MALDI-TOF MS upon optimized sample preparation and instrument settings and analyzed by a cluster analysis for strain or ecotype level differentiation. Furthermore, we investigated the effect of different culture conditions on selected strains representative for different beer types in relation to specific propagation or fermentation stages in the brewery, e.g., varying sugar concentrations and availability of oxygen. The differentiation of top- and bottom-fermenting brewing yeasts was achieved by >95% of more than 400 samples. Top-fermenting *S. cerevisiae* strains could further be subdivided into ecotypes according to their application in the production of different beer types, like wheat or alt beer. Differences within *S. pastorianus* strains were also present, but not as distinctive as for *S. cerevisiae*. The status of yeast fermentation or respiration could be precisely discriminated, while differences resulting from low and high sugar concentrations were less decisive. These results enable fast classification of unknown strains, improvement of quality control, and pursuit of different physiological states in the yeast culture during the brewing process.

Julia C. Usbeck was born in 1984 in Wuppertal, North Rhine-Westphalia, Germany. In 2009 she finished her studies in food chemistry at the Westfälische Wilhelms-Universität, Münster, followed by a mandatory practical year to accomplish the second state examination. Currently she is working on her Ph.D. thesis on the ability to detect beverage spoiling yeasts using MALDI-TOF mass spectrometry at Technische Universität München under the supervision of Rudi F. Vogel at the Chair of Technische Mikrobiologie in Weihenstephan.

238. Direct supplementation of yeast with lipids as a means to reduce sulfur dioxide formation. Presenter: Michael James, MillerCoors, Milwaukee, WI, USA.

In traditional brewing practice, yeast is cropped and repitched in subsequent fermentations. However, at the end of fermentation yeast is lipid depleted and requires lipid levels to be restored in order to initiate cellular growth. Adverse effects on biochemical fermentative processes are experienced if lipid levels are not restored to functioning levels. In such a situation, high levels of sulfur dioxide are produced, which may cause issues for the brewery, necessitating a warning on the label if the level is not kept below 10 mg/L. This study investigates the supplementation of cropped brewer's yeast with a mixture of lipids, its effects on certain fermentation parameters, and its ability to reduce sulfur dioxide produced during fermentation of a synthetic media. Successful results were observed using this method, as a 64.3% reduction in total sulfur dioxide was obtained using the supplemented yeast. Cellular growth, under anaerobic conditions, was also improved with growth rates nearly four times that of the control. While comparable final concentrations of ethanol were achieved in both fermentations, the supplemented fermentation produced ethanol at a faster rate, reducing overall fermentation time. Utilization rates of tested amino acids were increased as well. The results indicate that direct yeast supplementation with lipids can be used as an effective means to reduce the amount of sulfur dioxide produced during fermentation while stimulating overall yeast growth.

Michael James received his M.S. degree in brewing science from Heriot-Watt University in Edinburgh, U.K. He began his career working in the craft sector for four years before joining MillerCoors in 2009. Since 2011, he has been a part of MillerCoors' Corporate Brewing Group as a staff brewer, reporting to Bob Taylor II. He is currently obtaining his Ph.D. from Heriot-Watt University.

239. Experiences with new fermentation test-tubes—Standardized small scale fermentation from wort to bottle. Presenter: Thomas Tyrell, Versuchs- und Lehranstalt für Brauerei, Berlin, Germany. Co-author(s): Steve Eglin, Technische University, Dresden, Germany; Rolf Exner, Eckert & Wellmann GmbH, Berlin, Germany; Roland Folz and Christoph Uhde, Versuchs- und Lehranstalt für Brauerei, Berlin, Germany.

Testing of yeast properties is mainly done in so called EBC fermentation test tubes. There dimension allows, in comparison to other fermentations in laboratory scale, a relatively high liquid column with only 2 L of volume. This simulates a certain sedimentation height and static pressure to the yeast. These dimensions come closer to a real fermentation then fermentations in other laboratory equipment. Nevertheless the well-established EBC test tubes have their limitations in further oxygen free processing of the fermented beer and test fermentations under higher CO_2 concentrations. Additionally the handling of these relatively fragile tubes is not very practical. For that reason at VLB Berlin new fermentation test tubes have been developed and were presented at the last EBC Conference in Glasgow in 2011. This poster presents standardized methods for yeast propagation before and after beer handling (filtration and oxygen free bottling) in fermentation trials and gives an overview of possible applications.

Thomas Tyrell apprenticed as a brewer and maltster at the Pott's Brauerei in Oelde, Germany, from 1991 to 1994. After his apprenticeship, from 1994 to 2001 he gained working experience in different breweries, including Saxer Brewing Company (Portland, OR), Wettringer Brauhaus (Wettringen, Germany), Weißbräu Brauhaus (Cologne, Germany), and Magister Cervecerías Artesanales (La Coruña, Spain). Studying in Berlin at the Technical University he received a Diplom Ingenieur degree in brewing science (2001–2006) (diploma thesis at Cervecería Polar, Caracas Venezuela). Since early 2007 he has been at VLB, and since 2008 part of the VLB Department of Brewing and Beverage Science & Applications. At VLB he is responsible for research/consulting projects. In addition coordination of the institute's activities for Iberoamerica are part of his responsibilities.

240. Exploring and exploiting the natural phenotypic landscape of yeast. Presenter: Jan Steensels, CMPG Laboratory for Genetics and Genomics, Belgium. Co-author(s): Kevin Verstrepen, CMPG Laboratory for Genetics and Genomics, Belgium.

Although yeast has been used for more than 7,000 years for the fermentation of foods and beverages, the yeasts used are often suboptimal. In terms of biodiversity, industrial yeasts only represent the tip of the proverbial iceberg. Many industrial yeasts are genetically related to each other, and many share similar traits. In many cases, the particular yeast used for a specific industrial application is not the best possible yeast. This is especially true in the beer brewing industry, where brewers often use a particular yeast because of historic rather than scientific reasons. To explore the phenotypic landscape of yeasts and investigate the full potential of *Saccharomyces cerevisiae* and other species, we examined a collection of over 500 different yeast strains from different origins. These yeasts were subjected to a plethora of industrially relevant, high throughput assays. These experiments focused on tolerance to different stressful environments, sugar assimilation, production of certain enzymes and fermentation efficiency. Secondly, we also measured the production of about 20 of the most im-

portant aroma compounds. Together, we have now obtained more than 150 measurements for each yeast strain. This huge dataset provides an excellent tool for selection of yeast strains with very specific properties. In other words, producers can now select the yeast that best suits their needs. Moreover, we have also identified several non-conventional yeasts with a clear potential for industrial applications. Last but not least, our results provide a good basis for further breeding of novel, superior yeasts that are ideally suited for specific applications.

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, major in cell and gene technology, minor in industrial microbiology< from the same university in 2010. He did his master 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.

241. Formation of styrene in wheat beer dependent on fermentation management and the release of cinnamic acid during mashing. Presenter: Frank-Jürgen Methner, TU Berlin, Germany. Co-author(s): Katrin Schwarz, TU Berlin, Germany.

