MBAA TQ vol. 49, no. 2 2012 pp. 39–69

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 suboptimal 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.