Styrene is a harmful component with a low carcinogenic potential. Depending on type, the content of styrene in commercial wheat beers ranges between 0 and 24 ppb. The formation of styrene, derived from cinnamic acid (CA), occurs as analog with the decarboxylation of ferulic- and p-cumaric acid to 4 vinylguaiacol (4VG) and 4 vinylphenol These reactions can proceed both as an enzymatic and as a thermal decarboxylation. The enzymatic decomposition of CA to styrene is encoded by the same phenyl acrylic acid decarboxylase (PAD1) and ferulic acid decarboxylase (FDC1) gene, as the decomposition of phenol carboxylic acids to the corresponding phenols. Only phenolic off-flavor positive (POF+) yeast strains, e.g., top-fermenting *Saccharomyces cerevisiae*, which are used for wheat beer production, possess these enzymes. Styrene is quantified with a headspace GC-FID. The hydroxycinnamic acids and 4VG are determined with HPLC-DAD. The fermentation temperature was set at 16 and 25°C. The influence on the release of CA during mashing is determined for the parameter's temperature (25–80°C), pH (4.2–6.8), and time (10–300 min). This work should present a practical aid for brewers to help minimize the content of styrene in wheat beer by optimizing different fermentation parameters. Higher fermentation temperatures and an open fermentation management lead to a rapid decrease in styrene. Fortunately, these two parameters also lead to higher formation of 4VG. In the case of widely used bottle fermentation, it can be assumed that the use of POF+ yeast strains will lead to a higher styrene content in the final beer. Furthermore it could be shown that all available CA in wort was converted to styrene within a few hours. In addition to the improvement in fermentation management there is also the possibility to minimize the formation of styrene by a targeted selection of low-CA malt and an optimized mashing process. A screening of different malt types and the influence of temperature, time, and pH on the release of CA during mashing is also part of this research. These results will be compared with the existing publications on the release of ferulic acid during mashing.

Frank-Jürgen Methner conducted studies in brewing science at Berlin Institute of Technology (TU) from 1975 to 1981. After the studies, he began working as an operating supervisor at the Schlösser Brauerei, Düsseldorf. From 1982 to 1986, he was a scientific assistant with teaching duties. Research projects and Ph.D. thesis, "Aroma Formation of Berliner Weissbier with Special Focus on Acids and Esters," were further tasks. For 18 years, starting in 1987, he held a leading position as a director at the Bitburger Brauerei, Bitburg, Germany, with responsibilities in fields such as research and technology, as well as quality assurance. Beginning with the winter semester 2004/2005 he took over the Chair of Brewing Science within the Department of Biotechnology at Berlin Institute of Technology (TU Berlin).

242. High throughput evaluation of industrial growth conditions for industrial *Saccharomyces* yeasts. Presenter: Anita Van Landschoot, University College Ghent, Ghent, Belgium. Co-author(s): Sylvie Vandoorne and Dana Vanderputten, University College Ghent, Ghent, Belgium; Gary Prescott, BioTek Instruments Inc., Luzern, Switzerland.

Saccharomyces strains are industrially important yeasts in the production of many beverages. The ability to obtain the desired product reliably and repeatedly in the same amounts requires careful monitoring of not only the material inputs, but also the growth of the yeast strain during propagation or hydration/revitalization, in the case dried yeast, that is used for the fermentation process. This often requires monitoring of the growth of these strains under various conditions to optimize industrial conditions. The Synergy H1 hybrid multi-mode microplate reader was used to provide temperature control, suspension agitation, and monitor cellular yeast growth using light scattering in 96-well microplates at 600 nm. Measurements were made every 2 min, and data were collected using Gen5 data analysis software. The system was used to explore the optimum conditions for propagation and hydration/revitalization of 13 industrial yeast strains. These *Saccharomyces* yeasts were used to study the effect of temperature, pH, and density of industrial media (wort or must of white grapes) on yeast growth/biomass production. Lowering the pH of the wort to an industrially acceptable value of 4.8 had almost no effect on yeast growth for the different densities of the wort. Lowering the density of the wort or must to about 10°P extract of malt or grapes always had a positive effect on yeast growth. This proved that such medium contains sufficient nutrients for the industrially necessary yeast growth during propagation or hydration/revitalization. If it is industrially relevant, a higher yeast growth temperature can be used. At 30°C the same final cell number can be obtained in half the time compared to room temperature. The final conclusion is that the Synergy H1 microplate reader is a useful high throughput system to screen for optimal industrial growth/revitalization conditions for industrial yeasts. The system has also been tested for fast screening of yeast fermentation conditions.

Anita Van Landschoot is a professor at University College Ghent and Ghent University and lecturer in brewing technology and industrial microbiology. The research of Anita's group is related to applications of microbial and enzymatic biotechnology: industrial yeast starter cultures, microbial contaminants, microbial populations, antibacterial activity, industrial fermentation processes, glycobiology, and isolation and characterization of microorganisms and some enzymes. The group has the technology and know-how for extraction and fermentation of biomass to ethanol and for the brewing of most Belgian beer types. Most of the research is done in collaboration with industry. The brewing lab represents the oldest Belgian brewing institute.

243. Impact of hops and yeast strains on production of hydrogen sulfide during fermentation: H_2S production from five hop varieties with lager and ale yeast. Presenter: Seung Park, Kyung Hee University, Yongin-Si, Korea. Co-author(s): Beom Seon Lee and Joo Seok Bang, Kyung Hee University, Yongin-Si, Korea.

Hop is an essential ingredient in brewing; however, there is a lack of understanding as to how hop addition affects hydro-

gen sulfide (H_2S) production during fermentation. In this study, five dried leaf hop varieties were investigated for their H_2S production during fermentation by lager and ale yeasts. Laboratory scale fermentations were conducted in a 2 L bottle with a commercial malt extract. Fermentation temperatures were 10 and 15°C for lager and ale, respectively. Quantitative measurements of H_2S were carried out at 24-hr intervals using an H_2S detection tube method (FIGASA sulfur stick) that was easy, convenient, and reliable for quantifying H_2S production throughout fermentation. A lengthy lag period was observed for lager fermentation at lower fermentation temperature, whereas ale yeast fermented at 15°C started fermentation immediately after yeast pitching. Lager yeast produced much higher levels of H_2S, while ale yeast produced only trace levels or none. Two or three distinctive H_2S peaks were observed from lager fermentation during the course of fermentation, and the highest levels of H_2S were produced when the yeasts were actively fermenting the wort. Different hop varieties exhibited various levels of H_2S production, with the levels between 33 and 46 µg/L for lager yeast; ale yeast produced trace levels or none regardless of hop varieties. This result indicates that hops and yeast strains are the potential sources of H_2S overproduction. Accordingly, identifying sources of H_2S production such as yeasts and hops in brewing can significantly reduce H_2S levels.

Seung Park obtained a B.S. degree in food science and technology in 1981 from Kyung Hee University in Korea and then joined the Technical Research Institute of Dong Suh Foods Corporation (a joint venture of Kraft Foods in the United States), where he has worked on coffee flavor chemistry, technology, and process engineering for instant coffee manufacturing. From 1986 tot 1993, Seung attended the University of California at Davis, where he received M.S. and Ph.D. degrees in food chemistry and biochemistry, specializing in wine flavor chemistry. He was a post-doctoral researcher at Ernest and Julio Gallo winery research laboratories in Modesto, CA. Currently, Seung is a professor of food chemistry and analysis at Kyung Hee University in Korea, and his major research interests include wine and beer flavor chemistry and biochemistry, especially development of new analytical methods that are important for the quality improvement of wine and beer products.

244. Impact of hops on production of hydrogen sulfide during fermentation: H_2S production from different levels of elemental sulfur. Presenter: Seung Park, Kyung Hee University, Yongin-Si, Korea. Co-author(s): Beom Seon Lee and Joo Seok Bang, Kyung Hee University, Yongin-Si, Korea.

To study the potential impact of hydrogen sulfide (H_2S) production from residual sulfur on leaf hops, increasing levels of elemental sulfur were spiked into the wort and fermented by lager yeast. One representative dried leaf Perle hop selected from our previous studies was washed with tap water in order to remove any residual sulfur that might be left on the leaf hop at harvest. Six increasing levels of elemental sulfur (0, 1, 2, 3, 4, and 5 mg/L) were spiked into wort that had been hopped with washed hops previously. Fermentations were conducted at 15°C in laboratory scale fermentors. Non-washed hops were used as a control to compare the H_2S production with the sulfur-spiked wort samples. H_2S production was quantitatively measured at 24-hr intervals using a H_2S detection tube method developed in our lab. Residual H_2S levels in the finished beers were also determined using the H_2S detection tube method. Yeast produced the highest levels of H_2S during the active fermentation period, and a high level (>3 ppm) of sulfur spiking produced higher levels of H_2S during fermentation, and the H_2S concentration (>5 ppb) remained high in the resulting beers. Increasing addition of elemental sulfur to certain levels, however, did not proportionally produce increased levels of H_2S, indicating that specific yeast-sulfur interactions during fermentation were apparent.

Seung Park obtained a B.S. degree in food science and technology in 1981 from Kyung Hee University in Korea and then joined the Technical Research Institute of Dong Suh Foods Corporation (a joint venture of Kraft Foods in the United States), where he has worked on coffee flavor chemistry, technology, and process engineering for instant coffee manufacturing. From 1986 tot 1993, Seung attended the University of California at Davis, where he received M.S. and Ph.D. degrees in food chemistry and biochemistry, specializing in wine flavor chemistry. He was a post-doctoral researcher at Ernest and Julio Gallo winery research laboratories in Modesto, CA. Currently, Seung is a professor of food chemistry and analysis at Kyung Hee University in Korea, and his major research interests include wine and beer flavor chemistry and biochemistry, especially development of new analytical methods that are important for the quality improvement of wine and beer products.

245. Investigating the influence of wort amino acid composition on fermentability using a model solution. Presenter: Blanca Gómez G., Laboratorio Tecnológico del Uruguay (LATU), Uruguay. Co-author(s): Aaron MacLeod, Grain Research Laboratory, Canadian Grain Commission, Canada; Tania De León, Laboratorio Tecnológico del Uruguay (LATU), Uruguay; Michael J. Edney, Grain Research Laboratory, Canadian Grain Commission, Canada.

Fermentability is an important malt quality parameter as it predicts potential beer production. The most important process in brewing fermentation is the assimilation of carbohydrates and nitrogenous compounds by yeast. An adequate level of free amino acids in wort ensures efficient yeast growth and, hence, appropriate fermentation performance. Wort is a very complex medium containing a range of nutrients, and this complicates research on relationships of fermentability with micronutrients. To study the relationship between individual amino acid profiles and fermentability, a controlled medium (broth) was created with high maltose syrup and pure amino acids. The broth simulates a standard wort (12°P), and pure amino acids were added to the solution at concentrations similar to what is expected in a standard 12°P malt wort. Four amino acids (glutamine, asparagine, arginine, and lysine) were studied in depth by varying their concentration in the broth while maintaining the same total amino acid content. Each amino acid was studied separately by preparing three model solutions. The first contained the standard concentrations for all amino acids; the second increased the proportion of the one amino acid being studied; and the third contained a decrease in the proportion of the one amino acid being studied. Each solution was fermented using three different yeast strains (lager, ale, and Fleischman). Results showed a significant effect of yeast strains on fermentability level. Furthermore, changes in lysine and glutamine concentrations showed a significant effect on fermentability for some of the yeast strains, but asparagine and arginine presented no significant effect on fermentability for any of the yeasts. Similar significant reductions in pH during fermentations were also found. It was concluded that individual yeast strains have varying requirements for amino acids, and these requirements are not the same for each essential amino acid.

Blanca Gómez received a B.S. degree in biochemistry from Universidad de la República in Montevideo, Uruguay. She began employment with Laboratorio Tecnológico del Uruguay (LATU) in 1998 as a chemist in the analytical laboratory of the Malting Barley Unit. She worked in quality assurance and became a quality manager for ÖVQ.

In 2009 Blanca received an M.S. degree in food science from the University of Manitoba and is working on her research project at the Grain Research Laboratory, Canadian Grain Commission, in Winnipeg, MB. Since 2009 she has functioned as a scientist in the Cereals Department of LATU and is in charge of research projects. She belongs to the Uruguayan Barley Board, serving as executive secretary from 2010 to 2011.

246. Methods and applications for the appropriate characterization of microorganisms. Presenter: Konrad Müller-Auffermann, Forschungszentrum Weihenstephan, Freising, Germany. Co-author(s): Friedrich Jacob, Forschungszentrum Weihenstephan, Freising, Germany.

Thousands of different microorganisms are applied in the beverage industry worldwide. In order to obtain more information about their characters, small scale fermentations are necessary. These laboratory sized fermentations unfortunately do often not reflect realistic situations, as they can be found in the industry. The reasons are multicausal. For instance, distortions can be caused by the substrate used, which can vary in quality and composition. Also the fermentor itself, the number of fermentations, the physiological condition of the organisms, and the appropriate statistic interpretation can lead to difficulties that impede adequate scale up. This presentation therefore is devoted to this important and practical relevant theme. Different types of fermentors, natural and artificial worts, necessary requirements, and process and interpretation methods are discussed as they were reviewed and tested. Subsequently a new concept for appropriate small scale fermentations is illustrated. This construction contains numerous tanks; is comparatively relatively inexpensive and easy to clean and handle; and allows a certain scale up. Finally test results generated in this plant under the variation of applied biomass, substrate aeration, fermentation temperature, and system pressure for one of the most common yeast steams (TUM 34/70) is presented.

Konrad Müller-Auffermann had two years of international experience before he began studying drinking and brewing technology at the Technical University of Munich. During his studies in Weihenstephan he worked for several mayor construction companies, partly in other countries. In 2009 Konrad was employed at the Research Center Weihenstephan for Brewing and Food Quality as a consulting engineer. In 2010 Konrad became the head of the Research and Development Department of the institute.

248. Modern brewery yeast management. Presenter: Helmut Kuhnl, Esau & Hueber, Schrobenhausen, Germany.

Yeast is the single most precious asset that a brewery owns and our most important operator. Propagation conditions should ensure that the "best" possible amount of yeast is produced, which provides an optimal fermentation performance when pitched. Yeast can survive and grow in the presence or absence of oxygen. In terms of the yeast cell, its survival and growth is optimal in the presence of oxygen. Only in an aerobic environment is yeast able to synthesize unsaturated fatty acids and sterols, which are important components of the cell membrane and responsible for cell growth. Aerobic propagation creates most vital cells with a high fermentation power and helps to suppress potential bacteriological issues. Modern systems use an aeration device that ensures a uniform distribution of air and a homogeneous mixture in the yeast suspension. Ideally the design of propagation vessels should be without intrusions, i.e., agitators/stirrers, to accomplish the highest microbiological safety. Hence observations on prop systems have proved that aeration outside the propagator with a two-component jet in the loop is the better choice in terms of hygiene compared to built-in aeration equipment. External aeration provides further process benefits like the creation of tiny bubbles, less foaming, homogeneous yeast suspension, perfect air distribution, and minimal and efficient air flow. The generated vital cells incorporate high fermentation power and suppress potential bacteriological issues. Typical process control parameters are the scheduled time of propagation, temperature, duration of aeration and frequency of airflow per hour, pitching startout cell count resp, the residual propagator volume, and wort volume added. In addition to propagation modern yeast management includes the possibility of crop yeast revitalization to guarantee a perfect fermentation performance. This is achieved by circulation with simultaneous aeration of the yeast before re-pitching, using the same loop as for propagation for removing the fermentation CO_2, homogenization of the crop yeast in the yeast storage/pitching vessel, adding fresh O_2 to the crop yeast, and supplying nutrient fluid (wort) if required. Rigid receipt control systems for yeast propagation are not flexible enough to run yeast propagation without observation and manual interaction of an operator. This belongs now to the past. In-line measurement of cell count and gravity in the loop allow an optimized adjustment of temperature and aeration rates according to yeast growth and residual extract with the new yeast propagation manager YPM with the objective to achieve the best yeast vitality at point of pitching. YPM is a self-learning fuzzy logic software application acting like an experienced operator. This virtual expert observes nonstop all the process parameters, detects trends and reacts, operates 24/7 without breaks, holiday, and pay slip, releases operators from routine jobs, increases plant efficiency, flexibility, and reliability, and last but not least saving money. YPM is the first fully automatic on-line yeast propagation system.

In 1974 Helmut Kuhnl apprenticed in brewing and malting at the Spaten-Franziskaner-Bräu Munich before moving on to study at TU Weihenstephan. He received the Diplombraumeister degree in 1980 and worked in leading sales positions for several process equipment suppliers. Taking evening classes he obtained his BBA. In 1998 Helmut joined Esau & Hueber as a sales manager and was significantly involved in the development of the company into one of the world's leading suppliers of yeast management systems during the last decade.

249. Organic acids in the brewing process—A new approach in "drinkability." Presenter: Thomas Tyrell, VLB Berlin, Germany.

Organic acids had been of minor interest due to relatively high flavor thresholds in beer. For that reason relatively little is known about technological possibilities to influence their concentration. Due to new knowledge from medical research about salubriousness a rising interest in organic acids has been observed. A few years ago a paper concerning gastric acid output introduced by alcoholic beverages was published. It was observed that alcoholic beverages from fermentation lead to a higher output of gastric acid then alcoholic beverages from distillation. As responsible substances organic acids, which are formed during fermentation, were described. From these findings it was assumed that it could present an improvement for fermented beverages to reduce the concentration of special organic acids. In this way the salubriousness of the beverage could be enhanced, and finally the drinkability could be improved. The presentation gives an overview of technological possibilities to influence the concentration of organic acids in brewing, pathways of their formation re discussed, and the latest research results of a VLB project are presented.

Thomas Tyrell apprenticed as a brewer and maltster at the Pott's Brauerei in Oelde, Germany, from 1991 to 1994. After his appren-

ticeship, from 1994 to 2001 he gained working experience in different breweries, including Saxer Brewing Company (Portland, OR), Wettringer Brauhaus (Wettringen, Germany), Weißbräu Brauhaus (Cologne, Germany), and Magister Cervecerías Artesanales (La Coruña, Spain). Studying in Berlin at the Technical University he received a Diplom Ingenieur degree in brewing science (2001–2006) (diploma thesis at Cervecería Polar, Caracas Venezuela). Since early 2007 he has been at VLB, and since 2008 part of the VLB Department of Brewing and Beverage Science & Applications. At VLB he is responsible for research/consulting projects. In addition coordination of the institute's activities for Iberoamerica are part of his responsibilities.

250. Practical yeast culturing for brewpubs to productions brewing. Presenter: Derek Stepanski, The Saint Louis Brewery, St. Louis, MO, USA.

Practical ideas and techniques for culturing yeast. Covering techniques for the brewpub as well as for production brewing. These techniques include culturing from pitchable tubes, slants, and cryotubes.

Derek Stepanski has been the quality manager at The Saint Louis Brewery for the last four years. He is responsible for all things quality related. Along with his team he maintains and develops all the quality programs that help the Schlafly brand be successful. Derek received his B.S. degree in fermentation science from the University of California Davis.

251. Stress tolerance in group 1 and 2 lager brewing strains. Presenter: Chris Powell, University of Nottingham, UK. Co-author(s): Tobias Fischborn, Lallemand Inc., Canada.

Saccharomyces pastorianus strains are natural hybrids of *S. cerevisiae* and *S. bayanus* yeast and display properties particularly suited to the production of lager type beers. While it is accepted that lager strains arose approximately 200 years ago following a mating reaction between these two species, recent evidence has suggested that such an event may have occurred more than once in the evolution of this yeast. It has been suggested that the *S. pastorianus* species may contain two subgroups, designated Saaz (Group 1) and Frohberg (Group 2), as a result of separate mating events. These groups have been broadly differentiated according to a number of criteria including genome rearrangements, gene copy number, DNA sequence polymorphisms, and differences in ploidy. Despite detailed genetic analysis, only limited studies have previously been performed to characterize their phenotypic properties. The purpose of this study was to investigate the similarities and differences between the physiological characteristics of Saaz (Group 1) and Frohberg (Group 2) yeast. A number of strains belonging to each group were selected and assessed for their growth characteristics and their capacity to assimilate sugars. Furthermore, each strain was analyzed for its ability to withstand stress factors associated with the production of alcoholic beverages, including temperature and osmotic and oxidative stress. The data obtained and presented here indicate that there are some fundamental differences between the capacity of each yeast group to respond to their immediate environment. While this is particularly interesting for the understanding of lager brewing yeast strain variation, it may also be significant for associated industries such as the biofuel industry, where investigation into the properties of industrial yeast strains is of particular importance, or the active dried yeast industry, where robust brewing yeast strains are required to withstand stress factors associated with desiccation, in addition to favorable properties during fermentation.

Chris Powell holds a Ph.D. degree on the subject of yeast cellular aging and fermentation performance from Oxford Brookes University, U.K. Chris has also occupied research positions at Bass Brewers (now Coors UK) and more recently at Lallemand, based in Montreal, Canada. During his six years at Lallemand, Chris was responsible for the R&D laboratory for the molecular identification and characterization of micro-organisms utilized within the food and beverage industries, in addition to research focused on brewing yeast. In 2010 Chris returned to the United Kingdom to take up his current position as lecturer in yeast and fermentation at the University of Nottingham. Chris is presently involved in research in the areas of both brewing science and sustainable bioenergy. Chris is the author or co-author of more than 40 scientific publications and is a regular reviewer for several scientific journals. Chris has also served on the ASBC Technical Committee since 2005 and the ASBC Board of Directors since 2010. Outside of work, Chris is a keen soccer player and spends a significant portion of his time running, hiking, and exploring different parts of the world.

252. The evolution of the yeast monitor as a critical process control instrument within modern breweries. Presenter: John Carvell, Aber Instruments, Aberystwyth, UK. Co-author(s): Christopher Boulton, University of Nottingham, Nottingham, UK.

The concept of using a radio-frequency impedance (RFI) probe for measuring the concentration of live yeast cells was first published back in 1987, and in this paper we review how this invention has evolved over the last 20 years to become a critical process control instrument in the modern brewery. The first biomass sensor that detected cells, by virtue of their dielectric properties, was designed for applications in bioreactors and could be used with a wide range of eukaryotic and prokaryotic cells at relatively high cell densities. In order to operate in breweries with packed yeast cell densities at low and often varying conductivities, the original "biomass monitor" instrument had to be substantially modified before the first "yeast monitor" was introduced into the brewing market in 1991. The justification for installing a yeast monitor is usually based on the ability of the probe to provide precise and repeatable control of yeast pitching rates. It follows that procedures that lead to precise and repeatable control of yeast pitching rate will result in consistent fermentation performance. The traditional methods of taking a grab sample from the yeast storage vessel, diluting, and then quantifying the live yeast concentration in the laboratory are time-consuming and require skilled personnel to ensure precision and repeatability. Most of the large international brewing groups have now installed the Aber yeast monitor, and in recent years some of the larger microbreweries are now operating with the system. The actual improvements to a process after installation of the instrument were first published by the Bass Brewing Group, Miller, and Guinness in the early 1990s, but much of the data in this area are still not public. A comprehensive study by SAB Miller in 2011 shows the improvements in fermentation rates and the real degree of fermentation for certain brands after the installation of the yeast monitor. Although the vast majority of applications of RFI in the brewing industry are in-line applications with the probes installed around the yeast storage vessels, the probe is being used to monitor and in some cases control the fermentation process. The most comprehensive study with brewing fermentors involved following the spatial distribution of live yeast within a production vessel using a series of submergible RFI probes. We also show how the probe has become a critical control parameter for some continuous fermentation in bioethanol and other non-brewing fermentations. This review of the development of the yeast monitor for the brewing process concludes with the introduction of a compact version of the instrument where the electrodes and the electronics are all built

into an IP 65 stainless steel housing. We describe the rationale behind the new design and show how the same components can be utilized within an off-line instrument and a version for mounting on propagators and fermentors.

John Carvell is a graduate in biochemistry and received his Ph.D. degree at Newcastle University, U.K. He held roles as production manager at yeast manufacturer and senior sale roles within APV and Alfa Laval before joining Aber Instruments Ltd. as a director. With the business over 90% export and split between both the brewing and biotechnology industries, he spends a large proportion of his time visiting key customers involved in a diverse range of applications. John has presented posters at many of the major brewing conferences and also presented papers at the SIM (Society of Industrial Biotechnology), RAFT (Recent Advances in Fermentation Technology), ACS, ASBC, and MBAA annual meetings. When time permits John enjoys a number of activities, including badminton and fly fishing, as well as coaching a junior cricket team.

253. The Nalco yeast activity monitor: Brewing applications. Presenter: Michael Bradley, Nalco Company, Naperville, IL, USA.

Inconsistent yeast management practices can negatively impact the consistency of fermentation and the quality of beer produced. A new laboratory instrument for automated yeast activity measurements is now offered by Nalco Company. The fundamental basis of the technology is a fast, fluorescence generating reaction that targets native yeast enzymes. The instrument's interactive touch screen computer guides the user through the sample preparation steps, which are performed on a digital balance connected to the instrument and require about 30 sec of hands-on time. Several key features differentiate this new technology from the industry standard practices of microscopic cell counting and viability staining. The sample preparation and reaction monitoring procedures employed are completely non-subjective and automated, meaning that anyone can perform the measurement without introducing operator-to-operator variability. The results are automatically logged by the instrument and made immediately available over a network for integration into reports, databases, and control systems. Finally, the activity levels measured with the system depend on the number of viable cells in the sample, as well as the metabolic activity (vitality) of the population. Examples will be presented of successful application of this technology for optimizing propagation, yeast pitching from a slurry, and fermentation monitoring in the brewing industry.

Mike Bradley lives in the suburbs of Chicago and works as an R&D scientist at Nalco Company, where he develops microbial detection and control technologies to improve process efficiencies for Nalco's customers. Prior to working at Nalco, Mike trained at the University of Chicago and the University of Florida, where his research combined elements of bioinformatics, evolutionary theory, and wet-lab approaches to explore biomolecular structures and functions. Mike received his Ph.D. degree at the University of Illinois Chicago for his thesis on yeast prion proteins.

254. Threshold detection of premature yeast flocculation inducing malt using the miniature fermentation assay. Presenter: Joshua Adler, Dalhousie University, NS, Canada. Co-author(s): Alex Speers, Dalhousie University, NS, Canada.

Premature yeast flocculation (PYF) is a burden on the malting and brewing industries. It causes production difficulties and quality issues characterized by high sugar concentrations and low yeast cell counts post-fermentation. This results 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 industry relies on a variety of fermentation assays. These methods can indicate if a sample displays PYF, but they do not determine the threshold at which PYF occurs. This study used modified miniature fermentations to investigate how a concentration of known PYF malt can influence the fermentation. Using wort prepared from varying ratios of control and PYF malts the change in absorbance and Plato was monitored. In addition, a PYF factor was extracted and used in varying amounts in a synthetic wort (i.e., malt extract) and fermented. While it is difficult to specify the actual amount of PYF factor extracted, this "PYF solution" was used in a second experiment to make up synthetic worts at levels of 0–100%. These trials were conducted using a 15 mL fermentation with a consistent temperature and pitch rate (21°C, 1.5×10^7 cells/mL). At designated intervals throughout each fermentation, yeast in suspension was monitored spectrophotometrically at 600 nm, and the apparent extract was measured. It was found that varying the ratio of either PYF malt or the amount of PYF factor could influence fermentation behavior. In the first experiment, low PYF malt levels (i.e., 20 and 40%) had no significant difference ($P > 0.05$) on absorbance. However, significantly different absorbance measurements ($P < 0.05$) were found when using concentrations of 60% PYF malt or higher. In the second series of experiments no difference from the control to the PYF solution at strengths of 20, 40, and 60% was observed ($P > 0.05$). But, significantly different fermentations ($P < 0.05$) were noted when malt extract was prepared with 80 and 100% PYF solutions. These findings agree with previous work but also suggest that PYF malt may be blended with typical malt as long as a critical threshold is not exceeded. This technique provides the brewer with another tool for making informed decisions when managing their process stream. Further statistical analysis of extracted measurements will also be presented.

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 gain 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" and hopes to contribute innovative findings on the fermentability of malt, as well as pass on valuable knowledge as a teaching assistant in product development and quality assurance courses. When outside the laboratory, Joshua can usually be found in the boxing ring training for an upcoming bout 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. He recently returned from the Lowland region of Scotland, where he visited a variety of breweries and distilleries.

255. Understanding and evaluating the effect of wort boil time and trub levels on malt fermentability with the miniature fermentation. Presenter: Ankita Mishra, Dalhousie University, Halifax, NS, Canada. Co-author(s): Alex Speers, Dalhousie University, Halifax, NS, Canada.

The basic aim of barley malt breeders and maltsters is to produce malt with optimum fermentability levels, which enables maximum alcohol yield from fermentable wort dissolved solids (extract). This challenge includes understanding and assessing the effects of physical processes involved in beer preparation such as wort boiling and trub formation. The intent of this research was to understand and evaluate the effect of wort boiling and autoclaving at varying time periods (30, 45, 60, 90, and 120 min) on malt fermentability. The effect of trub content was also analyzed. Small-scale fermentations were carried out using a "reference malt" and the now standard SMA yeast strain. The apparent degree of fermentability (ADF), turbidity (absorbance

at 600 nm) and density (°P) was measured at specific time intervals over the 3-day fermentation period. The ADF relates to the relative percentage or the extent to which wort is fermented. The decline in density was modeled with the logistic equation that predicts a sigmoidal shaped decline in density. Turbidity measurements reflect the relative amount of yeast cells present in the fermenting wort and can be modeled with a tilted Gaussian fit. The data sets were then modeled and compared with ANOVA type analysis using PRISM software. From the results obtained, we suggest that upon boiling the wort without trub (at 100.2°C) for a range of times significant differences in ADF are obtained ($P < 0.05$). All fermentation runs, with and without trub, at lower boiling or autoclaving times were faster than longer boiling or autoclave treatments showing steeper extract curves when modeled. Malt fermentability also considerably declined upon treatment of wort with high autoclave temperature and pressure levels (121.1°C, 2 atm). The amount of trub formed after boiling or autoclaving was found to be 0.2–0.32 g in 500 mL of wort. The presence of trub gave mixed results. When wort was autoclaved, trub significantly lowered ($P < 0.05$) the fermentation rate. However, when boiled wort containing trub was fermented, no significant difference in the fermentation rate were noted ($P > 0.05$). Free amino nitrogen (FAN) level and wort color (EBC units) were also determined and compared after each specific wort treatment.

Ankita Mishra received her bachelor of technology degree in biotechnology from the Vellore Institute of Technology, India, in 2009. She began her master of science degree in food science at Dalhousie University in 2009. Ankita received Industrial training from Dabur Industries (India) in plant biotechnology and quality control in 2008. She has previously worked with Biostadt (India Ltd.) in quality assurance in manufacturing bio-pesticides and related manufacturing processes.

256. Use of structured problem solving methodology to improve acid wash yeast process. Presenter: Sarah Willis, MillerCoors LLC, Milwaukee, WI, USA. Co-author(s): Vince Coonce, Liana Turner, Amina Daar, Kamran Amini, Daniel Pearson, and Gustavo Charry-Parra, MillerCoors, Milwaukee, WI, USA.

This poster illustrates the use of the DMAIC process for improving the consistency of acid washed yeast pH. A focused improvement team consisting of a cross-functional group of brewery employees looked for a sustainable improvement in the current acid washing process with the goal of removing the variability that can lead to poor yeast health or poor microbial control, and thus improve the quality of the yeast crop for pitching. Using a statistical model to determine the level of impact to the process, measurement of the effects of variables in the acid washing process (i.e., gauge R&R of instrumentation, acid-yeast contact time, variability of acid concentration, post-acid sample times) led to a review of operator SOPs and the procedure for determining the volume of acid used for washing. Improvements made to the acid washing process reduced post-acid addition pH variability.

Sarah M. Willis is a senior quality assurance engineer at the MillerCoors Milwaukee brewery. She holds a degree in chemistry from Alcorn State University in Lorman, MS, and has been employed with MillerCoors for 13 years.

Symposia

BCOJ Symposium: Technology for the Future

S-1. Yeast comprehensive analysis system for evaluating fermentation performance. Presenter: Hiroyuki Yoshimoto, Kirin Brewery Company, Limited, Yokohama, Japan.

In Japan, alcoholic beverages such as beer and low- and no-malt brews, are produced in line with the Japanese taxation system. After the mid-1990s, a lot of new types of low- and no-malt brewed beverages were launched in response to changes in the tax system. In these beverages, various ingredients were used in place of malt to lower applicable taxes. Because the performance of brewer's yeast is unpredictable under these variable low- and no-malt conditions, many problems can arise, such as those related to delayed fermentation and off-flavor production, which are difficult to resolve using only traditional yeast analysis methods. The bottom fermenting yeast *Saccharomyces pastorianus* is reported to have arisen as a natural hybrid of two *Saccharomyces* strains, *S. cerevisiae* and *S. bayanus*. The *S. pastorianus* genome includes *S. cerevisiae* (Sc)-type genes and orthologous lager-fermenting-yeast specific (Lg)-type genes derived from *S. cerevisiae* and *S. bayanus*, respectively. As the genome structure of *S. pastorianus* is complex, it was difficult to solve these problems using analytical approaches commonly applied to *S. cerevisiae*. Therefore, a need exists for the development of novel analysis systems suitable for the bottom fermenting yeast *S. pastorianus*. Here, we developed a comprehensive analysis system for evaluating yeast fermentation performance using not only established methods, but also newer comprehensive methods in the bottom fermenting yeast *S. pastorianus*. Our system analyzes DNA, gene expression, protein, and metabolite levels, as well as phenotype, and thus comprises a powerful tool with which to improve the fermentation performance of the bottom fermenting yeast *S. pastorianus*. DNA level analysis by PCR, single nucleotide polymorphisms (SNPs), and chromosomal structure was used to distinguish between bottom fermenting yeast *S. pastorianus* strains. In addition, DNA copy number profiling using array-based comparative genomic hybridization was able to detect gene copy number aberrations, which are useful for determining the stability of DNA to evaluate fermentation performance. Analysis of gene expression level using oligonucleotide microarrays for expression profiling of orthologous genes demonstrated that the expression of particular Lg-type genes differed from that of orthologous Sc-type genes, suggesting that certain Lg-type and Sc-type genes may have different functional roles. For analysis at the protein level, DNA identification from partially purified proteins using genome information and gene disruption methods was an effective approach to understand a mechanism. Analyses of metabolites and phenotypes levels by determining intracellular metabolite concentrations using CE-TOFMS and by quantifying cell morphogenesis using the image processing program CalMorph were performed for evaluating yeast physiological status to find clues to solve problems. In this presentation, we focus on our yeast comprehensive analysis system, which was specifically applied to predicting the physiological state of *S. pastorianus* by combination analyses of intracellular metabolite concentrations and quantitative cell morphogenesis.

Hiroyuki Yoshimoto received a Ph.D. degree in engineering from Hiroshima University, Japan, in 1992. He began his career researching yeast in the Central Laboratories for Key Technology with Kirin Brewery Company, Limited. He also studied yeast technology at Stanford University, United States, from 1999 to 2001. Since 2007, he has been working in the Brewing Technology Development Center at Kirin Brewery Company, Limited.

S-2. Research of brewer's yeast based on genome information. Presenter: Tomoo Ogata, Asahi Breweries, Ltd., Moriya, Japan.

Brewer's yeast has unique characteristics different from other yeasts, such as high wort fermentation ability and flocculation. We investigated these characteristics of brewer's yeast by genome analysis, which has rapidly advanced in recent years, to contribute to the construction of more adequate yeast strains. Bottom-fermenting yeast, *Saccharomyces pastorianus*, is a natural hybrid between *S. cerevisiae* (SC) and *S. bayanus* (SB). Therefore, it is different from *S. cerevisiae*, which laboratory yeast strains and top-fermenting yeast belong to. This fact has been confirmed by Southern hybridization and other experiments (Yamagishi and Ogata, System. Appl. Microbiol. 22:341-353, 1999). Bottom-fermenting yeast has both SC- and SB-types of genes and chromosomes. But, our genome analysis revealed that bottom-fermenting yeast has some characteristic chromosomes, and some of these chromosomes have SC and SB sequences crossing each other (Ogata et al, J. Applied Microbiol. 107:1098-1107, 2009). One of the characteristic chromosome structures of bottom-fermenting yeast is SC-type chromosome VIII. The Lg-*FLO1* gene, which encodes the specific agglutinin, is located on the right arm end of SC-type chromosome VIII. In the case of the laboratory yeast *S. cerevisiae* S288C, *FLO5* is located on this region. In most bottom-fermenting yeast strains, this region is heterozygous. In the case that LOH (loss of heterozygosity) occurs and Lg-*FLO1* is missing, these yeast strains lose flocculation ability (Ogata et al, J. Applied Microbiol. 105:1186-1198, 2008). We constructed new yeast strains using the genome information of bottom-fermenting yeast. Bottom-fermenting yeast has both an SC- and SB-type *SSU1* gene that encodes for the sulfite efflux pump. We successfully constructed a brewer's yeast with high *SSU1* expression that enhanced the sulfite-excreting ability and diminished the production ability of hydrogen sulfide, MBT, and 2M3MB. This new brewer's yeast strain would contribute to the production of superior quality beer (Iijima and Ogata, J. Applied Microbiol. 109:1906-1913, 2010).

Tomoo Ogata received an M.S. degree in pharmacology science from Chiba University. After graduation in 1985, he worked on brewing microbiology at the Research Laboratories for Brewing, Asahi Breweries, Ltd. He received a Ph.D. degree in microbiology science from the University of Tokyo in 1997 and an award from the Brewing Society of Japan in 2001.

S-3. Study on the attractive hop aroma for beer. Presenter: Takako Inui, Suntory Liquors Limited, Osaka, Japan.

Aroma characters derived from hops are very important for the quality of beer, as they can give an attractive sensory impact to beer by choosing suitable hop varieties, hop boiling conditions, fermentation conditions, and so on. In order to develop strategies to facilitate an attractive hop aroma in beer, we have been trying to clarify the relationship between hop aroma qualities in beer and brewing conditions. Many researchers have been studying hop aroma focused mainly on the essential oils in hop such as terpenoids. But these compounds are not necessary brought directly into beer products as they are. When they go through thermal reaction, oxidation, revelation from their glycosides, and biotransformation by yeast, there are chemical changes to different odor active compounds that are destined for final beer products. Such complexity makes the profiling of hop aroma in beer for mining the key compounds very difficult. In order to estimate the influencing compounds, the idea of "food metabolomics" was introduced for analyzing the data. Five hopped beers were brewed using different hop varieties, as well as unhopped beer. The aroma compounds were analyzed comprehensively by GC×GC/TOF-MS, and sensory evaluation was also performed. Both results, chemical analysis and sensory data, were subjected to multivariate analysis to correlate chemical components and sensory evaluation. As a result, influencing compounds were distinguished from other numerous compounds. For suitable boiling conditions to give preferable hop aroma quality and intensity, linalool, a floral note component in beer, was used as one of the indicators for the hopped beer, because controlling the concentration of linalool in beer is essential for achieving target quality. In order to rationalize the factors that may affect the concentration of linalool during the boiling process, we have carried out brewing experiments using model solutions, as well as pilot scale brewing under various conditions, such as the timing of hop addition, temperature and amounts of late hopping, gravity of the wort, and so on. We also have focused on other hop aroma compounds, in addition to linalool, to explain the differences in organoleptic characteristics of beers. For the fermentation conditions, two different brewing yeast strains were used to examine the resulting hop aroma characteristics and hop derived components. Although the same cold wort was used for the fermentations, significant differences in hop aroma portraits were perceived. The obtained results, thus, are attributable to differences in metabolites generated from the components derived from hops by different yeasts. Further detailed investigations on the effects of hop on beer aroma, particularly on the changes during brewing, are awaited.

Takako Inui graduated from Kyusyu University. She started her research career with Suntory Ltd. in 1989 at the Institute for Fundamental Research. Since 2002, she has been conducting research at the Institute for Beer Development on the development of brewing technology and flavor science of beers, including hops.

S-4. The effects of insufficient nutrition on flavor compounds production, propagation and fermentation of yeast. Presenter: Masahide Sato, Sapporo Breweries Ltd., Shizuoka, Japan. Coauthors: Atsushi Tanigawa, Sapporo Breweries Ltd., Shizuoka, Japan; Takeshi Arai, Sapporo Breweries Ltd., Oita, Japan; Tatsuro Shigyo, Sapporo Breweries Ltd., Shizuoka, Japan.

In Japan, beer-flavored beverages brewed with no malt have been available since 2003. The raw materials used in beer-flavored beverages with no or less malt compared with regular beer generally contain fewer nutrients than malt, which sometimes leads to sluggish fermentation and the production of off-flavors. In this paper, we review our studies on the effects of insufficient nutrients on off-flavor compound production, propagation, and fermentation of yeast. Among the off-flavors, sulfur-containing compounds, such as hydrogen sulfide (H_2S), are of particular interest for many brewing scientists and brewers, because they have an unpleasant flavor and very low threshold. We have extensively studied the production of hydrogen sulfide (H_2S) and its precursor, sulfite (SO_2), during fermentation in lager yeast. Upon the addition trials of three concentrations of methionine, we found that five genes (*MET3*, *MET5*, *MET10*, *MET6*, and *CYS4*) were regulated by our microarray analysis system. Further gene expression analyses revealed that the gene expression balance of *MET3* and *MET10* led to the production of a higher level of sulfite (SO_2) in the lager yeast. We also found that metabolism from SO_2 to H_2S by yeast occurred depending on the pH value during the secondary fermentation. The amount of H_2S was higher at the lower pH value during secondary fermentation. Furthermore, in order to reduce the risks in brewing new products, we investigated the possible production of other off-flavors that might

be important in brewing new raw materials. During the investigation, we detected several off-flavors that are different from sulfur-containing compounds. GC-MS-olfactometry analysis revealed that one of these flavors was indole. In brewing, indole has been recognized as an off-flavor and is thought to be produced by microorganism contamination during fermentation. It has not been reported that brewing yeasts produce indole during fermentation. We concluded that the lack of vitamin B_6 in wort led to the accumulation of indole in yeast due to the inhibition of tryptophan synthase reaction. Further study on the fermentation and propagation of yeast in the case of nutrient deficiency also shows that the lack of inositol induces a strange budding. In reviewing both of these findings, we discuss the essential combination of nutrients for brewing in order to reduce the risk of off-flavors and poor fermentation and propagation.

Masahide Sato is a general manager for Frontier Laboratories of Value Creation Sapporo Breweries, Ltd. He joined Brewing Research Laboratories Sapporo Breweries, Ltd. in 1990 after receiving an M.S. degree in applied microbiology from Tohoku University, Japan. From 1990 to 2008 he studied the genetic instability of flocculation of lager yeast and the sulfur amino acid metabolism of lager yeast. In 2002 he received a Ph.D. degree in applied microbiology from Tohoku University, Japan. In 2008 he moved to the Shizuoka brewery, in 2009 to the Kyushu Hita brewery (present name), and in 2010 to his present position.

EBC Symposium: Resources for the Future

S-7. Four years past the merger with The Brewers of Europe: What's new at EBC. Presenter: John Brauer, EBC

No abstract available.

S-8. The EBC Brewing Science Group: A different concept of scientific exchange. Presenter: Carsten Zufall, Cerveceria Polar Los Cortijos, Caracas, Venezuela.

No abstract available.

S-5. Visualizing fermentation in living yeast cells. Presenter: Sebastian Meier, Carlsberg Laboratory, Copenhagen, Denmark. Co-authors: Magnus Karlsson, Pernille Jensen, and Mathilde Lerche, Albeda Research, Copenhagen, Denmark; Jens Duus, Carlsberg Laboratory, Copenhagen, Denmark.

The visualization of biotechnologically relevant metabolic pathways in living microbial cells provides direct insight into cellular biochemistry and facilitates the optimization and control of cells in production. Special emphasis and interest is placed on the optimization of yeast fermentation with assays on intact cells with the hope of obtaining improved cell factories for fermentation. Classical studies of intracellular enzymes in isolation do not reconstruct the complex composition and macromolecular crowding of the intracellular milieu. In addition, enzymes do not necessarily operate in isolation inside the cell, but are often subpartitioned into functional complexes. The complexity of functional and structural organization inside the cell therefore makes the direct detection of cellular processes in their natural surroundings desirable for advancing the biochemical understanding and biotechnological control of metabolism. Direct observations of cellular reaction chemistry in living organisms call for noninvasive methods that resolve reactant signals and ideally detect natural substrates rather than chemically introduced reporter groups. The combined need for chemical detail on molecular transformations and sufficient time resolution to detect transient reaction intermediates is not met by conventional spectroscopic methods. Instead, the time course of cellular reactions in vivo is commonly approximated using isotope labeling patterns of metabolic products extracted from cells grown on defined substrates. We describe a novel methodology termed dynamic nuclear polarization, which yields substrate solutions with an approximately millionfold enhanced nuclear magnetic resonance (NMR) spectral signal relative to the complex cellular background to make real-time observations of fast metabolic reactions and short-lived pathway intermediates in vivo realistic. We show that the enhancement of nuclear spin polarization allows us to directly follow the flux of the glucose signal through rather extended reaction networks of central carbon metabolism in living fermentations. Experiments are conducted as real time assays of a few minutes duration that detect metabolic bottlenecks, pathway use, reversibility of reactions, and reaction mechanisms in vivo with subsecond time resolution.

Sebastian Meier received his diploma in biochemistry from the University of Regensburg (Germany) in 2000 and his Ph.D. degree in biophysics from the University of Basel (Switzerland) in 2004. He began his employment with the Carlsberg Laboratory (Copenhagen, Denmark) in 2007 as a staff scientist and was named senior scientist in 2010, serving as an expert on spectroscopy of complex systems.

S-6. Influence of different hop products on the *cis*/*trans* ratio of iso-alpha-acids in beer and changes in key aroma and bitter taste molecules during beer aging. Presenter: Martin Biendl, Hopsteiner HHV m.b.H., Mainburg, Germany. Co-authors: Christina Schmidt and Andreas Stephan, Bitburger Braugruppe GmbH, Bitburg, Germany; Christian Vogt and Thomas Hofmann, Chair of Food Chemistry and Molecular Sensory Science, Technische Universität München, Germany.

Isomerization of alpha-acids from hops can result in different ratios of *cis*- to *trans*-iso-alpha-acids. Whereas wort boiling in the brewhouse usually gives a ratio of approx. 2.5:1, it can be considerably higher in the case of isomerization outside the brewery when using suitable catalysts. In contrast to oxidative degradation processes, the conversion of iso-alpha-acids to tri- and tetracyclic compounds during beer aging is only possible in the *trans* form. Therefore it might be an advantage to use pre-isomerized hop products in order to maximize the *cis* form in beer. For this study beers conventionally hopped with regular pellets were compared to beers produced with isomerized pellets or isomerized extracts respectively (dosages at the beginning of wort boiling for each of the variations). In addition in-line pre-isomerization (isomerization of conventional hop products in the brewery prior to addition to the kettle) was investigated as another alternative. All hop products used were from the same variety (Hallertau Magnum, crop 2009). Their dosages were calculated in order to achieve identical bitter units in the final beers (target: 30 IBU). All of the four variations were produced in duplicate in a pilot brewery (20 hL scale). Bottled beers were stored at 5 and 28°C. Analysis of fresh and aged samples after 4, 8, and 12 months targeted bitter compounds (e.g., *cis*/*trans*-iso-alpha-acids, allo-isohumulones, allo-isohumulonhydroperoxides, allo-isohumulonhydroxides, tricyclohumols, tricyclohumenes, and tetracyclohumols by means of HPLC-MS/MS) and volatile aroma compounds (e.g., linalool and strecker aldehydes by means of GC-MS/MS). General wort and beer parameter analysis showed no significant differences between the various brews. Both the addition of regular pellets at the beginning of wort boiling and their in-line pre-isomerization in the brewery resulted in almost the same *cis*/*trans* ratio. As expected, beers pro-

duced with isomerized hop products showed higher ratios of *cis*- to *trans*-iso-alpha-acids, and their concentrations of tricyclohumols, tricyclohumenes, and tetracyclohumols were accordingly lower after aging. However, all of the four types of beers hopped in different ways showed very similar volatile aroma compound profiles, even during aging. Moreover sensory evaluation of aged beers showed no preference for the variations higher in *cis*/*trans* ratios of iso-alpha-acids.

Martin Biendl received a Ph.D. degree in organic chemistry from Regensburg University in 1990. He is head of the R&D/Analytical Department at the German branch of the Hopsteiner Group, one of the largest international hop growing, trading, and processing firms. His research experience is in the field of hop-related needs for the brewing industry and beyond. He is the representative of the International Hop Industry Cooperation in the EBC Analysis Committee and, since 2001, chair of the Hops Subcommittee. As an EBC representative he is also co-chair of the International Hop Standards Committee.